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Horticulture plays a crucial role in ensuring food security, enhancing nutritional diversity, and contributing to the economy through agricultural exports and job creation. With the global population projected to reach nearly 10 billion by 2050, the importance of horticulture in meeting the increasing demand for fruits, vegetables, and ornamental crops cannot be overstated. Moreover, horticulture contributes to environmental sustainability by promoting practices such as agroecology, organic farming, and urban agriculture, which minimize the use of chemical inputs and reduce environmental impact. However, despite these advancements, horticulture faces several challenges that need to be addressed to ensure its sustainable development. Climate change poses a significant threat to horticultural production, leading to unpredictable weather patterns, increased pest and disease pressure, and water scarcity. Additionally, the globalization of trade has brought about new challenges such as invasive species, market competition, and food safety concerns. Furthermore, ensuring equitable access to resources, promoting sustainable land use practices, and addressing socio-economic issues such as labour shortages and rural poverty are critical for the long-term viability of horticulture. Thus, continued research, innovation, and collaboration are essential for overcoming these challenges and advancing the field of horticultural science to meet the needs of future generations.

Journal of Horticultural Sciences attempts to attract the knowledge gained from the scientific community and share with the peers. The number of articles has increased to 39 including two reviews published in this issue. The editorial team gratefully acknowledge the authors, subscribers as well as members of the Society for Promotion of Horticulture for their constant support.

A review on heat stress in vegetable crops by ***Parveen et al.*** *highlighted the significant challenges posed by heat stress in vegetable crops and advocates for the development of heat-tolerant varieties. It comprehensively examines the impacts of heat stress on crop morphology, physiology, and molecular dynamics, while also evaluating genomic approaches aimed at breeding resilient varieties. In another review, focusing on date palm cultivation,* ***Faleiro and Krishna Kumar*** *highlight the pivotal role of date palm cultivation in arid regions, emphasizing its contribution to the economy and nutritional security. They also delve into the global significance of date palms, discussing their cultivation, nutritional value, and the challenges faced in this sector.*

The article on wild mango diversity by ***Ruchika et al.*** *investigates the range of wild mango varieties in coastal Karnataka, analysing various morphological traits across different accessions. It offers valuable insights into the diversity of mango germplasm in the region.* ***Perveen et al.*** *demonstrated the utility of volatiles as biochemical markers for characterizing potential mutants in mango genotypes, aiding in their validation and characterization.* ***Vishwakarma et al.*** *characterized wild Psidium species for morphological and biochemical traits, revealing significant diversity and highlighting correlations among traits with implications for guava improvement.*

Varsha et al. *studies heterosis in bell pepper and identified promising crosses that exhibit favourable growth and yield traits, thereby assisting in the selection of superior genotypes.* ***Chethan Kumar et al.*** *elucidate relationships among various traits, aiding in the selection of traits that contribute to yield through correlation and path analysis in tomato populations, which provides valuable insights into trait associations crucial for tomato breeding efforts. The investigation into the floral biology of wild melon by* ***Kalyan Chakravarthi et al.*** *sheds light on aspects such as flower longevity, pollen viability, and pollination dynamics, thereby contributing to a deeper understanding of the reproductive biology of wild melon species.*

In the study on parental selection in marigold hybridization by ***Sumalatha et al.****, the use of BLUP and GCA methods facilitates the strategic selection of male sterile and fertile parents based on yield and*

related traits, enhancing hybrid seed production in marigolds. Similarly, ***Gurung et al.*** *assessed the genetic diversity in chrysanthemum utilizing Mahalanobis D^2 statistics, and categorizes genotypes into six distinct clusters, providing valuable insights for chrysanthemum improvement.* ***Safeena et al.*** *evaluated Heliconia genotypes, revealing significant variations in growth, flowering and yield-related traits, aiding in the selection of superior genotypes. The genetic diversity studies in turmeric by* ***Raghuveer et al.*** *unveils distinct clusters and pinpoints superior genotypes associated with higher fresh rhizome yield, offers valuable insights into the genetic variation within turmeric and underscores its potential for enhancing yield through targeted breeding efforts. The study on microsatellite marker development in Garcinia morella by* ***Ravishankar et al.****, not only facilitated genetic diversity analysis and molecular characterization but also offers valuable resources for advancing genetic studies and trait mapping in this fruit-bearing medicinal tree.*

Nutrient management studies in guava by ***Adak et al.****, revealed that both soil and foliar applications of $ZnSO_4$ and borax significantly improved yield and quality of guava fruits.* ***Wise and Selby-Pham's*** *demonstrated that fish emulsion supplementation enhances the functional value of tomato fruits without perceptibly affecting sensory perception and underscores the potential of fish emulsion as a fertilizer supplement for improving the nutritional quality of tomato.* ***Rishitha et al.*** *reported that alfalfa meal and MiracleGro® effectively enhanced the growth, development, and yield of Anaheim pepper, suggesting organic supplement as a viable strategy for boosting pepper productivity.* ***Nair et al.*** *concluded that application of water soluble fertilizers @ 125:100:125 kg $N:P_2O_5:K_2O$ ha^{-1} during the cropping period through fertigation at bi-weekly intervals resulted in higher yield, net income and B: C ratio kharif grown chilli.*

Bharathi et al. *reported significant variation in corm and cormel production of Gladiolus varieties under aeroponic systems and identified suitable genotypes and nutrient regimes for efficient corm multiplication.* ***Sangeetha Priya et al.*** *demonstrated soil-less production systems for gerbera cultivation showed significant impact on the growth, quality, and water use efficiency of gerbera plants, and recommended aggregate wick system with pots on the ground as a superior alternative for gerbera cultivation. Another study by* ***Sangeetha Priya et al.*** *assessed different land configurations and fertilizer doses for African marigold cultivation. The raised bed configuration with reduced fertilizer dose exhibited enhanced vegetative growth, flower yield, and economic viability, rendering it suitable for commercial production. The study by* ***Prasanth et al.*** *investigated osmotic stress and plant growth promotion using osmotolerant plant growth-promoting bacterial strains, revealed that while osmotic stress impacted hormone production, all strains still managed to produce plant hormones even under stress conditions.* ***Ramachandrudu et al.*** *recommended 75% shade for optimal seedling development in oil palm cultivation.*

In the study on value addition to cashew apple by ***Preethi et al.,*** *osmo-dehydrated products prepared with ripe fruits demonstrated suitability for product development based on biochemical and organoleptic qualities. In the research on optimizing osmotic dehydration of dragon fruit by* ***Ranjith et al.,*** *response surface methodology was employed to determine optimal osmotic dehydration parameters for dragon fruit slices, identified conditions that led to optimal water loss and solid gain during osmotic dehydration, resulting in enhanced product quality.* ***Ranjitha et al.*** *assessed various packaging materials and modified atmosphere conditions to prolong the shelf life of fenugreek microgreens and found that polypropylene effective in preserving quality of microgreens during storage. Additionally,* ***Bhatt et al.*** *explored the use of food dyes for to enhance their visual appeal and market value without compromising postharvest life of tuberose and found that immersion in lemon yellow food dye for one hour yielded the most favourable results.*

Machuca et al. *conducted a study on fungicide susceptibility testing for tomato pathogens in two Cladosporium spp. isolated from tomato plants. The study offers valuable insights into effective control strategies against tomato pathogens. In another research,* ***Vanlalneihi et al.*** *compared in vitro pin-prick*

and non-wounding spray methods for screening anthracnose resistance in chilli accessions, identified variations in resistance patterns among accessions and inoculation methods, shedding light on effective screening techniques. ***Ghotbi and Shahraeen*** *identified Impatiens necrotic spot virus (INSV) infection in ornamental plants and characterized its genetic diversity at the molecular level. This research contributes to understanding the epidemiology and genetic makeup of INSV.* ***Sridhar et al.*** *determined the optimal dose of gamma irradiation for inducing sterility in male South American tomato moths and revealed that sterile males exhibited reduced mating competitiveness and fertility, suggesting the potential application of sterilization techniques for pest control.*

Atheequlla et al. *conducted a study on the challenges and strategies for capsicum cultivation, identified a spectrum of constraints, encompassing production, market, financial, technological, institutional, weather-based, health, and labour-related challenges. It underscores the necessity for interventions to address these constraints and ensure the profitability and sustainability of capsicum cultivation. In a similar vein,* ***Poornima et al.*** *investigated the information needs for salad cucumber production, focused on various technical aspects associated with polyhouse construction, maintenance, and repair, as well as the requirements for fertilizers and fertigation systems.*

Venugopalan et al. *conducted a statistical analysis of mango rootstock trials, proposing a statistical method grounded in robust ANOVA to address outliers and high coefficient of variation (CV) encountered in the analysis of long-term mango rootstock trials. The study's findings concluded that the proposed method offers accurate estimates of treatment effects in perennial crop experiments characterized by high CV.*

Muralidhara et al. *characterized a unique avocado accession (PA-026), which has distinct yellow-coloured fruits and high carotenoid content, rendering it valuable for breeding aimed at enhancing the carotenoid content.* ***Richardson and Arlotta*** *evaluated various sweet potato varieties for greens production, assessed the impact of harvesting greens on storage root yield and nutrient content. Although the study found no significant differences in greens production among varieties, it observed an increase in nutrient content in storage roots after greens harvesting.* ***Ramteke et al.*** *investigated crossability studies in cashew genotypes, revealing variations in crossability among genotypes, with certain combinations demonstrating higher crossability rates. The research sheds light on the hybridization potential of different cashew genotypes.*

Singh et al. *investigated the effect of various coloured shade nets on Dianella tasmanica and Pleomele reflexa on the growth and quality parameters, revealed that red-coloured shade nets significantly enhanced the performance of both species, suggesting the potential utility of red shade nets in commercial cultivation to optimize growth and quality.* ***Hsiang et al.*** *conducted a study on sugar changes during flowering stages of phalaenopsis inflorescences. Their research unveiled dynamic alterations in glucose, fructose, and sucrose levels throughout the flowering stages, indicating their pivotal roles in floret opening, blooming, and senescence processes.*

Salehi et al. *studied essential oil content and composition in German chamomile revealed that while wild chamomile exhibited slightly lower essential oil content, both genotypes contained similar major components, implying the potential utilization of wild genotypes in domestication programs.*

I profusely thank the Editorial Board and secretarial assistant, authors, readers, reviewers and executive committee members of Society for Promotion of Horticulture (SPH) for their continuous support for bringing out this issue. Hope the New Year 2024 will bring cheers to all stakeholders of Horticulture Science. Wish you all a very Happy New Year 2024.

Rajiv Kumar
Editor in Chief

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Review

Understanding heat tolerance in vegetables: Physiological and molecular insights, and contemporary genomic approaches for enhancing heat stress resilience

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ABSTRACT

The increasing threat of heat stress in agriculture, fueled by the relentless rise in global temperatures, presents a formidable challenge for vegetable crops. High-temperature stress instigates intricate morphological, anatomical, and physiological changes in vegetables, resulting in a noticeable decline in yield and an overall compromise in quality. Mitigating these challenges necessitates the imperative development of heat-tolerant vegetable varieties, underscoring the need for a nuanced understanding of crop responses to the rigors of high-temperature stress. This comprehensive review systematically explores the multifaceted impacts of heat stress on vegetable crops, spanning morphological traits, physiological processes, and molecular dynamics. Beyond the identification of challenges, the review explores into the intricate adaptive mechanisms employed by vegetables to counteract the stresses imposed by elevated temperatures, besides exploring in detailed how these crops navigate and respond to the physiological disruptions caused by heat stress. Further, it also assesses the efficacy of diverse genomic approaches in the development of heat-tolerant vegetable varieties. In addition, the review explores genomic tools such as genomic selection, transgenic approaches, and genome editing technologies, which hold promise in expediting the development of vegetable varieties endowed with enhanced thermo-tolerance and heightened productivity. By synthesizing insights from diverse scientific realms, the review aspires to provide a comprehensive and integrative perspective on mitigating the adverse impacts of heat stress on vegetable crops, paving the way for sustainable agricultural practices in the face of escalating global temperatures.

Keywords: Abiotic stress, climate change, genome editing, genomics, heat tolerance

INTRODUCTION

Human-induced greenhouse gas emissions have indisputably driven a 1.1°C global surface temperature increase from pre-industrial levels (1850–1900) to 2011–2020, and despite the consistent expansion of mitigation policies since Assessment Report 5 (AR5), the projected emissions for 2030 suggest a trajectory that is likely to surpass the 1.5°C warming threshold, making it increasingly challenging to limit global warming below 2°C (IPCC, 2023). This escalating temperature trend has significantly impacted the growth and development of nearly all crops, leading to substantial losses in yield and quality on a global scale. Heat stress exerts detrimental effects on essential physiological processes such as photosynthesis, respiration, and membrane stability. Exceeding the crop-specific threshold temperature induces morphological and physiological injuries, severely affecting seed germination, as well as reproductive and vegetative growth. Vegetables play a crucial role in addressing micronutrient deficiencies, offering income prospects for small landholding farmers, and creating more employment opportunities per hectare compared to staple crops; however, the substantial reductions in yield and quality resulting from heat stress pose a significant threat to livelihoods. The biochemical, metabolic, and physiological processes crucial for vegetable growth are temperature-dependent, and elevated temperatures negatively impact these vital processes, resulting in diminished vegetable production.

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Temperatures surpassing 25°C contribute to fruit-setting failure due to factors such as bud drop, reduced pollen production, low pollen viability, abnormal flower development, ovule abortion, inadequate carbohydrate supply, and other reproductive abnormalities (Hazra et al., 2007). Addressing the adverse effects of global warming on vegetables necessitates the development of heat-tolerant varieties. In response to heat stress, various mechanisms, including the activation of heat stress-related genes, metabolite production, and signal transduction, come into play. Vegetables have evolved intricate biochemical, physiological, cellular, and molecular systems to adapt and respond to heat stress. Breeding heat-tolerant vegetable varieties requires a deep understanding of the genetic basis of heat tolerance and the response mechanisms to heat stress. Modern genomics plays a crucial role in vegetable heat stress breeding, encompassing techniques such as marker assisted selection (MAS), quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), genomic selection (GS), transgenic approaches, and genome editing tools. These advanced genomic tools enhance the efficiency of developing heat stress-tolerant genotypes with improved yield. This comprehensive review elucidates the impact of elevated temperatures on vegetables, explores the adaptation mechanisms employed by these crops, and provides an in-depth discussion on the use of both conventional breeding approaches and modern genomic tools to effectively combat heat stress in vegetables.

High temperature effects on different stages of vegetables

Over the past two decades, research has consistently demonstrated the adverse impacts of increasing temperatures on various types of vegetables across different growth phases.

Seed germination: The germination of seeds represents the initial phase of plant development, and this critical stage is significantly impacted by elevated temperatures, albeit with variations in the optimal temperature range for different vegetables. Heat stress negatively influences seed germination across various crop species, manifesting in reduced germination percentages, delayed emergence, weakened seedlings, and impaired growth of radicles and plumules (Sehgal et al., 2018). Studies by Besma & Mounir (2010) noted diminished germination of okra at 40°C. Heat stress adversely affects the germination and development of cucumbers (Kurtar, 2010), in carrot >35°C (Nascimento et al., 2008), in cucumber and melon 42°C and 45°C, respectively, while, seeds of pumpkin, summer squash, winter squash, and watermelon fail to germinate at 42°C (Kurtar & Balkaya, 2010), and in spinach, germination unexpectedly decreases between 25 and 30°C, and it ceases completely at 35°C (Chitwood, 2016). These findings underscore the vulnerability of seed germination to elevated temperatures across diverse vegetable species.

Vegetative growth and quality: In tomato, lyconene degradation initiated above 25°C and complete destruction occurred at 50°C (Hackett et al., 2004). Heat stress also impacts the development of red color in ripening chili fruits. Heat stress in tomatoes manifests in various undesirable outcomes, including the occurrence of green shoulders, fruit cracking, sunburn, and blossom-end rot. Wien (1997) observed that temperatures above 17-28°C in lettuce led to issues such as tip burn, leaf chlorosis, loose and puffy heads, and the accumulation of bitter compounds. Dark red lettuce plants exposed to a temperature of 33°C exhibited induced bolting and bitterness, ultimately diminishing the yield and quality of dark red lettuce (Ilić et al., 2017). High temperatures contribute to issues such as tip burn, bracting, hollow stem, and loose heads in broccoli (Kałużewicz et al., 2012). Cabbage experiences decreased leaf expansion above 32°C, while, Chinese cabbage is susceptible to tip burn, and cauliflower may exhibit bracting under elevated temperatures (Nievola et al 2017). At 40°C, Tekalign et al. (2012) observed alterations in onion bulb size accompanied by an increase in sulfur content. Heat stress in pepper (Valles-Rosales et al., 2016), manifested in fruit cracking, sunburn, and blossom-end rot, ultimately diminishing the quality of the produce. For potatoes, temperatures exceeding 22°C led to a reduction in tuber development, with tuberization inhibited at 25°C and above, while, Suzuki et al. (2014) documented responses to heat stress in okra, including a reduction in plant shoot length, burning of leaf tips, necrosis, yellowing, and scorching of leaves.

Reproductive growth: Kałużewicz et al. (2012) reported a decrease in bud size in broccoli attributed to rising temperatures, specifically highlighting the adverse impact on flower and fertilization aspects. Bell

peppers experienced flower drop at temperatures surpassing 32°C, while hot peppers exhibited a reduction in both flower and fruit numbers at temperatures above 38°C (Erickson & Markhart, 2001). Watermelon and cucumber shows reduced flowering at temperatures exceeding 35°C, while, tomatoes faced a decline in the number of fruits and flowers at temperatures >25°C. Notably, heat stress induced stigma elongation in tomatoes, hindering the process of self-pollination (Pan et al., 2019). Several studies had shown that elevated day temperatures have been observed to negatively impact anthesis, dehiscence, and fruit setting in both tomato and capsicum. Screening studies conducted under heat stress conditions for tomatoes indicated a decrease in pollen viability, ovule viability, and the number of released pollens (Singh et al., 2015). Furthermore, high temperatures contribute to pollen abortion, consequently reducing seed viability (Chaudhary et al., 2022).

Effects of heat stress on physiological processes

Various physiological processes, including photosynthesis, respiration, and membrane stability, undergo disruption in the presence of heat stress.

Photosynthesis: Elevated temperatures have a significant impact on the photosynthetic process in plants. Moderate heat stress, typically ranging from 35-40°C, induces the enlargement of chloroplasts and an increase in plastid vesicles within them, indicating a substantial alteration in the structural composition of chloroplasts and thylakoids (Zeng et al., 2021). Both heat-sensitive and heat-tolerant potato cultivars show a decrease in photosynthetic activity under heat stress conditions (Aien et al., 2011). Similarly, studies on okra genotypes reveal a reduction in photosynthetic activity at 45°C, with heat-sensitive genotypes experiencing more adverse effects than heat-tolerant ones (Hayamanesh et al., 2023). The net photosynthetic rates (A) of Chinese cabbage and radish exhibit a decline at temperatures surpassing approximately 25°C, which corresponds to their optimum temperature for photosynthesis (Oh et al., 2015). This decrease is attributed, at least in part, to elevated rates of respiration observed above 25 or 30°C. The vulnerability of PS II electron transport to heat stress can be attributed to two primary factors: firstly, the elevated fluidity of thylakoid membranes at higher temperatures leads to the displacement of PS II light harvesting complexes from the membrane; and secondly, the integrity of PS II is reliant on the dynamic behavior of electrons (Mathur et al., 2014).

Membrane stability: The normal functioning of cellular membranes is indispensable for vital processes such as photosynthesis and respiration. However, biological membranes are highly sensitive to heat stress, and their stability can be compromised. Elevated temperatures intensify the molecular movements within biological membranes, disrupting the chemical bonds between molecules. Heat stress induces alterations in the tertiary and quaternary structures of membrane proteins, leading to increased membrane permeability and subsequent electrolyte loss. Oxidative stress induced by heat stress leads to the peroxidation of membrane lipids, proteins, and nucleic acids (Mittler et al., 2004). This results in reduced membrane stability, leading to an increase in electrolyte leakage and exacerbating membrane injuries (Wahid et al., 2007). In tomatoes, subjected to heat stress, ion leakage from the cell membrane was found to be negatively associated with inflorescence number, pollen germination, and fruit setting (Xu et al., 2017). All reported findings on the relationship between thermo-membrane stability and heat stress tolerance consistently indicate that crop genotypes with higher thermo-membrane stability exhibit greater heat stress tolerance.

Oxidative stress: Plants experiencing heat stress undergo the generation of reactive oxygen species (ROS), including singlet oxygen (O_2), superoxide radical ($O_2^{"}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{"}$), leading to oxidative stress (Choudhury et al., 2013), results in cell injury through the oxidation of lipids, nucleic acids, and proteins. Notably, lipids, crucial components of cell membranes and organelles, are primary targets for these active oxygen species. The autocatalytic peroxidation of unsaturated fatty acids in membrane lipids, triggered by reactive oxygen species, induces membrane permeability loss, electrolyte leakage, and ultimately cell death (Wahid et al., 2007). Investigations reveal that plants when exposed to higher temperatures accumulate more of H_2O_2 due to reduced activities of ROS scavengers such as catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), and dehydroascorbate reductase (DHAR).

Impact of heat stress at molecular level

In response to heat stress, there is a dynamic modulation in the expression of genes crucial for safeguarding vegetables from the adverse effects of elevated temperatures. These genes encompass those associated with the synthesis of osmoprotectants, detoxification enzymes, heat shock proteins, and various regulatory proteins (Slama et al., 2015). Elevated temperatures induce enzyme deactivation, inhibit protein synthesis, and cause aggregation in chloroplasts and mitochondria. Specific cellular proteins or transcription factors regulate genes linked to stress.

Under high-temperature stress, various heat shock factors (HSFs), transcription factors (TFs), chaperones, and osmoprotectants become activated (Jacob et al., 2017). Studies have underscored the efficacy of specific genes in enhancing plant resilience to heat stress. For instance, the overexpression of the StnsLTP1 gene in potatoes enhances cell membrane integrity during heat stress (Gangadhar et al., 2016). In potato plants subjected to high-temperature stress, overexpression of the APX, Cu/Zn SOD, and NDPK2 genes improves oxidative stress tolerance (Kim, 2010). Similarly, in tomato plants, the LeAN2 gene, when overexpressed during heat stress, boosts fresh weight production, antioxidant activity, and photosynthesis rate (Kumar et al., 2012). Overexpression of the cAPX gene in tomato plants enhances tolerance to heat stress (Gerszberg & Hnatuszko-Konka, 2017). Research on chilli genotypes revealed that susceptibility to heat stress is linked to low expression of HSP70, while, tolerant genotypes exhibit HSP70 overexpression, contributing to increased thermo-membrane stability (Usman et al., 2015). In onions, under heat stress, overexpression of genes related to the synthesis of osmoprotectants, ROS scavengers (TRX, ANX, GRX, CAT2, and FDX), transcription factors (NAC-domain, WRKY, MYB, and WD-40), genes controlling hormone production (Ethylene, Auxin, and ABF3), genes governing the production of signal transduction molecules (CDPK, CaBP, CBP, CBL, MAPK20, and RAN), and heat shock proteins (HSP 40, HSP 60, HSP 70, HSP 90, and HSP 101) confers heat tolerance compared to outer scales (Galsurker et al., 2018).

Physiological and molecular mechanisms of heat stress tolerance

To counter the challenges posed by heat stress, vegetables employ a diverse array of physiological and molecular mechanisms to enhance heat tolerance. These mechanisms include the stabilization of thermos-membranes, scavenging of ROS, accumulation of osmolytes, production of antioxidant enzymes, activation of mitogen-activated protein kinase (MAPK) and (CDPK) cascades, chaperone signaling, and transcriptional activation. When exposed to heat stress, the plasmalemma is initially affected, resulting in increased lipid fluidity, which induces Ca2+ influx, leading to the upregulation of MAPK and (CDPK). The signaling cascades activated by these kinases contribute to the synthesis of osmolytes and antioxidants (Wahid et al., 2007).

To counteract the damage caused by reactive oxygen species during heat stress, plants have evolved an antioxidant defense mechanism involving the production of antioxidant enzymes. Reactive oxygen species, when maintained at optimum levels, function as signaling molecules in response to stress perception by stress sensors. These signaling molecules are disseminated throughout metabolically active plant tissues and are regulated by the ROS gene network. During heat stress, signaling ROS are mediated by calcium or the activation of NADPH oxidases at the plasma membrane, acting as heat stress signal transducers (Devireddy et al., 2021). Reactive oxygen species, in conjunction with other signals like Ca^{2+}, are integral to long-distance systemic signaling necessary for the activation of systemic acquired acclimation in response to heat or other abiotic stresses. For instance, plant hormones such as abscisic acid (ABA) and jasmonic acid (JA) trigger ROS production, initiating a systemic signal known as the ROS wave (Devireddy et al., 2021). This hormone-triggered ROS move through cell-to-cell propagation, forming an amplification loop and triggering systemic acquired acclimation responses. Studies have indicated that the local application of heat or cold stimuli induces similar stress transcriptional responses in both local and systemic tissues, dependent on the ROS wave. Moreover, ROS activate calcium channels, which then activate two pore channel 1 (TPC1), a vacuolar calcium channel transporting vacuolar-stored Ca2+. This activation leads to the activation of respiratory burst oxidase homolog D (RBOHD) proteins, creating a feedback loop that activates ROS and calcium, inducing a comprehensive acclimation response to high temperature (Devireddy et al., 2021). In sweet potatoes, the expression of heat-responsive

genes, such as abscisic acid-responsive elements binding factors (AREB) and CBF TFs, plays a pivotal role in its robust adaptation to heat stress. The involvement of Heat Shock Proteins (HSPs) in signal transduction during heat stress is crucial. The enhanced high-temperature tolerance conferred by HSPs contributes to improvements in photosynthesis, nutrient uptake, and membrane stability (Momcilovic & Ristic, 2007).

Hormones also play a significant role in plants' adaptation to heat stress. Under high-temperature conditions, there are alterations in the synthesis, stability, quantity produced, and distribution of hormones. Stress hormones, such as ethylene (C_2H_4) and ABA, function as signaling molecules in response to heat stress, contributing to the plant's adaptive mechanisms. Research indicates that ABA activates heat shock proteins (HSPs), suggesting it as a potential mechanism for imparting heat tolerance to plants (Pareek et al., 1998). When plants experience a sudden or gradual increase in temperature, specific proteins called heat shock proteins (HSPs) are synthesized and accumulated. In tomato plants, HSPs form granular structures in the cytoplasm, protecting the protein biosynthesis machinery. HSPs/chaperones not only facilitate signal transduction but also activate genes involved in heat tolerance mechanisms, including the production of osmolytes and antioxidants. Thermotolerance alters gene expression by activating HSPs and inhibiting the expression of other genes. Destabilization of mRNAs encoding non-heat-stress-induced proteins occurs during heat stress, and reactive oxygen species (ROS) activate heat shock factors (HSFs) (Hu et al., 2015).

Salicylic acid (SA) hormones play a crucial role in heat stress tolerance, participating in signaling pathways. SA facilitates the binding of heat shock elements to the promoter of heat shock proteins. Treatment with sulphosalicylic acid (SSA) has been shown to mitigate the adverse effects of reactive oxygen species by removing H_2O_2. SSA treatment effectively eliminated H_2O_2 from cucumber seedlings by inducing the production of catalase enzymes (Snyman & Cronjé., 2008). Recent findings indicate that brassinosteroids hormones confer thermotolerance in tomato and oilseed rape. Studies stress that xanthophylls contribute to heat tolerance by enhancing thermo membrane stability. Xanthophyll molecules interact with membrane lipids, reducing their fluidity and thereby safeguarding against lipid peroxidation in response to heat stress. Endogenous synthesis of isoprene protects biological membranes and PS II by directly interacting with reactive oxygen species generated during high-temperature conditions (Velikova et al., 2008).

Heat tolerance through conventional breeding

Plants exhibit varying degrees of thermotolerance, and considerable diversity exists in the responses to heat stress both between and within plant species, which provides an opportunity to enhance heat stress tolerance in vegetables through strategic exploitation. The development of heat-tolerant varieties via conventional breeding programs emerges as an economically viable solution. Employing recurrent selection procedures enhances the frequency of desirable alleles by selecting superior genotypes from the base population (Benites & Pinto, 2011). Figueiredo et al. (2015) utilized the recurrent selection to establish high-temperature stress tolerance in potato plants. Plant breeders have successfully developed heat-tolerant potato varieties, including Kufri Surya (Minhas et al., 2006), Haruka (Kobayashi et al., 2009), and Konyu (Iwama, 2008), utilizing conventional breeding methods. Although, heat tolerance is a complex, multi-genic trait influenced by environmental factors and various other characteristics, breeders have managed to develop commercially acceptable, heat-tolerant open-pollinated varieties and hybrids.

In cowpea selection for high pod set and abundant flower production resulted in the development of high-temperature-tolerant and high yielding genotypes (Ehlers et al., 2000). CIAT breeders in 2006 utilized heat-tolerant lines to develop a heat-tolerant bean genotype (Blair et al., 2006). The high-temperature-bearing chickpea line 'ICCV-92944' was developed through selection (Gaur et al., 2019). The Asian vegetable research and development center (AVRDC) identified thirty-nine heat-resistant lines in tomatoes through selection. Some of these lines were incorporated into the tomato breeding program of AVRDC, leading to the development of heat stress-tolerant lines such as 'Equinox' and 'Sun Leaper' (Gardner, 2000).

Genomic approaches for heat tolerance

Improving vegetable crops through conventional breeding methods faces limitations, as these

approaches involve long breeding cycles and often yield a low success rate (Parent & Tardieu, 2012). Challenges arise due to the difficulty in transferring genes from only closely related species, resulting from pre- and post-fertilization failures. Traditional breeding strategies may not effectively isolate and transfer desirable single genes, leading to the inadvertent transfer of unwanted genes alongside the targeted ones. Since, heat tolerance is a quantitative trait influenced by environmental factors, traditional breeding experiments are susceptible to failure. Advancements in plant genomics and biotechnology have significantly enhanced scientists' understanding of the molecular, biochemical, and physiological responses of plants to heat stress. This knowledge has enabled the successful and efficient selection, transfer, and expression of target genes. In the contemporary genomic era, various tools, such as molecular marker technology, QTL mapping, genome-wide identification of molecular markers, genomic selection, genetic transformation, and genome editing tools, are being employed to develop vegetable cultivars with enhanced heat tolerance. These modern genomic approaches offer more precision and efficiency in breeding practices compared to traditional methods.

Marker assisted selection for heat tolerance: Molecular marker-assisted selection (MAS) has the potential to augment traditional breeding methods, although the current application of molecular markers in developing heat-resistant vegetable varieties is limited. The advent of genome sequencing, genome resequencing, and transcriptome sequencing has led to the discovery of an extensive array of molecular markers. In the future, these markers are expected to play a pivotal role in genetic mapping, trait recognition, and the enhancement of genetic makeup through MAS to confer high-temperature tolerance in vegetable crops. Given the intricate mechanisms of high-temperature tolerance and the challenges in phenotypic trait selection, MAS emerges as a promising technology for improving the heat stress tolerance of vegetable crops (Foolad & Panthee, 2012).

The successful application of MAS relies on the identification of genetic markers linked to genes/QTLs responsible for heat stress tolerance at the whole plant level or affecting traits related to heat stress tolerance. Marker technology enables the detection and characterization of quantitative trait loci affecting high-temperature tolerance at various plant growth stages (Foolad et al., 2008). Various MAS schemes have been developed, including selection for multigenic traits by applying markers at multiple loci across successive selection cycles, enrichment of desirable allelic frequency in early selection cycles, and the use of markers in backcrossing with foreground and background selection. MAS can be employed for introgressing desirable alleles into an elite background and incorporating innate characters into a breeding line in progress (Bassi et al., 2016). To implement MAS effectively, markers proximal to the target locus are essential, making it particularly useful for traits with a quantitative mode of inheritance, such as high-temperature stress tolerance (Tayade et al., 2018).

For enhancing heat tolerance in vegetable crops, single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSR) are now widely employed. Among vegetables, the application of MAS is predominantly confined to tomatoes. In tomatoes, 15 yield-related markers were identified, of which 13 were deemed more reliable for identifying genes that confer tolerance to high-temperature stress (Ruggieri et al., 2019). Dominant sequence characterized amplified region (SCAR) based markers were developed to distinguish high-temperature stress-tolerant genotypes from susceptible ones in tomatoes. During the evaluation of tomato genotypes, one SSR and two random amplified polymorphic DNA (RAPD) markers were found that could be applied to differentiate susceptible and tolerant genotypes.

Quantitative trait loci mapping for heat tolerance: Utilizing molecular biology and molecular markers to identify quantitative trait loci (QTLs) associated with high-temperature tolerance is crucial for comprehending the genetic basis of heat tolerance. Various QTLs contribute to heat stress tolerance in vegetables. In tomato, multiple studies have focused on mapping QTLs linked to traits associated with high-temperature tolerance, including style length, style protrusion, pollen viability, number of pollens, number of inflorescences, and fruit setting (Panthee & Gotame, 2020). Reliable QTL information is indispensable for successful marker-assisted breeding. While few studies have mapped QTLs associated with high temperature tolerance in vegetables, the majority of QTL mapping work has been concentrated in tomato. A search across

various search engines revealed six QTL mapping experiments related to high temperature tolerance at different growth stages in tomato (Alsamir et al., 2021). QTL mapping was applied to different genotypes of tomato, revealing significant marker relationships between the number of flowers, fruit setting, number and weight of fruits, electrolyte leakage, and dry weight of the plant.

QTL mapping has been instrumental in identifying new genetic variability as a source of heat tolerance for crop breeding. Although QTLs may encompass numerous genes, the significance of various QTL mapping studies is sometimes limited due to low marker density. In cowpea, four QTLs linked to the number of pods set/peduncle under heat stress were mapped and utilized in its heat tolerance improvement program (Pottorff et al., 2014). Nine QTLs associated with internal necrosis in potato tubers, explaining 4.5% to 29.4% of the phenotypic variability, were identified (McCord et al., 2011). QTL mapping in potato led to the identification of the HSC 70 gene, whose enhanced expression under moderate heat stress resulted in increased tuber yield (Trapero Mozos et al., 2018). In tomato, six QTLs linked with fruit setting under heat stress were identified using fluorescent AFLP markers, aiding in the rapid selection of heat-tolerant plants. Additionally, 21 QTLs related to fruit traits such as fruit weight, number of fruits, percentage of fruit setting, and brix value were mapped on four chromosomes at temperatures above 35 degrees Celsius. In Chinese cabbage, three QTLs, and in broccoli, five QTLs, explaining 17.6% to 41.1% and 62.1% variability under high-temperature stress, were detected (Branham et al., 2017). In lettuce, the main QTL Htg6.1 (high temperature germination) was identified and strongly linked to thermotolerance during sprouting (Driedonks et al., 2016).

Genome-wide identification of molecular markers in vegetable crops

Cucumber marked a pioneering achievement in vegetable genomics as the first vegetable to be fully sequenced (Huang et al., 2009). The abundance of molecular markers, such as simple-sequence repeats and single nucleotide polymorphisms, has notably enhanced the efficiency of breeding programs, genetic mapping, and trait identification (Gao et al., 2012). Whole-genome analysis in vegetables has identified a substantial number of genetic markers, providing new avenues for germplasm characterization and the development of heat-resistant genotypes. In the genomes of potatoes, over 3.67 million, and in tomatoes, approximately 5.4 million SNPs have been detected (Tomato Genome Consortium, 2012).

The application of transcriptome sequencing technology in vegetables allows for the rapid generation of many expressed sequence tags (ESTs), facilitating the identification of molecular markers such as SNPs and SSRs. Genotyping by sequencing (GBS), a contemporary approach, has increased the number of markers, especially SNPs, evenly distributed across the genome (Spindel & Iwata, 2018). This enables the creation of genetic maps with high resolution, accurate mapping of quantitative trait loci (QTLs), and the identification of candidate genes associated with quantitative traits. Genome-wide association studies (GWAS) provide a means to identify specific haplotypes in natural and wild populations by narrowing down candidate genomic regions (Verdeprado et al., 2018). In modern crops, the emphasis on yield and uniformity, a consequence of the green revolution and conventional breeding, has resulted in a narrowed genetic diversity. Despite this, considerable genetic variability still exists in various crops. GWAS proves to be a valuable tool for understanding the genetic basis of phenotypically complex traits. Compared to conventional genetic mapping techniques, GWAS offers high genetic mapping resolution, although information on its application in vegetables remains limited.

Genomic selection: Genomic selection (GS) proves highly valuable when a trait is influenced by numerous minor quantitative trait loci (QTLs). Unlike Marker assisted selection (MAS) and marker-assisted backcrossing (MAB), GS does not necessitate QTL mapping or detailed information about trait inheritance. GS leverages various molecular markers, incorporating them into prediction models to unveil variability resulting from minor QTLs (Shamshad & Sharma, 2018). This technique has been implemented in various crops, including tomato, and is particularly advantageous for traits governed by many QTLs, providing more comprehensive insights into genetic gain (Adlak et al., 2019).

Research affirms that when a trait is controlled by numerous QTLs, GS outperforms traditional breeding techniques and MAS in terms of time efficiency and

precision in plant breeding programs. In the context of enhancing high-temperature tolerance in tomatoes, genomic selection emerges as a promising strategy. In crops, such as pepper (*Capsicum* spp.), where fruit-related traits significantly influence quality and are quantitative in nature, GS utilizes genotypic and phenotypic data from a training population to predict the phenotypes of a test population with only genotypic information. GS has found successful application in staple crops like potato, maize, wheat, and barley, focusing on quality traits, yield, and disease resistance (Habyarimana et al., 2022; Stich & Van Inghelandt, 2018). The versatility of GS across various crop species is attributed to the availability of genome-wide, high-performance, cost-effective molecular markers that can be applied to large population sizes, both in model and non-model crop species, whether possessing a reference genome sequence or not (Bhat et al., 2016). GS serves as a pre-breeding tool for identifying germplasm with valuable variations, predicting the breeding value of plants within a breeding population. This application efficiently enhances genetic gain for quantitative traits, making GS an integral part of breeding programs, particularly in the private sector where it is widely utilized for developing new varieties in different crops within a short timeframe (Tayade et al., 2022). One notable advantage of GS over MAS and marker-assisted recurrent selection lies in its ability to detect alleles with minor effects, which can be crucial in the selection process.

Transgenic approaches for heat tolerance: Through recombinant DNA methods, the creation of heat-tolerant transgenic lines can be achieved swiftly, allowing for the transfer of potential genes from diverse species to target crops. One study focused on the overexpression of the AtDREB1A gene in transgenic potatoes, revealing changes in the metabolite composition of the lines under high-temperature stress conditions (Iwaki et al., 2013). The analysis of gene sequences, transcriptomes, and proteomes provides extensive information for identifying valuable genes associated with heat stress in vegetables (Zhuang et al., 2014). Increased thermo-tolerance in transgenic lines is often attributed to elevated levels of Heat Shock Protein (HSP) chaperones. For instance, the MT-sHSP of tomatoes demonstrated molecular chaperone functionality *in vitro*. The development of transgenic lines is considered an efficient and reliable method for crop improvement, with examples including heat-tolerant transgenic potato lines that have been successfully developed (Trapero Mozos et al., 2018). Introducing stress-related genes, such as StnsLTP1, into potatoes has shown increased tolerance to abiotic stresses and enhanced expression of genes related to other stresses. Similarly, the insertion of AtCBF3 from Arabidopsis into potatoes increased tolerance to high temperatures by improving photosynthetic efficiency and antioxidant defense (Dou et al., 2015). Transgenic potatoes with the insertion of the heat-tolerant gene sHSP17.7 from carrots demonstrated elevated high-temperature tolerance.

In major vegetable like tomato, genetic modifications have been implemented. Wheat, tomatoes, and maize have seen the development of transgenic lines with heat tolerance by targeting heat shock proteins (HSPs) and heat shock factors (HSFs) (Wang et al., 2019).

Genome editing methods: Genome editing technologies, particularly CRISPR/Cas9, have demonstrated successful performance in the Solanaceae family, surpassing earlier techniques like ZFN and TALENs due to its high specificity (RNA-DNA hybrid) and cost-effectiveness (Kim et al., 2017; Yamamoto et al., 2018). In tomatoes, the SlMAPK3 gene, belonging to the mitogen-activated protein kinase family, plays a role in responding to various environmental stresses. Mutants of SlMAPK3, created through CRISPR/Cas9-mediated genome editing, exhibited increased thermo-tolerance compared to wild-type plants, suggesting its negative regulatory role in high-temperature tolerance (Yu et al., 2019). Similarly, the BRZ1 gene, which positively regulates the production of reactive oxygen species in the apoplastic region of tomatoes for heat tolerance, was edited using CRISPR/Cas9. Mutants of bzr1 exhibited impaired H_2O_2 production in the apoplast, indicating its significance in temperature tolerance (Kumar et al., 2023). In lettuce, CRISPR/Cas9 was used to knock out the LsNCED4 gene, resulting in increased germination of lettuce seeds under heat stress (Devi et al., 2022). The successful application of genome editing relies on two essential prerequisites: the availability of the complete genome sequence and active transformation techniques. In tomato, both conditions are fulfilled, making it an ideal candidate for genome editing. The technology has unveiled insights into genes and gene networks controlling pollen viability, ovule fertility, and photosynthetic

system efficiency under high-temperature stress. CRISPR/Cas9 has proven effective in enhancing heat tolerance in tomatoes by targeting genes involved in jasmonic acid production, invertase activity, and ethylene response (Wang et al., 2019). Notably, CRISPR/Cas9 has emerged as a rapid and precise tool for studying molecular pathways related to heat stress, allowing the creation of gene knockout mutant lines to investigate specific gene functions.

CONCLUSION AND FUTURE PROSPECTIVE

As global air temperatures continue to rise, heat stress poses a significant threat to vegetable production worldwide, impacting various aspects of quality, growth, reproduction, photosynthesis, and respiration. Addressing this challenge, requires the development of heat-tolerant vegetable varieties, necessitating a comprehensive understanding of vegetable responses and adaptive mechanisms to heat stress - a quantitative trait influenced by intricate molecular, biochemical, and physiological pathways. Conventional breeding alone falls short in improving this complex trait. Identifying specific genes and understanding the pathways governing heat tolerance are crucial. Therefore, a combination of traditional breeding, marker-assisted selection (MAS), quantitative trait locus (QTL) mapping, Genome-Wide Association (GWA) studies, and genomic selection is employed to develop heat stress-tolerant vegetable varieties, with target genes spanning different vegetable species. Advancements in genome editing technologies offer a precise means of identifying and understanding gene functions. Transgenic approaches facilitate the transfer of heat-tolerant genes across species and genera. In tomatoes, genome editing tools like TALENs, ZFNs, and CRISPR are employed to develop heat-tolerant genotypes. With increasing availability of genome sequences, these modern technologies hold the promise of delivering vegetable varieties with enhanced heat tolerance and expedited production in the future.

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Review

Date palm-A gift for health and nutrition: national and international scenario

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ABSTRACT

Date palm (*Phoenix dactylifera* L.), family Arecaceae or Palmae, is cultivated mostly in the arid regions of the world. The crop has played a significant role in the economy of these countries, provides nutritional security, besides helping to mitigate the adverse effects of desertification and climate change over centuries. Date palm personifies human civilization in the arid countries. It is estimated that there are 150 million date palms worldwide, and 75% of these in the Near East and North Africa region. According to the Food and Agriculture Organization (FAO) of the United Nations, the global production of dates has increased from just 1.8 million tons in 1962 to nearly 9.75 million tons in 2022. In India, commercial dates are cultivated mainly in the states of Gujarat and Rajasthan which are emerging as major producers of fresh dates. Besides, local and wild dates are abound across the country. Dates are consumed fresh or in dry form and considered a complete food, providing food and nutrition security through a wide range of essential nutrients that have beneficial effects on human health. This article presents an overview of dates on nutrition and human health besides giving an insight on propagation, production, protection, processing, marketing, and associated challenges plaguing the sector.

Keywords: Date palm, health, agro-technique, crop protection, value addition

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious species of the palm family Arecaceae or Palmae, predominant in the arid regions of the world and one of the oldest cultivated foods in human civilization, grown in several globally important agricultural heritage sites (FAO). Archaeological evidence suggests that the crop was cultivated 6000 years ago and is currently important to the agrarian economy of many countries (Zohary & Hopf, 2000). In context of the Indian subcontinent, the *Veda* and *Ramayana* period refer to the cultivation of date palm (Shah, 2014). In India, cultivation of the date palm goes back to the fourth century BC (Pareek, 2015). During the early eighth century, the subcontinent witnessed the accidental introduction of Arabian dates by foreign invaders in Western India (Blatter, 1926), which increased with the Arab traders (up to 13th century). Most of the seedling progeny of date palm in India could probably be traced back to this period. The genus *Phoenix* is derived from the ancient Greek bird, while, *dactylifera* originates from the Greek word '*daktulos*', meaning finger.

Date palm thrives in the Near East and North African (NENA) region between 24^{o} to 34^{o} N but is distributed between 10^{o}-39^{o} N latitude (Johnson et al., 2013). Earlier focus was on selecting superior female palms, propagation through offshoots, crop and water management, segregation by gender, artificial pollination, naming of cultivars and characterization of fruit development stages, texture etc. Of late crop improvement using molecular breeding and tissue-culture for higher productivity and resistance to pests is the focus (Johnson, 2011). Entire genome of cultivar '*Khalas*' has been sequenced (Al-Dous et al., 2011; Al-Mssallem et al., 2013) facilitating incorporation of desirable traits for yield, quality, and stresses (El-Hadrami & Al-Khayri, 2012). Date palm has the ability to withstand severe arid conditions, and salinity up to 2000 ppm.

Area, production and yield

Local populations of the NENA region (Table 1) depend on date palm as a source of income, nutrition and to mitigate the adverse effects of climate change and desertification. In the last three to four decades, the crop is also gaining importance in Australia, India, Indonesia, Mexico, Namibia, Southern Africa, South America, Pakistan, and the United States (Chao & Krueger, 2007).

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Table 1 : Date producing countries

Continent	Country
Asia (18)	Bahrain, China, India, Indonesia, Iran, Iraq, Israel, Jordan, Kuwait, Oman, Palestine*, Pakistan, Qatar, Saudi Arabia, Syria*, Turkey, UAE and Yemen
Africa (16)	Algeria, Benin*, Chad, Cameroon, Djibouti*, Egypt, Kenya*, Libya, Mauritania, Morocco, Namibia*, Niger, Sudan, Somalia, Swaziland* and Tunisia
America (4)	Colombia*, Mexico*, Peru* and USA
Europe (2)	Albania* and Spain*
Oceania (1)	Australia

Updated from Siddiq & Greibi (2013); *produce <10,000 tonnes/ annum

The wide genetic diversity in date palm can be attributed to out breeding (Popenoe, 1992). Worldwide, it is estimated that there are 3000 cultivars though, many are synonyms (Johnson et al., 2013). The original source of most of the present established cultivars in several countries is seedlings, which are raised for breeding, germplasm conservation and for desirable traits (Johnson et al., 2013). Date palm is a dioecious plant which is artificially pollinated for enhancing yield. Male date palms constitute an important genetic resource and pollination is carried either manually by inserting pollen strands in individual female flowers or mechanically using pollen dusters to ensure fruit set (Al-Wusaibai et al., 2012).

The date fruit is botanically a 'drupe' typically characterizing the variety (El-Hadrami & Al-Khayri, 2012; Johnson et al., 2013). The texture, shape, color, and chemical composition of the date fruit exhibit high diversity depending on the genotype, environment, season, and cultural practices. *Hababouk*, *kimri, khalal, rutab* and *tamar* are the five development stages of the date fruit in Arabic, where moisture levels progressively decrease from 80% at the *kimiri* stage to 20 % at *tamar* stage (Fayadh & Al-Showiman, 1990; Al-Shahib & Marshall, 2002).

In the last five decades, there has been a significant increase in the production and productivity of dates. The global production of dates has increased from mere 1.8 million tons in 1962 to 9.75 million tons in 2022 (FAO). Currently, the crop is cultivated over 1.40 million ha (FAOSTAT, 2019) with ~150 million date palms worldwide, 75 % which exist in Near East and North Africa region. Nearly 90 per cent (8.14 million tons) of the global date production comes from the top ten date producing countries (Fig. 1). Though, major date producing countries are in the Northern hemisphere, it is also cultivated in the Southern hemisphere in Namibia, Indonesia, and Australia. India is emerging as a major producer of fresh dates, with new plantations in Gujarat and Rajasthan (Pareek, 2015) and to a limited extent in Tamil Nadu and Andhra Pradesh (Shah, 2014). Atul Rajasthan Date Palm Ltd. (ARDP), a public private-cooperative venture has taken up date palm cultivation in Rajasthan in a big way.

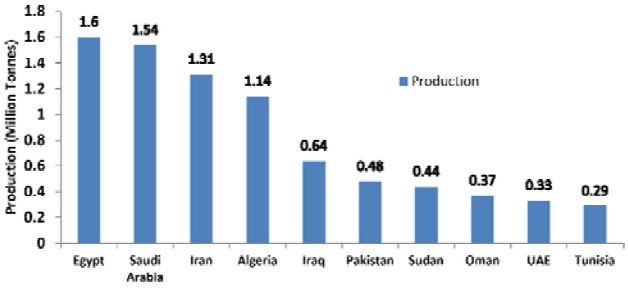


Fig. 1 : Top ten date producing countries (FAOSTAT, 2019)

A 2018-19 report by the Gujarat Horticulture, Agriculture and Farmers Welfare and Co-operative Department reveals that 19,379 ha is under date palm in Gujarat producing 180,255 metric tons of *Khalal* dates. Over 90% of the production is from the Kutch district (Personal communication by Dr. C.M. Muralidharan).

Nutrition and health

Date is called a miracle food and consumed fresh or dry is considered as almost a complete diet. It is a rich source of carbohydrates, dietary fibres, some essential vitamins, and minerals, and a variety of phytochemicals, *viz*., phenolics, carotenoids, anthocyanins, and flavonoids. Furthermore, date pits (seeds) are also an excellent source of dietary fibre, minerals, lipids, and protein (Ahmed et al., 2013). The medicinal value of dates in terms of therapeutic implications in diseases control through antioxidant, anti-inflammatory, anti-tumour, and ant-diabetic effect are summarized (Rahmani et al., 2014). Date seed extracts have medicinal properties to treat neuro-degenerative diseases (Sirisena, et al., 2015; Abdul Afiq et al., 2013). Date fibres have high antioxidant

and antimicrobial activities due to associated lignin and tannins (Shafiei et al., 2010). In general dates have several medicinal properties (Vayalill, 2002; Al-Farsi et al., 2005; El-Hadrami and Al-Khayri, 2012) and are known to;

- Promote digestion and bowel movement
- Boost heart health
- Facilitate healthy pregnancy and childbirth
- Reduce blood pressure
- Provide instant source of energy
- Anti-inflammatory properties
- Promote healthy nervous system
- Strengthen bones
- Help maintain body weight

Dates have a low glycaemic index as date fruit is high in fructose and low in glucose and sucrose making dates suitable for consumption by diabetic patients. Dates are a good source of calcium which contributes to bone strength and rich in iron which is beneficial to pregnant women and anaemic patients. Kurdi et al. (2017) reported that consumption of dates during late pregnancy facilitates easy labour. Dates being rich in oxytocin help to accelerate milk production and therefore recommended for consumption by lactating mothers. Consumption of dates is known to control blood cholesterol levels there by contributing to heart health. The high fibre content in dates plays an important role in digestion and regulation of bowel movement. Dates are low in fat and protein, but rich in healthy sugars, dietary fibre, and minerals (Al-Farsi et al., 2005). Al-Shahib & Marshall (2003) reported that dates including date seeds contain carbohydrates, fat, minerals, protein, vitamins, and a high percentage of dietary fibre and several minerals (boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc) with significant levels of potassium. Dates are a source of fluorine that is useful in protecting against teeth decay. Cancer fighting properties due to the presence of selenium in dates is another important dietary attribute (Al-Farsi et al., 2005; Al-Shahib & Marshall, 2003; Sirisena et al., 2015). Al-Shahib & Marshall (2003) also reported that dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A. Consumption of male flowers and spathe is reported to have invigorating properties (Popenoe, 1973). Furthermore, Bahmanpour et al. (2006) reported that dates can help in the treatment of male infertility. These nutritional attributes make dates a complete food providing the essential nutrition security especially to the rural communities living in the arid regions of the world where the crop is cultivated since long. Dates are a holistic food that are light and can be easily preserved, and ideally suited for consumption on long distance travel. In providing nutritional security, dates could have a major role to play in the mid-day meal programme for school children in India as well as being a source of instant energy boosting food for the armed forces in the border regions. Date as holistic food, which can be included the National Nutrition Mission of Govt. of India under the '*Poshan Abhiyan*' scheme, as it provides instant energy.

Climate and soil

Date palm withstands significant fluctuations in temperature, at <7°C, vegetative growth stops leading to a resting period and at <0°C for a certain period of time, metabolic disorders set in leading to partial or total leaf damage. While, date palms are sensitive to cold and frost, date fruits need consistent period of high heat from pollination to maturity, which decides the suitability of the variety for a particular region (Sirisena et al., 2015).

Dates are cultivated generally in regions where precipitation is scarce. Rainfall during pollination and maturity is detrimental to productivity and fruit quality. As high relative humidity has significantly impact on incidence of diseases and fruit quality, it may be difficult to produce high quality *Tamar* dates in India where fruit maturity coincides with the monsoon. Further, enhanced in-groove humidity in young date plantations predisposes the palms to attack by red palm weevil (*Rhychophorus ferrugineus*) (Aldryhim & Al-Bukiri, 2003). On the contrary, low relative humidity coupled with increasing summer temperatures and water stress favors the occurrence and development of mites *Oligonychus afrasiaticus* (McGregor) and the stem borer *Jebusea hammerschmidti* Reich (Al-Deeb, 2012; El-Shafie, 2012). Date palm is reported to withstand high velocity wind. Strong winds may, however, uproot newly planted offshoots and therefore under open desert conditions it is recommended to plant wind break trees along the borders. Wind break trees will

also protect *Rutab* and *Tamar* stages of the fruit from dust.

The crop is mostly cultivated in sandy soils but can adapt to heavy clay soils. Date plantations are characterized by saline and alkaline soils which have a high concentration of soluble salts and sodium, respectively. Addition of organic manures to the soil could be useful to facilitate root formation of newly planted date palm offshoots besides enhancing the water holding capacity of the soil and restrict the adverse effects of alkalinity (Zaid & de Wet, 2002a).

Date palm cultivars

Globally, there are nearly 3000 date palm cultivars endemic to a country or region, some popular cultivars in different countries (Siddiq & Greibi, 2013) are presented in Table 2. The popular date palm cultivars of today are known to have originated from seedling progenies (Johnson et al., 2013). In India, the role of State Agricultural Universities and ICAR institutes/ regional stations, especially of the National Bureau of Plant Genetic Resources (NBPGR), Central Institute for Arid Horticulture (CIAH) and Central Arid Zone Research Institute (CAZRI) located in the date palm predominant states of Gujarat and Rajasthan is important to identify, and conserve non-descript seedling cultivars of date palm. Cryo-conservation of pollen as a source of conservation of diversity, marker assisted selection, use of superior genotypes for date sugar and alcohol hold promise. Three to five years old date palm offshoots are currently widely used in modern commercial date plantations as these are true to type with a known genetic makeup. Besides offshoots, tissue cultured palms are also used to establish new commercial plantations.

Agro-techniques

Date palm cultivation needs special attention when it comes to selection of planting material, palm nutrition, irrigation, pollination, and tree and bunch management.

Propagation and planting

Seed propagation is not true to type, and date palm is widely cultivated through offshoots, mainly produced during the early life of the palm (20 years). Zaid & de Wet (2002b) recommend 3-5 year old offshoots that weigh 12-25 kg with a girth of around 25 cm to be preferred and offshoots that originate high on the palm should be avoided. Besides offshoots, tissue cultured

Table 2 : Popular date palm cultivars in different date producing countries*

Country	Cultivars
Algeria	Deglet Nour, Iteema, Thoory
Egypt	Amhat, Hayani, Siwi, Samany, Zoghloul
Morocco	Medjhool
Tunisia	Deglet Nour
Saudi Arabia	Khalas, Sheshi, Reziz, Sukhari, Sugai, Anbarha
UAE	Lolo, Khalas, Bahri
Iran	Estamaran, Shahani, Kabkab, Mazafati
Israel	Medjhool
India	Bahri, Halawi, Khadrawy, Kuneizi, Zahidi
Oman	Zabad, Hilali Omani, Nashukharma, Khalas, Barni
Pakistan	Zaidi, Mobini, Shakri, Khadrawi, Dhakki
Palestine	Medjhool
USA	Medjhool, Deglet Nour, Empress, Zahidi, Khadrawy, Halawy

*updated from Siddiq & Greibi (2013)

Farmers *Khalal* dates collected from elite non-descript seedling progeny (DPRC, Mundra, Kutch, Gujarat, India)

Dates from elite seedling palms in Sudan (Agricultural Research Centre, Merowe, Sudan)

Tissue culture date palm cv. Khalas

palms are also used to establish new commercial plantations and should be transplanted at 4-plus pinnate leaf stage after hardening (Zaid & de Wet, 2002b). Micro-propagated date palms enable farmers to grow verified varieties instead of depending on off-shoot selections (Sirisena et al., 2015).

It is recommended to plant date palm offshoots/tissue cultured plantlets on the surface of a pit (hole) of 1 m^3 and mixed well with the organic manure and planted in 10 x 10 m (100/ha), 9 x 9 m (123/ha) or 8 x 8 m (156/ha). In areas with high humidity, spacing of 10 x 10 m and for dwarf cultivars 8 x 8 m is recommended (Zaid & de Wet, 2002b).

To avoid stress to the offshoots / plantlets, planting should be carried out in the early morning. The heart of the offshoots / plantlets should be above the soil surface avoiding deep planting in the pit (hole). A basin of about 3 m diameter and 20 to 30 cm deep should be prepared around the palm soon after transplanting to prevent run-off and to ensure sufficient availability of irrigation water. The basin should have a slight downward slope towards the plant to allow the water to reach the root system of the young plant (Zaid & de Wet, 2002b).

The stem does not come in direct contact with the irrigation water in the basin as this is known to pre-dispose the palms to attack by red palm weevil, *R. ferrugineus* (Faleiro & Al-Dawood, 2020). Mulching of the basin around the palm with organics after planting is highly recommended.

Date palm nutritio n

Global averages requirement of N, P and K per yielding date palm is 650 g, 650 g and 870 g of N, P and K, respectively needs to be applied/date palm/ year. Young palms up to four years old are fertilized at 262-525 g N, 138 g P and 540 g K/palm/year (Klein & Zaid, 2002a).

Irrigation

Date palm responds well to irrigation. Although the crop is known to withstand high levels of salinity, it is important that irrigation water is not highly saline, and the crop is not over irrigated (Liebenberg & Zaid, 2002*)*. In Saudi Arabia, lysimeter based studies on the daily water requirement of date palm ranged between 87 and 297 L during January and July, respectively with a daily average of 182 L through the whole year depending mostly on weather and quality of the irrigation water (Dewidar et al., 2015). The response of date palm to trickle irrigation is better in comparison to the basin method (Al-Amoud, 2000).

Pollination in date palm

Date palm is a dioecious crop where male (pollen bearing) and female (fruit bearing) inflorescences are on separate palms (Popenoe, 1992). To ensure good fertilization, overcome disadvantages of dichogamy and reduce the number of male palms, artificial pollination is carried out, where pollen harvested from staminate flowers are used for artificial manual pollination (as traditionally practiced throughout the Middle East) or mechanically using dusters. Pollination in date palm is influenced by several factors *viz*. pollination time, flowering period of male palm, the type, amount, viability and availability of pollen and receptivity of female flowers (Johnson et al., 2013; Ben Abdallah et al., 2014). A mature male spathe is soft in texture and attains a brown colour before splitting. Immediately after the spathe breaks,

Date palm pollen applicator

the male inflorescence reaches its maturity and male flower clusters must be cut at this stage (Zaid & de Wet, 2002b).

In commercial plantations, the female flowers are usually pollinated by hand cutting the strands of male flowers and inserting them (2-3 strands) between female flower clusters during the first few days of its opening when the female flowers are receptive, which are loosely tied to ensure successful pollination.

Frond Pruning

Date palm cultural practices recommend the pruning of dry fronds and old fruit stalks (Zaid & Klein, 2002b). Fronds that are nearing maturity become dry, lose their photosynthetic efficiency, and must be removed annually by pruning the fronds at the base close to the trunk. With the growing threat of red palm weevil (*R. ferrugineus*) where freshly cut frond bases attached to the trunk emit volatiles that attract gravid female RPW for oviposition, is vital to protect freshly cut frond bases with insecticide immediately after pruning.

Truck mounted platform to perform operations in the crown of date palm

De-thorning

Date spines on the base of the fronds pose a potential hazard and can result in injury to the workers during different operations, particularly during pollination and bunch management. These spines are usually removed from the new growth of fronds in the crown of the palm just before the pollination season to allow easy access to the date spathe as they emerge.

Protecting pollinated female inflorescences and bunch management

Bunch breaking can be prevented by securing (tying) the fruit stalk to the midrib (leaf rachis) of one of the lower leaves, for support after pollination. This practice of securing the bunches, also makes the bunch easily accessible for subsequent operations *viz*. thinning, bagging and/or pesticide application. Bagging with paper bags of newly pollinated inflorescences protects from low temperatures and enhances fruit set. During the early *Khalal* stage fruit bunches are bagged using plastic mesh (Zaid & de Wet 2002b).

An adult date palm could produce 20 or more fruit bunches. Reducing fruit bunches after fruit set ensures sustained production in subsequent years. The recommended ideal bunch to leaf ratio is 1:10 (Al-Salman et al., 2012). Thinning of fruits can be achieved through bunch removal, removing strands in a bunch or removal of fruits in the strands. Thinning is practiced increasing the fruit size, besides ensuring early ripening. A 25% bunch removal coupled with 25% strand cutting ensured least number of shrivelled dates in the cultivar Gaar (Al-Darwish & Ben Abdallah, 2010). In commercial plantations, bunch covers are commonly used to protect fruits from birds, insect infestation, high humidity, and rain.

Crop Protection

Date palm is attacked by a wide range of pests and diseases (Table 3). The pest complex of date palm has significantly increased to 12 species of insects and mites and 22 species attacking stored dates (El-Shafie, 2012). Insect pest like red palm weevil (RPW) (*Rhynchophorus ferrugineus* Olivier), long horn stem borer (*Jebusea hammerschmidti* Reich), rhinoceros beetle (*Oryctes* spp.), mites (*Oligonychus afrasiaticus*) (McGregor), dubas bug (*Ommatissus lybicus* de Bergevin) and the lesser date moth (*Batrachedra amydraula)* Meyrick are of key importance in the NENA region. A framework strategy formulated for eradication of RPW at the Rome meeting aims to support national programs to control RPW (FAO, 2019). New fumigation technologies for storage of dates, using modified atmosphere with ethyl formate in liquid CO_2 are gaining importance (Wakil et al., 2015).

Sedra (2018) described the prevalence and management of 12 fungal, two phytoplasmic and three diseases of undetermined causal agents in date palm. Millions of palms have been lost to soil borne Bayoud or *Fusarium* wilt of date palm caused by *Fusarium oxysporum* f. sp. *Albedinis* (Kill. & Maire) in North

Table 3 : Major pests and diseases of date palm*

Pest/Disease	Scientific name
Pests	
Red palm weevil	*Rhynchophorus ferrugineus* Olivier
Long horn stem borer	*Jebusea hammerschmidti* Reich
Rhinoceros beetle	*Oryctes* spp.
Mites	*Oligonychus afrasiaticus* (McGregor)
Dubas date bug	*Ommatissus binotatus lybicus* de Berg
Green pit scale	*Palmaspis phoenicis* Ramachandra Rao
Carob moth	*Ectomyelois ceratoniae* (Zeller)
Lesser date moth	*Batrachedra amydraula* (Meyrick)
Termites	*Microcerotermes diversus* Silvestri
Almond moth	*Cadra cautella* (Walker)
Diseases	
Bayoud or *Fusarium* {XE "Bayoud"} or Fusarium wilt	*Fusarium oxysporum* {XE "*Fusarium oxysporum*"} f. sp. *Albedinis* ({XE "*Fusarium oxysporum* f. sp. *albedinis*"} Kill. & Maire)
Black scorch	Thielaviopsis (Chalara) paradoxa (Dade)
Diplodia disease	Diplodia phoenicum (Sacc.) Fawc. & Klotz
Leaf spots disease	Cladosporium herbarum, Alternaria alternata, Drechslera australiensis, Pestalotia palmarum Cooke (Pestalotiopsis palmarum (Cooke) Steyaert), Helminthosporium sp. and Thielaviopsis paradoxa (Dade)
Bending head disease	Thielaviopsis paradoxa (De Seynes Hohn)
Heart and trunk rot disease	Fusarium spp., Botryodiplodia theobromae (syn. Lasiodiplodia theobromae), Chalara paradoxa (syn. Thielaviopsis paradoxa Dade), C. Moreau (asexual stage) and Gliocladium spp.Belaat disease Phytophtora spp.
Apical drying of leaves	Alternaria sp. Phoma sp. and Fusarium solani
Graphiola leaf spot or false smut	Graphiola phoenicis (Moug) Poit.
Inflorescence's rot	Mauginiella scaettae Mich. & Sabet

*El-Shafie (2012); Sedra (2018)

Africa. Black scorch, *Thielaviopsis* (*Chalara*) *paradoxa* (Dade), the phytoplasma borne Al-Wijam and diseases of unknown causal agents are also of concern. The role of National Plant Protection Agencies in strengthening pre and post entry quarantine regimes by deploying appropriate phytosanitary measures will go a long way in preventing the spread of pests and diseases in date palm.

El-Saeid & Al-Dosary (2010) indicate a hazardous trend in the date palm cultivation due to the detection of pesticides in date fruits and seeds above the acceptable limits. This is largely due to the over dependence on preventive chemical treatments to deal with the problem of insect pests like red palm weevil.

Harvesting and sorting

Depending on the cultivar and market, dates are harvested in the *Khalal*, *Rutab* and *Tamar* stages. Fruit harvested at *Tamar* stage do not perish if processed well and with modern vacuum packing facilities can be stored and consumed over a long period of time. In the Northern hemisphere, harvesting of *Tamar* dates takes place at the end of summer (early cultivars) in September and ends in the middle of November (late cultivars). In the Southern hemisphere, dates are harvested between February and April. Thus, if date production picks up in the Southern hemisphere in countries like Australia, there is a good potential to meet the off-season import needs of many countries (Sirisena et al., 2015). High quality dates are harvested (handpicked) individually in several pickings, while bulk harvesting is carried out by removing the entire bunch. Ladders and vehicle mounted platforms are used to harvest the bunches carefully. Harvested bunches are transported to the packaging house for sorting, processing, and packing. In India *Khalal* dates are harvested during June and July. To maintain high quality of dates in the market, it is essential to clean and sort the fruit. Harvested dates are initially graded as per size/weight and subsequently sorted to eliminate defects (blemishes, shrivelled fruit, dates with embedded dirt, mould, decay, fruit damaged by insects/ mites etc.)

Processing, packaging, and value addition

In general, fruit size, shape, colour, texture, moisture, and skin separation are important criteria to judge fruit quality of dates which must also be without defects. Processing and packing of dates demand maintaining

Securing the fruit on the palm by covering with mesh

Harvested date bunches

high quality standards that meet norms set by Codex Alimentarius Commission and other quality assurance agencies set by the European Union, USDA and date exporting countries of North Africa (Morocco and Tunisia) are also important. Regarding fresh dates in India, sweetness followed by colour, size of the fruit and fruit weight are considered important (DPRC, Mundra, India). Aleid et al. (2012) extended the shelf life of *Khalal* and *Rutab* stages of fresh Khalas dates through modified atmosphere packaging (MAP). Treatment using CO_2 levels of 20% or below demonstrated promising results for firmness and sensory scores even 18 days after treatment, compared with the control cardboard packaging. Also, magnetic freezing (cell alive system or CAS) showed positive results as compared to conventional blast freezing of fresh dates. These findings hold promise for extending the shelf life of fresh dates.

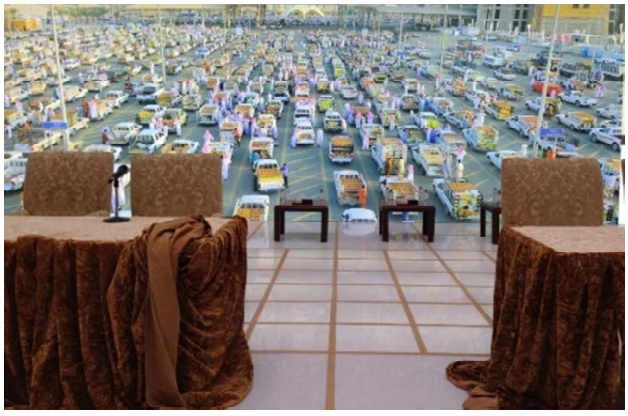
Chocolate coated dates are an ideal after dinner dessert. Date palm sap jaggery, date seed coffee, compressed furniture boards from pulverized date palm fronds and stem are other possibilities. Date palm waste is also very well-suited for the composting while low quality date fruit and date seed is an ideal resource for incorporating into animal feed. Date seed oil is a rich source of antioxidants (Barreveld, 1993). Fronds and tree stems as raw materials for producing charcoal (Zafar, 2021), use of dates to brew wine goes back to ancient Egypt (Gatley, 2009), and production of ethanol from dates (Zohri & Etnan, 2018). Date palm based handicraft items like traditional baskets, trays, purses, coasters etc., are also made by local artisans in several date palm growing countries.

Palm jaggery from the wild date palm is an important ingredient of the famous traditional sweet (dessert) '*Mishti Doi*' popular in the Indian state of West Bengal. Sap tapped from the inflorescence of wild date palm in India is used in preparation of several alcoholic and non-alcoholic beverages.

Date paste and date syrup

Marketing and export of dates

According to the International Dates Council, Riyadh, Saudi Arabia, the absence of international standards and weak internal and external marketing strategies is adversely impacting the date industry (IDC, 2013). In India, fresh date's market is growing rapidly with farmers securing a price of Rs. 30-50/kg, while, the consumer pays around Rs. 150/kg of fresh Barhee dates. India is the world's largest importer of *Tamar* dates. In the high-end premium markets of the US and Europe, there is a good demand for Deglet Nour and Medjool varieties.



Wholesale dates market in Al-Qassim, Saudi Arabia

Challenges impacting the date palm sector

A recent study by the Arab Organization for Agricultural Development (Oihabi & AOAD team, 2018) indicates that several factors impact the advancement and growth of the date palm sector resulting in the low productivity of dates in many countries. The traditional date palm grooves are dense with palms where the trees are old and have by large exceeded the productive age.

CONCLUSION

With the increase in production and consumption of fresh dates in several countries, marketing norms for this niche category of dates needs to be developed and standardized. India is the largest importer of dates and there is a good potential to increase date production to meet the growing internal demand and tapping the export market. However, like other date producing countries the date palm sector in India is facing several challenges (Pareek, 2015). In India, State Agricultural Universities and ICAR institutes/regional stations *viz*., Central Arid Zone Research Institute, Jodhpur; Central Institute for Arid Horticulture, Bikaner and the National Bureau of Plant Genetic Resources-Regional Station, Jodhpur, located in the states of Gujarat and Rajasthan have an important role to play in strengthening the date palm sector in the country. Besides these two states there is potential to cultivate date palm in other dry and semi-arid agro-climatic pockets of the country prevalent in Tamil Nadu, Andhra Pradesh, and Maharashtra etc.

National date palm programmes worldwide would stand to benefit by establishing regional and international linkages in certain niche areas of the date palm value chain that could be facilitated through organizations like the FAO of the UN, International Centre for Agricultural Research in the Dry Areas (ICARDA), Arab Organization for Agricultural Development (AOAD), Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), International Dates Council (IDC) etc. In general, the date palm sector lacks adequate support of qualified human resource and dedicated research institutions that can address problems associated with the date palm propagation, production, protection, processing and marketing of dates.

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Original Research Paper

Qualitative and quantitative assessment of wild genotypes of mango (*Mangifera indica* L.) in coastal districts of Karnataka, India

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ABSTRACT

Western Ghats are known for wild mangoes known for their distinctive flavours, tastes, and scents. The exploration of wild mangoes of coastal districts of Karnataka was undertaken. A total of 45 mango accessions were assessed for morphological characters (leaf and fruit) using numerical approach. The 14 traits including leaf blade length, leaf blade width, petiole length, fruit length, diameter, weight, and breadth, pulp, TSS, peel, and fruit thickness were analyzed. Fruit weight (g), stone weight (g), pulp (%), peel (%) and leaf blade length (cm) showed most diversity. The Moodbidri accession had most fruit weight (109.55 g), whereas, the Dakshina Kannada district's Moodbidri accession had the lightest stone weight (12.72 g). It is the first documentation of the local mango germplasm variability in coastal Karnataka.

Keywords : Coefficient of variation, mango, morphological traits, wild genotypes

INTRODUCTION

Mangifera indica L., family Anacardiaceae, is often named king of fruits for its luscious flavor and taste. It is an evergreen tree with a variety of sizes and shapes, a tetraploid species (4x) with chromosome number 40. India contributes 40% of the world's total mango production and is the top country for mango cultivation (Makarabbi, 2023). India is the world's leading producer of mangoes, producing 21822.3 thousand MT annually over an area of 2258.1 thousand ha with a productivity of 9.7 MT/ha. India is also the source of the most mango germplasm accessions (Sridhar et al., 2022). The genus has over 27 species, several of which produce eatable, juicy fruits, most notably the common mango (*M. indica*). Others, like *M. foetida*, produce astringent fruits that may be consumed and preserved in pickles (Litz, 2009).

The monograph of Mukherjee (1949) listed 41 *Mangifera* species. The genus *Mangifera* has approximately 69 species and mostly restricted to tropical Asia. These are located mostly on the Malaya peninsula in the Indonesian archipelago in Thailand and in the Philippines (Mukherjee, 2009). Vavilov (1926) has identified Indo-Burma region as the center of Mango. As a result, India has a wide variety of mangoes, and it also contains the most mango germplasm in South East Asia.

Based on morphological traits that make up the morphological markers, wild mango cultivars have been identified using vegetative criteria such leaf size, leaf shape, shoot length, fruit size, fruit shape, peel colour, stone size, and stone weight. Therefore, there has been an effort to explore and study wild mango cultivars from identified areas of Dakshina Kannada and Udupi districts of Karnataka. The combination of tropical climate, abundant rainfall, and fertile soil creates an environment conducive to the growth of diverse mango varieties. However, the genetic variation of mango genotypes in these coastal regions remains largely unexplored. Understanding the wild genotypes in these areas is crucial as they might possess traits that are specific to coastal environments, such as tolerance to salt stress, which is essential for sustainable mango cultivation in these regions. The aim of the present study was to unravel their unique genetic traits and to evaluate for their productivity.

MATERIALS AND METHODS

Using biodiversity international descriptors, an assessment of the morphometric traits of 45 mango

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accessions were conducted (IPGRI, 2006). The investigation of wild mangoes was taken in the regions of Dakshina Kannada at 12° 48' 37" N latitude and 75° 06' 22" E longitude, Bantwal to Moodbidri, and coastal Karnataka at latitude 13° 26' 09" N and longitude 75° 01' 56" E, Karkala to Hebri. A random selection of eleven to twelve wild mango trees was made in each area. Plants from each accession in the field were picked at random for the visual observations. Data were gathered from January to May throughout the blooming and fruiting stages on the quantitative and qualitative attributes. Samples of the fruit and leaves were gathered. Under each replication, the average results were calculated as the treatment mean. The characteristics analyzed and the methods used to document the observations. There are 14 quantitative features, including leaf blade width, petiole length, fruit length, diameter, weight, and width, in addition to pulp %, TSS, peel percentage, fruit thickness, and stone length, thickness, and weight. Total Soluble Solids are measured in brix using a portable refractometer made by ERMA. Ten replications were used to examine each accessions/ genotype's unique qualitative characteristics. There are 11 qualitative traits total, including the form of the fruit's apex, the thickness of the skin, the height of the tree, the shape of the fruit, the kind of fruit beak, and the crown shape.

Data obtained in the study were statistically analysed, including standard deviation and ANOVA by SPSS (F:\softwares\INDOSTAT BASIC\INDOSTAT BASIC). Significance differences among factors at 5% level. The data collected based on Anonymous (2006) descriptor for mango and tabulated and analysed as per the method discussed by Gomez and Gomez (1976). The data were analyzed and the means compared for significance using CD at 5% level (Sheoran et al., 1998).

RESULTS AND DISCUSSION

Morphological traits (qualitative and quantitative) of leaves and fruits were examined with numerical methods in 45 mango accession. Data presented in Table S1 showed significant differences for 14 quantitative traits of different mango accessions of coastal Karnataka.

All the genotypes differed significantly in leaf blade width, petiole length, fruit length, fruit diameter, fruit weight, fruit width, pulp, TSS, peel, fruit thickness, stone length, stone thickness, and stone weight. The genotype K 2 exhibited significantly higher leaf blade length (27.58 cm), while, lowest was observed in H11 (8.56 cm). The genotype B11 exhibited significantly highest leaf blade width (6.56 cm) compared to the other genotypes. Petiole length has highest significant value (5.05 cm) and lowest (1.06 cm) in K2 and H10, respectively. Genotype B1 exhibited significantly highest fruit length (7.51 cm)., whereas, K3 recorded lowest fruit length (3.92 cm). The highest significant fruit diameter was recorded in K5 (28.7 cm), fruit weight B1 (134.103 g), fruit width M10 (6.13 cm), pulp percentage B6 (42.28%), TSS B5 (25.09), peel percentage K4 (37.87%), fruit thickness B6 (5.51 cm), stone length B1 (6.56 cm), stone thickness K8 (2.67 cm), stone weight B1 (60.93 g) and lowest significant value for the genotypes in different characteristics fruit diameter H9 (9.74 cm), fruit weight H9 (24.54 g), fruit width H9 (2.54 cm), pulp percentage H9 (8.175%), TSS H11 (11.58 ºBrix), peel percentage H3 (7.044%), fruit thickness M2 (1.76 cm), stone length K3 (2.83 cm), stone thickness M1 (0.12 cm), stone weight H9 (11.975g). Figure 1 showed coefficient of variation for quantitative traits of mango cultivars, showing each character's variability. The highest significance level was observed in fruit diameter, pulp percentage and petiole length.

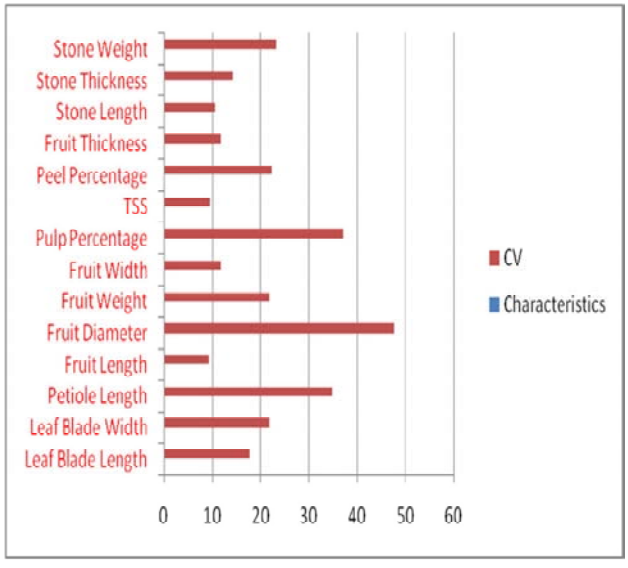

Fig. 1 : CV values of quantitative traits of mango accessions showing variability

On the basis of analysis of variance for 35 mango genotypes, Rathod and Naik (2007) found significant variation for ascorbic acid, fruit volume, fruit weight, acidity, peel percentage, TSS etc. The wild mango

Table 1 : Comparison between wild mango genotypes parameters* of coastal districts of Karnataka

Parameter	Udupi		Dakshina Kannada	
	Hebri	Karkala	Moodbidri	Bantwal
Leaf blade length (cm)	15.41± 3.07	17.64±4.78	20.97± 3.66	18.66±3.83
Leaf blade width (cm)	4.26± 0.88	4.59±0.94	4.83±0.52	5.08±0.85
Petiole length (cm)	2.28±0.67	3.01±0.94	2.98±0.47	3.26±0.80
Fruit length (cm)	5.36±0.69	5.61±0.91	5.22±0.48	5.31±0.90
Fruit diameter (cm)	12.82±1.67	15.32±4.75	12.58±3.66	14.29±2.10
Fruit weight (g)	56.75±19.55	73.57±27.52	60.69±15.31	72.86±30.96
Fruit width (cm)	3.89±0.57	4.38±0.89	4.59±0.68	4.69±0.70
Pulp (%)	11.08±6.35	14.68±5.85	16.41±10.48	18.46±12.32
TSS (ºBrix)	17.44±3.96	18.01±3.36	19.05±2.97	18.23±3.06
Peel (%)	17.49±6.78	23.59±9.08	19.49±4.88	20.44±7.04
Fruit thickness (cm)	3.80±0.46	4.10±0.57	3.45±0.88	3.55±1.15
Stone length (cm)	4.97±0.66	5.14±1.017	4.71±0.37	4.88±0.72
Stone thickness (cm)	2.02±0.23	2.11±0.26	1.33±0.81	1.73±0.68
Stone weight (g)	25.88±7.22	32.68±13.35	24.20±4.53	29.80±11.85

*Each parameter expressed as Mean±SD; N=5

genotype traits such as leaf blade length, fruit diameter, fruit weight, pulp percentage, TSS, peel percentage, stone weight length, were statistically significant and leaf blade width, petiole length, fruit length, fruit width, stone thickness, fruit thickness, stone length was statistically non-significant (Table S1). The fruit weight (109.55 g) showed significant superior and stone thickness (3.73) has lowest significance in comparison with other varieties. Singh et al. (2017) reported significant variations in fruit weight among varieties and found that Haathijhool was most superior variety according to fruit weight, while, Tamancha was identified to be most inferior and also added variations in surface morphology might be due to the inherent nature of particular varieties and the prevailing agro-climatic conditions.

The average mean value and standard deviation of 45 mango accessions from coastal districts (Dakshina Kannada and Udupi) of Karnataka showed variation for various morphological traits (Table 2). Parameters of mango accession in Dakshina Kannada and Udupi showed higher value such as leaf blade length (20.97± 3.66), leaf blade width (5.08±0.85), petiole length (3.26±0.80), fruit width (4.69±0.70), pulp percentage (18.46±12.32), TSS (19.05±2.97 and fruit length (5.61±0.91), fruit diameter (15.32±4.75), fruit weight (73.57±27.52), peel percentage (23.59±9.08), fruit thickness (4.10±0.57), stone length (5.14±1.01), stone thickness (2.11±0.26), stone weight (32.68±13.35), respectively.

Leaf blade length, fruit weight, peel percentage, pulp %, and stone weight showed clear variance between the sites. Another useful leaf descriptor that may be used to discriminate distinct mango cultivars were leaf length, breadth, and petiole length, as also observed by earlier workers (Bhamini et al., 2018; Igbari et al., 2019). Bhamini et al. (2018) found morphological variations in different mango genotypes. Ribeiro et al. (2013) showed three leaf blade shapes *viz.*, elliptic, lanceolate, and oblong, that allowed rapid and efficient characterization of mango cultivars. The leaf color varied from light-green to slightly brownish or purplish when plants are young, and acquires a dark green color as it develops and become mature.

The results of qualitative characters of different mango accessions from four locations indicated that the height of the tree varies from very tall, medium in different accession of mango (Table S3). Mango tree possess different types of canopies such as semi-circular in most of the mango genotype and spherical, circular and broadly pyramidal in other accessions. Majumder et al. (2011) observed ellipsoid plant shape in 23 genotypes and rest was spheroid. Leaf apex shapes

were largely acuminate and acute, whereas, leaf base shape predominantly acute and rarely obtuse. Leaf fragrance mostly mild and absent in the most of the genotypes but few showed mild leaf fragrance. According to Brazilian descriptors, Ierla et al. (2013) characterized 103 mango accessions for morphological traits and discovered that the pyramidal shape was most common among accessions.

Table S4 showed the variation in fruit shape, color and pulp color. Variation in skin color was mostly yellowish to greenish appearance, while, pulp color ranges yellowish to orange but less variation was observed in fruit characteristics, such as fruit apex which was roundish in nature. Fruit shape showed variation such as elliptic, obtuse, round, ovoid and fruit beak type is perceptible. The ripe fruits' skin and pulp colours ranged from green to yellow and from yellow to orange, respectively. Griesbach (2003) found that the fruits were light green or yellow skin becomes speckled with crimson blotches as it ripens. Sharma and Majumder (1989) emphasized that the fruit's red skin colour is dominant, is controlled by a duplicate gene, and progresses to a pink blush on the fruits in the population of offspring.

The current research is the first to describe mango accessions in coastal Karnataka districts in terms of structure. The results help the breeding programme to produce desirable and consistent quality mangoes across Karnataka. Significant variability exists for leaf blade length, fruit diameter, fruit weight, pulp percentage, TSS, peel percentage, and stone weight length in the wild variety of coastal Karnataka. Sridhar et al. (2022) found variations in morphological characteristics among the desirable wild mango varieties. Evaluation of variability by simple methods is also identified as one of the crucial steps for evaluating genetic diversity.

CONCLUSION

The meticulous examination of 45 mango accessions of coastal districts of Karnataka for morphological traits exhibited valuable insights into the inherent variability within the wild mangoes. The detailed analysis of key characteristics, encompassing various aspects such as leaf morphology, fruit dimensions, and pulp composition, has provided a comprehensive overview of the diversity present in these mango accessions. The variations observed in fruit weight, stone weight, and pulp percentage, peel percentage, and leaf blade length among the accessions highlight specific areas of interest for further investigation and targeted breeding efforts.

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Original Research Paper

Biochemical characterization of gamma-ray induced mutants in mango

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ABSTRACT

The volatile compound (VC) compositions of putative mutants were estimated and compared with the untreated seedlings and mother plants of mango genotype Bappakkai. Sesquiterpenes were the major VC detected in mother plants, control plants and putative mutant samples *viz.*, BM_4, BM_5 and BM_6, while, monoterpenes dominated the volatile fractions of other three putative mutant samples. A positive and significantly high correlation between all the mother plants, between mother plants and control seedlings as well as between the control seedlings, suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Hence, we conclude that volatile profiling can be used as a biochemical marker for characterization and validation of putative mutants in polyembryonic mango genotypes.

Keywords : Gamma irradiation, HS-SPME-GC/MS, mango, monoterpenes, sesquiterpenes, volatile profiling

INTRODUCTION

Headspace solid-phase microextraction coupled with gas chromatography/mass spectrometry (HS-SPME-GC/MS) is a method of choice for comprehensive analysis of volatile compounds (Shimizu et al., 2021). Leaf or fruits volatile organic compounds has extensively been used for investigating varietal differences in fig (Oliveira et al., 2010), mango (Shimizu et al., 2021), avocado (Ali et al., 2020) and apple (Roberts & Spadafora, 2020). The aromatic volatile profile of mango is dominated by monoterpenes, sesquiterpenes, esters, lactones, alcohols, aldehydes, ketones, volatile fatty acids and some carotenoid compounds (Pandit et al., 2009; Shimizu et al., 2021). Polyembryony is a peculiar trait in certain mango genotypes wherein multiple apomictic embryos develop from the maternal nucellar tissues along with a single zygotic embryo with the plants originating from apomictic embryos being identical to the mother plants.

Induced mutations have widely been used for widening the genetic base of mango (Rime et al., 2019; Perveen et al., 2022). Gamma irradiation has been reported to alter the concentration and composition of volatile compounds in rose (Ryu et al., 2020) and pistachio nut (Alinezhad et al., 2021) etc., and is emerging as a useful technique in characterization and validation of putative mutants. In the present study, an attempt was made to use volatile organic compounds as biochemical marker for validation of putative mutant progenies of polyembryonic mango genotype Bappakkai developed through gamma irradiation. This study was based on the hypothesis that any variation observed in the seedlings emerging from gamma irradiation treated kernels could be considered to have been resulted from mutation, while, the seedlings emerging from untreated kernels (control) should be similar to the mother plants owing to the nucellar origin of these plants.

MATERIALS AND METHODS

Generation of putative mutants

Fully matured fruits were collected from 30 years old genotype Bappakkai mother plants (BMP) being maintained in the field genebank of ICAR-Indian Institute of Horticultural Research, Bengaluru, for imposing irradiation treatment. The seed kernels were gamma irradiated with five doses (Gy) *viz.*, 15, 20, 25, 30 and 35. After 2-3 months of germination, seedlings emerging from each seed kernel were separated and transplanted in new polybags. Six months after germination, only one to two seedlings survived per seed, which were considered to be nucellar in origin due to the vigour taking cognisance of the previous study (Srivastava et al., 1988). On the basis of coefficient of variability, 30 Gy irradiation treatment was found to result in maximum

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morphological diversity (Perveen, 2022) which were used for the present study.

Extraction and analysis of organic volatile compounds

Recently matured leaves from mother plants, selected putative mutants (10 months old) and untreated (control) plants (10 months old) of polyembryonic mango genotype Bappakkai were collected, flash frozen in liquid nitrogen and brought to laboratory for further analysis. A total of 18 control seedlings were divided into three samples, *viz.*, BC_1, BC_2, and BC_3, each sample consisting of leaves from six seedlings. A total of 18 putative mutants were divided into six samples, each consisting of leaves from three putative mutant seedlings (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6). Volatile organic compounds were extracted using headspace-solid phase micro-extraction (HS-SPME) technique and identified with gas chromatography–mass spectrometry (GC-MS). Five-gram powdered leaf sample was transferred to 150 mL conical flask having a magnetic stirrer and the mouth of conical flask was sealed with a silicon stopper. A pre-conditioned SPME fibre was exposed to the sample headspace for 2 hours for adsorption of volatiles. The volatile compounds were desorbed from the fibre for 10 minutes in the injector (250°C) of a gas chromatograph, Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS-MS ion trap mass selective detector.

Statistical analysis

The total chromatogram for each sample was obtained by adding all the GC peak areas and each volatile compound was expressed as relative per cent area. The volatile compounds were identified by their retention times with references to standard compounds and the obtained mass spectra were compared with the spectra available in the Wiley and NIST-2007 libraries. After applying squared Euclidian cluster analysis to all of the characters' means, Ward's approach was used to create a dendrogram (Rencher, 1995). Principal components analysis (PCA) was then performed on the correlation matrix (SAS, 2012).

RESULTS AND DISCUSSION

Volatile compounds are low-molecular-weight, organic compounds, involved in plant defence against insects, adaption to abiotic stress and confers aroma and flavour to fruits (Vivaldo et al., 2017). In mango, volatile compounds have been used a biochemical marker for germplasm characterisation (Li et al., 2017; Shimizu et al., 2021) and investigating susceptibility to mango gall fly (Augustyn *et al.,* 2010). Analysis of leaf volatile compounds of Bappakkai allowed to differentiate between gamma irradiated and untreated plants. In the present study, monoterpenes and sesquiterpenes were found to be the major volatile compounds in all the samples. Earlier reports have also confirmed terpenes to be the most abundant volatile constituent of mango (Pino et al., 2005; Li et al., 2017). Sesquiterpenes were the major volatile compounds detected in mother plants as well as control seedlings, while, monoterpenes dominated the volatile fractions of three putative mutant samples (BM_1, BM_2 and BM_3) and sesquiterpenes were the most abundant volatile constituent in the remaining three putative mutants BM_4, BM_5 and BM_6 (Table 1). In general, the concentration of monoterpenes was more than sesquiterpene in all the samples except putative mutant seedling BM_4, where sesquiterpenes dominated the overall volatile profile being 59.14%. Beta-Selinene, was the most abundant volatile constituent (>30%) in the leaf samples of mother plants as well as control seedlings (Table 1). β-Pinene was detected in all the mother plants and control seedling while among the putative mutants it was present only in BM_4.

Among all the samples, BM_4 had the highest amount of sesquiterpenes (86.70%). Pandit et al. (2009) for the first time reported sesquiterpene dominated mango cultivars all of which were of Indian origin. In the selected putative mutant seedlings, the number of monoterpenes and sesquiterpenes was more as compared to mother plants and control seedlings. An increase in the number of volatile compounds with gamma irradiation has previously been reported in rose (Ryu et al., 2020) and pistachio nut (Alinezhad et al., 2021).

Correlation analysis

A positive and significantly high correlation was observed between all the mother plants, between mother plants and control seedlings as well as between the control seedlings. This suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Further, considerable amount of variability was observed among the selected putative mutants (Table 2).

Table 1 : Volatile composition (relative percentage) of leaf samples of mother plants, control seedlings and selected putative mutants of genotype Bappakkai

Compound	Mother plant			Control			Mutants					
	BMP_1	BMP_2	BMP_3	BC_1	BC_2	BC_3	BM_1	BM_2	BM_3	BM_4	BM_5	BM_6
Alcohols												
1-Hexyn-3-ol	ND	ND	ND	ND	ND	ND	0.25	ND	0.04	ND	ND	ND
Aldehydes												
trans-2-Hexenal	0.54	0.44	0.85	0.44	0.53	0.33	ND	ND	ND	ND	0.99	ND
Mono-terpenoids												
α-Thujene	ND	ND	ND	ND	ND	ND	0.21	ND	0.14	ND	ND	ND
α-Pinene	6.19	4.98	5.84	7.72	13.17	10.47	31.31	8.75	34.14	0.94	2.17	4.01
Camphene	ND	ND	ND	ND	ND	ND	0.45	ND	ND	ND	ND	ND
Sabinene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.72	0.26
trans-Ocimene	0.83	0.56	0.33	0.25	1.00	0.32	1.02	0.71	3.17	0.00	1.04	0.11
beta-Ocimene	ND	ND	ND	ND	ND	ND	2.45	1.10	2.38	ND	ND	ND
β-Pinene	0.90	0.61	0.57	0.41	1.50	4.35	ND	ND	ND	0.25	ND	ND
I-Phellandrene	7.11	17.66	20.43	11.76	9.18	10.22	21.25	18.82	31.22	0.00	0.00	5.34
α-Terpinene	ND	ND	ND	ND	ND	ND	1.63	ND	ND	ND	ND	ND
beta-Phellandrene	ND	ND	ND	ND	ND	ND	ND	1.17	ND	8.34	ND	ND
dl Limonene	14.07	ND	0.03	14.11	26.00	12.91	27.70	9.90	22.76	0.00	36.07	7.90
Cis-Ocimene	ND	ND	ND	ND	ND	ND	0.40	ND	ND	ND	ND	ND
trans-Ocimene	ND	ND	ND	ND	ND	ND	0.06	ND	ND	ND	ND	ND
γ-Terpinene	0.84	0.23	ND	ND	ND	ND	0.49	0.21	0.68	ND	ND	ND
α-Terpinolene	ND	ND	ND	ND	ND	ND	0.26	36.77	0.24	ND	ND	ND
Allo-Ocimene	ND	ND	ND	ND	ND	ND	0.12	ND	0.20	ND	ND	ND
Total	29.94	24.05	27.20	34.25	50.86	38.28	87.36	77.43	94.92	9.54	40.00	17.62
Sesqui-terpenoids												
alpha-Gurjunene	14.65	14.94	11.89	12.70	9.80	15.04	6.62	1.85	0.15	ND	18.47	17.41
alpha-Cubebene	ND	ND	ND	ND	ND	ND	ND	0.42	0.35	ND	ND	ND
trans-Caryophyllene	ND	9.99	13.90	5.92	7.37	6.45	2.40	1.10	0.21	22.90	7.01	6.49
Aromadendrene	ND	ND	ND	ND	ND	ND	0.03	ND	0.07	ND	ND	ND
β-Elemene	ND	ND	ND	ND	ND	ND	ND	ND	0.86	20.29	ND	ND
beta-Cadinene	ND	1.50	1.08	0.24	2.10	1.70	0.40	0.79	1.02	1.16	1.10	2.42
delta-Cadinene	ND	ND	ND	ND	ND	ND	0.05	0.07	0.13	ND	ND	ND
alpha-Copaene	ND	ND	ND	20.46	8.99	16.27	0.13	17.65	ND	ND	23.71	15.09
alpha-Humulene	16.01	7.65	13.98	4.52	5.56	3.39	0.57	ND	ND	12.96	4.36	4.73
beta-Selinene	39.39	41.44	32.00	21.91	15.33	19.19	1.89	ND	ND	29.39	3.68	31.37
alpha-Lonipinene	ND	ND	ND	ND	ND	ND	0.05	0.07	1.52	ND	0.67	2.57
Total	70.06	75.51	72.85	65.75	49.14	62.05	12.14	21.95	4.30	86.70	59.01	80.08

ND: not detected

Table 2 : Pearson correlation matrix of volatile profile for Bappakkai

Sample	BMP_1	BMP_2	BMP_3	BC_1	BC_2	BC_3	BM_1	BM_2	BM_3	BM_4	BM_5	BM_6
BMP_1	1											
BMP_2	0.884^{**}	1										
BMP_3	0.833^{**}	0.964^{**}	1									
BC_1	0.740^{**}	0.710^{**}	0.688^{**}	1								
BC_2	0.698^{**}	0.538^{**}	0.548^{**}	0.846^{**}	1							
BC_3	0.740^{**}	0.715^{**}	0.691^{**}	0.977^{**}	0.869^{**}	1						
BM_1	0.317^{NS}	0.222^{NS}	0.280^{NS}	0.479^{**}	0.769^{**}	0.544^{**}	1					
BM_2	0.039^{NS}	0.069^{NS}	0.107^{NS}	0.352^{*}	0.305^{NS}	0.324^{NS}	0.359^{*}	1				
BM_3	0.217^{NS}	0.198^{NS}	0.277^{NS}	0.393^{*}	0.634^{**}	0.442^{**}	0.960^{**}	0.396^{*}	1			
BM_4	0.575^{**}	0.651^{**}	0.666^{**}	0.383^{*}	0.298^{NS}	0.361^{*}	-0.069^{NS}	-0.125^{NS}	-0.106^{NS}	1		
BM_5	0.360^{*}	0.128^{NS}	0.124^{NS}	0.703^{**}	0.797^{**}	0.696^{**}	0.471^{**}	0.285^{NS}	0.285^{NS}	0.027^{NS}	1	
BM_6	0.859^{**}	0.855^{**}	0.779^{**}	0.908^{**}	0.696^{**}	0.898^{**}	0.224^{NS}	0.148^{NS}	0.118^{NS}	0.546^{**}	0.520^{**}	1

Cluster analysis

Volatile composition of different samples was used to generate a dendogram for understanding the relationship between them (Fig. 1). The studied samples were divided into two main clusters with three sub-clusters in the first cluster. Cluster 1 comprised of nine samples including mother plants, control and three putative mutants. Out of these, samples BMP_1, BMP_2, BMP_3, BC_1, BC_2 and BC_3 were present in the first sub-cluster, the second sub-cluster comprised of two putative mutants *viz.*, BM_5 and BM_6, while, putative mutant BM_4 formed the solitary member of sub-cluster 3. Cluster 2 comprised of putative mutants BM_1, BM_2 and BM_3 suggesting that these three putative mutants are more distant from mother plants and control seedlings as compared to the other three putative mutants (BM_4, BM_5 and BM_6).

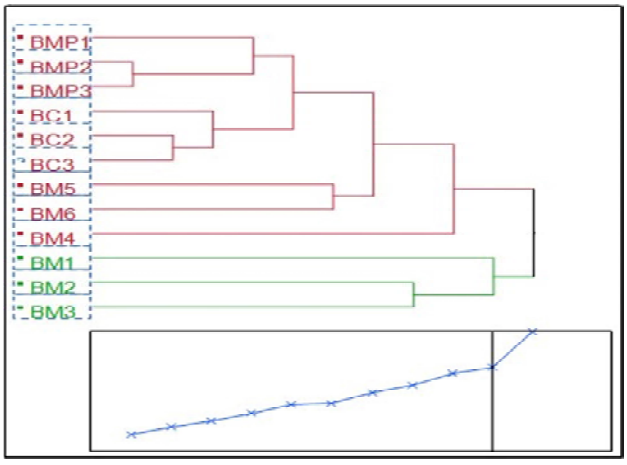

Fig. 1 : Cluster analysis of Bappakkai mother plants (BMP_1, BMP_2 and BMP_3), control sample (BC_1, BC_2 and BC_3) and selected putative mutant samples (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6)

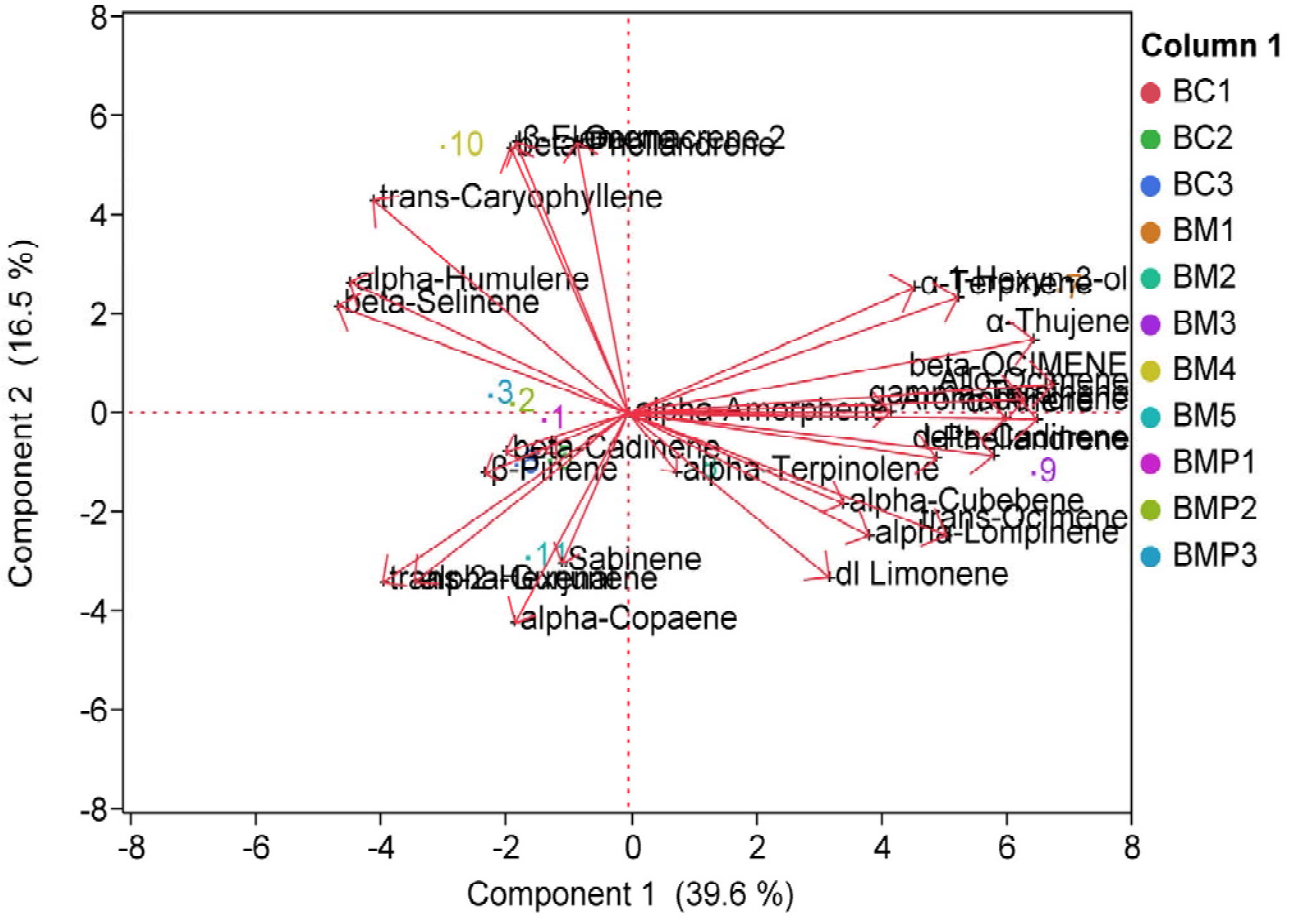

Fig. 2 : Two-dimensional bi-plot of individual VC in Bappakkai mother plants (BMP_1, BMP_2 and BMP_3), control sample (BC_1, BC_2 and BC_3) and selected putative mutant samples (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6)

Principal component analysis (PCA)

PCA results indicated that the total variability is being explained by 29 principal components out of which loading of more than 0.50 was considered significant for each factor. The first 2 principal components which collectively explained 56.14% of the total variability was depicted as two-dimensional biplot (Fig. 2). Out of these 39.63% of total variation was explained by the first principal component which had an eigenvalue of 11.89. Seventeen volatile compounds with loading (>0.5) significantly contributed to the variation in this Principal component (Table 3). Among these, 14 volatile compounds showed positive loadings wherein highest positive loading (>0.8) was exhibited by monoterpenes *viz.*, alpha-Thujene, alpha-Pinene, beta-Ocimene and Allo-Ocimene.

Table 3 : Loadings, eigen values and per cent of cumulative variance for the first two principal components

Parameter	Component	
	1	2
1-Hexyn-3-ol	0.764	0.340
trans-2-Hexenal	-0.569	-0.496
α-Thujene	0.941	0.215
α-Pinene	0.949	-
Camphene	0.662	0.368
Sabinene	-	-0.440
trans-Ocimene	0.738	-0.356
beta-Ocimene	0.986	-
β-Pinene	-0.333	-
I-Phellandrene	0.716	-
α-Terpinene	0.662	0.368
beta-Phellandrene	-	0.779
dl Limonene	0.466	-0.480
Cis-Ocimene	0.662	0.368
trans-Ocimene	0.662	0.368
γ-Terpinene	0.607	-
α-Terpinolene	-	-
Allo-Ocimene	0.921	-
alpha-Gurjunene	-0.496	-0.496
alpha-Cubebene	0.500	-
trans-Caryophyllene	-0.591	0.625
Aromadendrene	0.876	-
β-Elemene	-	0.795
alpha-Humulene	-0.645	0.384
beta-Selinene	-0.674	0.315
Eigen values	11.890	4.952
Cumulative variance (%)	39.634	56.141

CONCLUSION

Volatile profiling allowed us to differentiate between gamma irradiated and untreated plants of genotype Bappakkai. Sesquiterpenes were the major volatile compounds detected in mother plants, control plants and putative mutant seedlings, BM_4, BM_5 and BM_6 while monoterpenes dominated the volatile fractions of putative mutant seedlings, BM_1, BM_2 and BM_3. Further, the number and concentration of volatile components was found to remarkably increase with irradiation. A positive and significantly high correlation between all the mother plants, between mother plants and control seedlings as well as between the control seedlings suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Hence, study conclude that volatile profiling can be used as a biochemical marker for characterization and validation of putative mutants in polyembryonic mango genotypes. However, further study is needed for confirmation of volatile profile as biochemical marker for mutants by testing it on a sizable progeny of genotype Bappakai and other ployembryonic mango cultivars.

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Original Research Paper

Morpho-biochemical characterization of *Psidium* species

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ABSTRACT

Several *Psidium* species are available with many important traits, lack of intensive characterization limits their use in guava improvement. Therefore, the present study was carried out to characterize five wild *Psidium* species (*P. molle, P. chinensis, P. guineense, P. cattleianum* var. *cattleianum* and *P. cattleianum* var. *lucidum*) and two *P. guajava* genotypes (cv. 'Arka Poorna' and 'H 12-5'), based on morphological and biochemical traits. Among morphological traits, fruit weight was ranged from 5.22 g (*P. cattleianum* var. *cattleianum*) to 225.14 g ('H 12-5'), however, among biochemical traits, highest TSS (12.06 ºBrix) and total sugars (9.98%) were recorded in cv. 'Arka Poorna', while, lowest recorded in *P. cattleianum* var. *lucidum*. Highest ascorbic acid was recorded in *P. chinensis* (205.33 mg/100 g), whereas, lowest recorded in *P. guineense* (60.83 mg/100 g). A positive correlation was observed among wild *Psidium* species but none had correlation with *P. guajava* genotypes for quantitative traits.

Keywords: Ascorbic acid, correlation, guava breeding, *Psidium cattleianum*, *Psidium*

INTRODUCTION

Guava (*Psidium guajava* L.) is the 5th important commercial fruit crops in India. The fruits are rich in carotenoids, lycopene and phenolic compounds (Faraoni et al., 2012), besides having a high level of vitamin A & C, and B complex surpassing the levels found in other fruits (Pommer et al., 2006).

Wild *Psidium* species are having varied potential to be exploited in crop improvement programs. The species *P. guineense* Swartz is found in the coastal areas to high zones of Jalisco (Valera-Montero et al., 2018), while, strawberry guava (*P. cattleianum* var. *cattleianum* Sabine) and lemon guava (*P. cattleianum* var. *lucidum* Sabine) believed to be originated in the lowlands of eastern and southern Brazil to north-east Uruguay. The species *P. molle* is a shrub with profuse flowering, broad leaves with pubescence, small fruits, round with yellow pericarp, whereas, *P. chinensis* is a small shrub with narrowly elliptic leaves and fruits are rich in vitamin C (Banoth et al., 2017).

Moreover, guava scores over other fruits in ascorbic acid, pectin and other mineral contents (Banoth et al., 2017; Kumari et al., 2018). Present study was aimed to characterize wild *Psidium* species and genotypes using morphological and biochemical traits, which could be used in guava breeding.

MATERIALS AND METHODS

The study was carried out at Fruit Crops Laboratory, ICAR-Indian Institute of Horticultural Research, Bengaluru (13° 712 N latitude, 72° 2912 E longitude and 890 above mean sea level) during 2018 to 2020. Experimental material involved five wild *Psidium* species (*P. molle, P. chinensis, P. guineense, P. cattleianum* var. *cattleianum* and *P. cattleianum* var. *lucidum*) and two *P. guajava* genotypes *i.e.* 'Arka Poorna' and 'H 12-5'. Fruits were harvested from a uniform age group (10 to 12 years) of trees maintained in field genebank with uniform package of practices.

Morphological traits

The optimum ripe fruits of *Psidium* species and genotypes were evaluated for morphological traits *viz*., fruit weight (g), fruit volume (mL), fruit diameter (mm), fruit length (mm), fruit girth (mm), pulp thickness (mm), core thickness (mm), weight of 100 seeds (g), total number of seeds, pulp weight (g), seed hardiness (kgf), peel & pulp colour and number of fruits per plant based on guava descriptors (PPV & FRA, 2016). Hardiness of freshly extracted seeds was measured by Vinsyst Hardness Tester. The colour of fruit peel and pulp were described using RHS Colour Chart (RHSC, 2001). Morphological characters of trees were taken as an average of ten plants with three

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replications. Data on tree height (m) and plant spread (m) (north-south and east-west) were recorded using meter scale.

Biochemical traits

Biochemical traits *viz*., total soluble solids (°Brix), total sugar (%), reducing sugar (%), non-reducing sugar, ascorbic acid (mg/100 g), acidity (%), carotenoids (mg/100 g) and lycopene (mg/100 g) were estimated in fruit pulp. All biochemical traits were recorded from 10 fruits with three biological replications.

Fruit pulp TSS was measured using a hand refractometer (Anon., 2000) at room temperature (27±2 °C). Total and reducing sugars were analysed by the method of Nelson-Somogyi (1952). Percentage of non-reducing sugars was calculated by subtracting value of the reducing sugars from that of total sugars. The acidity was determined by titration method (Anon., 2000) and ascorbic acid content by DCPIP method (Anon., 2006), while, total carotenoids & lycopene by spectrophotometric method (Lichtenthaler, 1987) under low light condition.

Statistical analysis

Experimental design used was randomized block design with three replications. Mean data was compared by analysis of variance and the Fisher's least significance difference. Pearson's correlation (P=0.01) was performed by using SAS Institute Software (SAS, 2012).

RESULTS AND DISCUSSION

Morphological traits

The data revealed significant and wide variation among wild *Psidium* species and guava genotypes for fruit traits (Table 1 and 2). Highest fruit weight (225.14 g), diameter (85.01 mm), length (77.56 mm), volume (169.89 ml), core thickness (15.94 mm) and pulp weight (184.48 g), girth (314.8 mm), pulp thickness (56.56 mm) were observed in 'H 12-5' followed by 'Arka Poorna', whereas, lowest fruit size was recorded in *P. cattleianum* var. *cattleianum*. The fruits of cultivated guava were, at first sight, bigger, soft seeded and attractive in colour with a pleasant aroma and delicious taste compared to wild *Psidium* species. This difference could be due to selection pressure by human intervention and their genetic makeup (Banoth et al., 2017).

Significantly maximum seeds per fruit (211.60) and seed hardiness (13.04 kgf) was recorded in *P. guineense*, whereas, minimum seed per fruit found in *P. molle* (12.53) and soft seeds recorded in cv. 'Arka Poorna' (4.82 kgf). Maximum weight of 100 seeds was recorded in *P. cattleianum* var. *cattleianum* (4.40 g), whereas, it was minimum in 'Arka Poorna' (0.75 g). Seed number is known to be a function of fertility and effective fertilization (Vishwakarma et al., 2021).

Table 1: Fruit parameters of wild *Psidium* species and genotypes

Species/Genotype	Fruit weight (g)	Fruit volume (ml)	Fruit diameter (mm)	Fruit length (mm)	Fruit girth (mm)	Thickness		Weight of 100 Seeds (g)	Seeds per fruit (Nos.)
						Core (mm)	Pulp (mm)		
Psidium cattleianum var. *lucidum*	9.97^{E}	7.63^{E}	24.39^{D}	25.38^{D}	84.4^{D}	3.23^{D}	19.56^{D}	2.08^{C}	72.00^{D}
P. cattleianum var. *cattleianum*	5.22^{F}	4.79^{E}	20.54^{E}	22.72^{D}	70.0^{E}	2.39^{E}	14.34^{E}	4.40^{A}	26.33^{E}
P. molle	20.39^{C}	15.56^{D}	34.41^{C}	31.46^{C}	113.1^{C}	6.67^{C}	22.80^{C}	3.72^{B}	12.53^{F}
P. guineense	14.96^{D}	12.50^{D}	27.58^{D}	29.35^{C}	94.5^{D}	3.04^{D}	22.55^{C}	0.79^{D}	211.60^{A}
P. chinensis	53.47^{B}	49.09^{C}	44.49^{B}	40.55^{B}	151.1^{B}	6.37^{C}	36.65^{B}	0.78^{D}	188.13^{B}
P. guajava genotype 'H 12-5'	225.14^{A}	169.89^{A}	85.01^{A}	77.56^{A}	314.8^{A}	15.94^{A}	56.56^{A}	0.78^{D}	182.10^{BC}
P. guajava cv. 'Arka Poorna'	221.96^{A}	165.57^{B}	84.14^{A}	77.02^{A}	305.6^{A}	14.98^{B}	54.54^{A}	0.75^{D}	179.10^{C}
SEm±	1.193	1.039	1.062	0.995	0.337	0.160	0.767	0.041	2.489
LSD at 5%	3.674	3.218	3.217	3.065	1.095	0.524	2.190	0.120	7.898
CV (%)	2.82	2.99	4.23	3.97	3.60	3.68	4.09	3.705	3.46

Means with at least one letter common in a row are not statistically significant using Fisher's least significant difference.

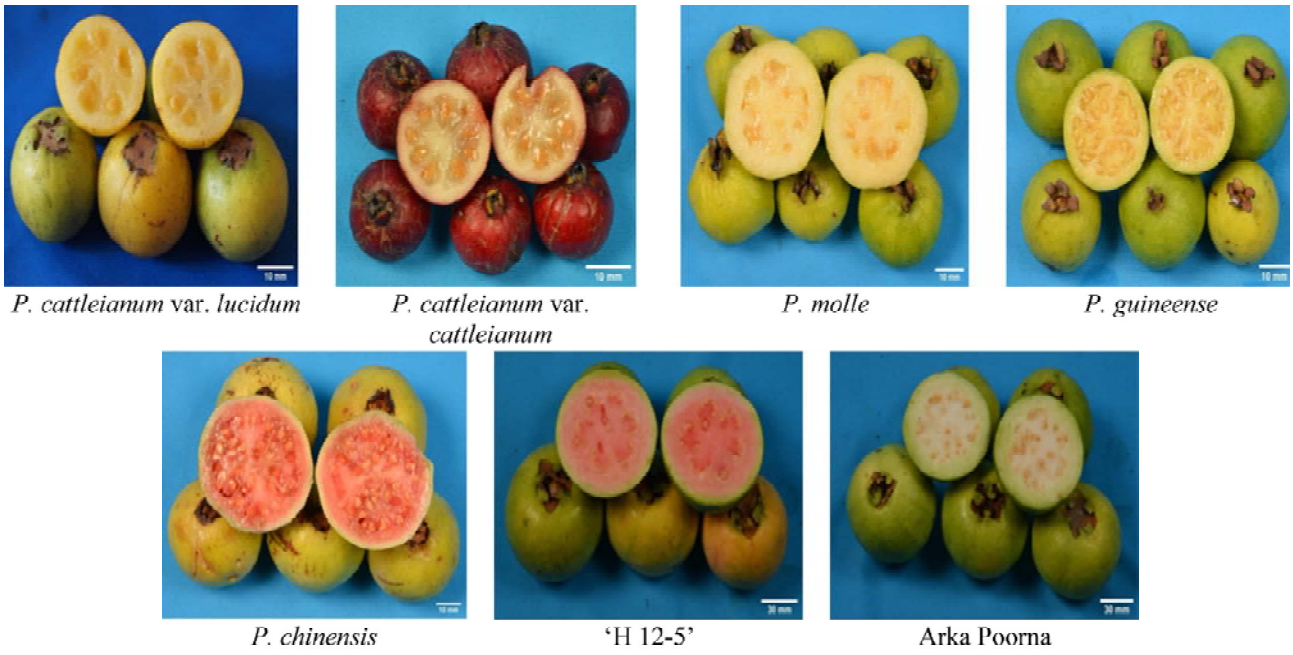

Fig. 1 : Fruits of wild *Psidium* species and genotypes; scale bar: a to e – 10 mm, f and g – 30 mm

Peel colour was yellowish-green in *P. cattleianum* var. *lucidum, P. guineense,* 'H 12-5' and cv. 'Arka Poorna', yellow in *P. molle* and *P. chinensis*, whereas, dark red in *P. cattleianum* var. *cattleianum* at optimum ripe stage (Fig. 1). The species *P. cattleianum* var. *lucidum* and *P. guineense* had yellow-green pulp, light red in *P. cattleianum* var. *cattleianum* and *P. chinensis*, whereas, *P. molle* had yellow colour pulp. 'H 12-5' comes under orange-red colour pulp category, while, 'Arka Poorna' with white pulp. Variation in pulp colour within *P. guajava* genotypes might be due to segregation in F_1 progenies developed from same parents as observed in 'Allahabad Safeda' with white pulp and 'Purple local' with red pulp. Similar variations in fruit characters were also observed by Damiani et al. (2011) and Valera-Montero et al. (2018).

Table 2 : Fruits and plant parameters of wild *Psidium* species and genotypes

Species/Genotype	Pulp weight (g)	Seed hardiness (kgf)	Peel colour	Pulp colour	Plant spread (m)			Fruits per plant (Nos.)
					N-S	E-W	Height	
Psidium cattleianum var. *lucidum*	7.72^{E}	11.31^{C}	YG 150B	YG 145C	2.06^{B}	2.03^{B}	3.00^{B}	2383.33^{B}
P. cattleianum var. *cattleianum*	4.08^{F}	11.83^{B}	Red 46A	Red 56D	2.21^{B}	1.98^{B}	2.77^{BC}	2573.33^{A}
P. molle	15.68^{D}	10.89^{D}	Yellow 11A	Yellow 11C	1.56^{D}	1.66^{C}	4.33^{A}	80.00^{F}
P. guineense	8.25^{E}	13.04^{A}	YG 144A	YG 149D	1.57^{D}	1.70^{C}	1.74^{F}	1946.67^{C}
P. chinensis	46.99^{C}	7.70^{E}	Yellow 11B	Red 47A	1.84^{C}	1.94^{B}	2.52^{D}	376.67^{E}
P. guajava genotype 'H 12-5'	184.48^{A}	4.94^{F}	Y G 150D	Orange Red 34C	2.53^{A}	2.56^{A}	2.24^{E}	770.00^{D}
P. guajava cv. 'Arka Poorna'	176.71^{B}	4.82^{F}	YG 149C	White 155C	2.62^{A}	2.52^{A}	2.74^{CD}	666.67^{D}
SEm±	1.188	0.096	-	-	0.069	0.044	0.073	37.329
LSD at 5%	3.637	0.320	-	-	0.177	0.141	0.228	113.64
CV (%)	3.24	1.81	-	-	5.77	3.74	4.584	5.145

Means with at least one letter common in a row are not statistically significant using Fisher's least significant difference.

Morphological traits

The guava genotypes and wild *Psidium* species showed significant variations for different plant characters. Among all genotypes, plant spread (N-S) ranged from 1.56 m (*P. molle*) to 2.62 m ('Arka Poorna') (Table 2) and plant spread (E-W) ranged from 1.66 m (*P. molle*) to 2.56 m ('H 12-5'). Plant height ranged from 1.74 m (*P. guineense*) to 4.33 m (*P. molle*), while, 'H 12-5' and 'Arka Poorna' recorded maximum plant spread in both directions (N-S and E-W), whereas, minimum plant spread (N-S and E-W) was recorded in *P. molle* with highest upright growth. The species *P. guineense* recorded lowest plant height showing drooping growth pattern. The strong apical dominance in *P. molle* might be a reason for maximum plant height (Deshmukh et al., 2013).

Significantly maximum number of fruits per plant was recorded in *P. cattleianum* var. *cattleianum* (2573.33) followed by *P. cattleianum* var. *lucidum* (2383.33), however, minimum recorded in *P. molle* (80.00). These variations in the bearing of fruits might be due to inherent genetic makeup of the species (Banoth et al., 2017).

Biochemical traits

'Arka Poorna' recorded significantly highest TSS (12.06 ºBrix), total sugar (9.98%), reducing sugar (8.44%) and non-reducing sugar (1.54%) over the other genotypes and wild *Psidium* species (Table 3). TSS was at par with 'H 12-5' followed by *P. molle, P. guineense* and *P. chinensis*. However, lowest TSS (8.83%), total sugar (5.08%), reducing sugar (4.24%) and non-reducing sugar (0.833%) were recorded in *P. cattleianum* var. *lucidum*. Cultivated genotypes might necessitate consumption of nutrients and sinking more carbohydrates into the fruits, thus producing larger fruits with more TSS and sugars. This confirms with the findings of Banoth et al. (2017) and Valera-Montero et al. (2018).

Significantly highest ascorbic acid (230.33 mg/100 g of pulp) was recorded in *P. chinensis* followed by *P. cattleianum* var. *cattleianum* and *P. cattleianum* var. *lucidum* while lowest in *P. guineense* (60.80 mg/100 g), however, lowest acidity was also recorded in *P. guineense* (0.290 %), while, highest in *P. cattleianum* var. *cattleianum* (0.819 %) (Table 3).

Among all guava genotypes and *Psidium* species, 'H 12-5' had higher total carotenoids (5.235 mg/100 g) and lycopene (4.064 mg/100 g) content followed by *P. chinensis*. While, lowest total carotenoids (0.694 mg/100 g of pulp) and lycopene (0.234 mg/100 g of pulp) content were recorded in *P. guineense.* Lycopene has been found to be higher in pink pulp types than white pulp guava which helps in reducing the risk of several chronic diseases (Omoni et al., 2005). The species *P. chinensis* could be explored for vitamin C in daily human diet (Corrêa et al., 2011). The larger variation in biochemical parameters might be due to phenotypic and genetic constitution of the species (Valera-Montero et al., 2018).

Correlation analysis of quantitative traits

P. cattleianum var. *lucidum* showed positive correlation with *P. cattleianum* var. *cattleianum*,

Table 3 : Biochemical parameters of fruits of wild *Psidium* species and genotypes

Species/Genotype	TSS (ºBrix)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ascorbic acid (mg/100 g)	Acidity (%)	Carotenoids (mg/100 g)	Lycopene (mg/100 g)
Psidium cattleianum var. *lucidum*	8.83^{D}	5.08^{G}	4.24^{F}	0.833^{E}	207.33^{BC}	0.632^{C}	0.756^{C}	0.317^{C}
P. cattleianum var. *cattleianum*	9.92^{C}	5.62^{F}	4.70^{E}	0.923^{D}	210.67^{B}	0.819^{A}	0.789^{C}	0.379^{C}
P. molle	10.88^{B}	5.76^{E}	4.83^{D}	0.923^{D}	88.93^{E}	0.326^{E}	0.618^{D}	0.358^{C}
P. guineense	10.84^{B}	7.94^{D}	6.97^{C}	0.967^{D}	60.83^{F}	0.290^{F}	0.449^{E}	0.234^{D}
P. chinensis	10.76^{B}	8.11^{C}	6.99^{C}	1.117^{C}	230.33^{A}	0.726^{B}	4.037^{B}	3.268^{B}
P. guajava genotype 'H 12-5'	11.76^{A}	8.63^{B}	7.44^{B}	1.190^{B}	189.47^{D}	0.467^{D}	5.235^{A}	4.064^{A}
P. guajava cv. 'Arka Poorna'	12.06^{A}	9.98^{A}	8.44^{A}	1.540^{A}	205.33^{C}	0.475^{D}	—	——
SEm±	0.136	0.031	0.027	0.018	1.304	0.005	0.009	0.013
LSD at 5%	0.439	0.103	0.085	0.058	4.185	0.016	0.038	0.072
CV (%)	2.194	0.73	0.75	2.904	1.326	1.602	0.807	1.512

Means with at least one letter common in a row are not statistically significant using Fisher's least significant difference.

Table 4 : Correlation analysis of *Psidium* species and guava genotypes

Species/Genotype	*P. cattleianum* var. *lucidum*	*P. cattleianum* var. *cattleianum*	*P. molle*	*P. guineense*	*P. chinensis*	*P. guajava* genotype 'H 12-5'	*P. guajava* cv. 'Arka Poorna'
Psidium. cattleianum var. *lucidum*	1						
P. cattleianum var. *cattleianum*	0.899**	1					
P. molle	0.724	0.933**	1				
P. guineense	0.854**	0.545	0.267	1			
P. chinensis	0.924**	0.699	0.526	0.926**	1		
P. guajava genotype 'H 12-5'	0.625	0.583	0.657	0.450	0.742	1	
P. guajava cv. 'Arka Poorna'	0.626	0.582	0.655	0.455	0.745	0.999**	1

P. guineense and *P. chinensis* (Table 4). Similarly, *P. cattleianum* var. *cattleianum* registered significant positive correlation with *P. mole*, while, *P. guineense* with *P. chinensis*. Wild species did not register any correlation with *P. guajava* genotypes ('H 12-5' and 'Arka Poorna') but registered high correlation with each other. Similarly, Kumari et al. (2018) also reported positive correlation in morphological parameters among cultivated varieties.

CONCLUSION

The wild species of *Psidium* are good source of ascorbic acid (*P. chinensis*) and heavy bearing (*P. guineense*). Strong correlation existed among wild species for quantitative traits but no correlation existed between *P. guajava* and wild *Psidium* species. The wild species could be exploited in crop improvement for imparting the traits *viz.*, heavy bearing, high ascorbic acid content and dwarf stature.

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Original Research Paper

Heterosis and heterobeltiosis in bell pepper (*Capsicum annuum* var. *grossum*) for growth and yield parameters

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ABSTRACT

The magnitude of heterosis over commercial check and better parent was estimated to identify best crosses for growth and yield traits in bell pepper. Experimental material included 21 F_1 hybrids developed by crossing seven diverse parents in half diallel mating design. The findings revealed that Arka Mohini X CW308 showed best heterotic cross combinations, recorded significantly higher heterosis and high *per se* value over commercial check with respect to number of primary branches, number of secondary branches, plant height, days to 50% flowering, days to first harvest, fruit length, fruit width, number of lobes per fruit, number of fruits per plant and average fruit weight. The cross Arka Mohini X CW308 also showed high heterobeltiosis for traits like fruit length, number of fruits per plant, average fruit weight and average yield per plant. Hence, this hybrid with high *per se* value and heterosis can be utilized to obtain desirable segregates for development of superior genotype for improvement of horticultural traits in bell pepper.

Keywords: Bell pepper, diallel, growth, heterosis, yield

INTRODUCTION

Heterosis breeding is known for increasing the productivity and quality of crop in the shortest possible time. The popularity of F_1 hybrids is mainly due to their uniformity, vigour, disease resistance, stress tolerance and good horticultural traits which in turn gives a stable yield (Khalil et al., 2014). *Capsicum*, being a highly polymorphic genus, both inter and intra-specific, bell pepper (*Capsicum annuum* var. *grossum*) is amenable for exploitation of heterosis. In India, bell pepper is grown in an area of 38,000 ha with the production of 563,000 MT (Anonymous, 2022). The main reason for their high price is due to non-availability of public institute bred hybrids in India. Hybrids ruling in Indian market are mainly imported from European and middle Eastern countries which increases the input cost for the farmers. Hence, there is an urgent need to strengthen the crop improvement programme in bell pepper in India for developing public institute bred varieties or hybrids capable of satisfying the needs of farmers as well as consumers.

Information on magnitude of heterosis in different cross combination is a basic requisite for identifying the crosses that exhibit high amount of exploitable heterosis (Sharma et al., 2013). There are different ways of estimation of heterosis. Heterobeltiosis is superiority of F_1 over the better parent; superiority of F_1 over the mean of two parents is mid parent heterosis; and superiority of F_1 over the mean of standard check is standard/ commercial heterosis. Superiority of F_1 over mid parent is non-practical for breeders since it does not provide any economic advantage. Therefore, the present study was carried out to estimate the magnitude of commercial heterosis and heterobeltiosis to identify the best cross combination for developing superior hybrids.

MATERIALS AND METHODS

The study was conducted at Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta lake post, Bengaluru during 2019-2020. The geographical location of the experimental farm is having a latitude of 13' 7′ N and 77 ' 29′ E. Seven diverse bell pepper genotypes *viz*., Arka Mohini (AM), Arka Gaurav (AG), Arka Basant (AB), Yolo Wonder (YW), California Wonder (CW), UHFBP-4 and CW-308 were crossed in half diallel design to obtain twenty-one F_1 hybrids. Hybrids along with parents and commercial check Indra were

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evaluated for growth and yield traits along with their parents in open field in randomized complete block design (RCBD) with three replications at a spacing of 60 x 30 cm during October, 2019 to March, 2020. The standard cultural practices were followed. Observations were recorded for growth and yield related traits *viz*., number of primary branches (NPB), number of secondary branches (NSB), plant height (cm) (PH), days to 50% flowering (DFF), days to first harvest (DFH), fruit length (cm) (FL), fruit width (cm) (FW), number of lobes per fruit (NLP), pericarp thickness (cm) (PT), average fruit weight (g) (AFW), number of fruits per plant (NFP), total yield per plant (g) (YP). Indostat software was used for statistical analysis.

Increase or decrease in performance of the F_1 hybrid was measured as the proportion of deviation of F_1 from better parent and standard check using following formulae.

$$\text{Heterobeltiosis} : \frac{F1\text{-}BP}{BP} \times 100$$

$$\text{Standard heterosis} : \frac{F1\text{-}C}{C} \times 100$$

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed that mean sum of squares due to treatments (including parents and hybrids) were highly significant (p=0.05) for all the traits means the hybrids and the parents have an inherent genetic variability which could be useful to make selection and genetic advancement. However, MSS due to parents vs hybrids were significant (p=0.05) for all the traits except fruit width and days to 50% flowering (Table 1) indicating similar performance of parents and hybrids for traits like fruit width and days to 50% flowering and chances of exploitation of heterosis is meagre.

Hence, heterosis for all other traits were estimated. The range of mean performance of parents, range of heterosis percentage of F_1 hybrids, number of heterotic crosses and two superior crosses with their heterosis over better parent and commercial check in all characters are presented in Table 3, 4 and 5a&b, respectively. Sufficiently high magnitude of standard heterosis and heterobeltiosis in desired direction were observed for all the growth and yield related traits indicating possible commercial exploitation of heterosis. Heterosis over the better parent and commercial check of the hybrids are summarized in Table 2a & b.

Table 1 : Analysis of variance (mean sum of squares) in half diallel analysis for various characters in bell pepper

Character	Treatment	Parents vs Hybrids	Error
df	27	1	54
Growth traits			
Plant height	75.5*	78.1*	15.09
Number of primary branches	0.15*	0.85*	0.08
Number of secondary branches	0.72*	5.73*	0.086
Fruit width	0.26*	3.08^{NS}	0.104
Fruit length	4.97*	23.30*	0.88
Number of lobes/fruit	0.16*	0.82*	0.08
Pericarp thickness	0.02*	0.11*	0.002
Days to 50% flowering	96.86*	47.15^{NS}	24.55
Days to first harvest	123.59*	785.81*	18.41
Yield traits			
Number of fruits per plant	3.99*	30.46*	0.17
Average fruit weight	418.82*	2516.88*	8.78
Total yield per plant	86207.41*	948017.06*	131.65

* indicated significance of values at p= 0.05; NS: non-significant

Plant growth characters indirectly influence yielding ability of the plant. For instance, in open field cultivation of bell pepper, more the number of primary branches, number of secondary branches and plant height indicates more number of flowers and fruits leading to more yield. In this study, number of primary branches ranged from 2.90 to 3.47 in parents, whereas, in crosses, it ranged from 3 to 3.77, and crosses YW×CW (17.8) and AM×YW (16.49) showed highest heterobeltiosis and AM×YW (20.21) and AM×UHFBP4 (20.21) showed highest standard heterosis (Table 3). Similarly, number of secondary branches ranged from 5.5 to 6.63 in parents and 6.13 to 7.47 in crosses. Crosses AM×UHFBP4 (16.58) and AG×CW (13.37) showed highest heterobeltiosis and AM×UHFBP4 (20.83) and AM×YW (16.67) showed highest heterosis. Plant height is another important growth trait determining yield potential along with other growth parameters. In the present study, range of heterobeltiosis was from -17.17 to 19.72 and the top performing hybrids which recorded highest heterobeltiosis were CW X UHFBP4 (19.72) and AG X AB (12.40). Similarly range of standard heterosis was -11.29 to 26.56 and top performing hybrids were CW X UHFBP4 (19.72) and AG X AB (20.39) for plant height. The obtained results are in agreement with the studies by Praveen et al. (2017)

Table 2a : Better parent and commercial heterosis of growth and yield traits

Crosses	Number of primary branches		Number of secondary branches		Plant height		Days to first harvest		Fruit length	
	BP	CC	BP	CC	BP	CC	BP	CC	BP	CC
AM X AG	3.09**	6.38**	6.53**	10.42**	-8.51**	-9.36**	-17.86 **	-9.80 **	5.49**	6.36**
AM X AB	11.34**	14.89**	9.05**	13.02 **	-17.17 **	-11.29**	-6.25	2.94	3.62**	4.05**
AM X YW	16.49 **	20.21 **	12.56* *	16.67 **	-7.58*	-9.30**	-12.80 **	-4.25	0.32	4.38**
AM X CW	-4.12**	-1.06**	-6.53**	-3.13**	-9.52**	-5.31	-9.82 **	-0.98	3.65**	-1.32
AM X UHFBP4	8.65**	20.21 **	16.58 **	20.83 **	-15.64 **	-10.82**	-14.58 **	-6.21	9.81**	4.55**
AM X CW308	8.25**	11.70**	-2.01**	1.56**	11.99**	16.60 **	-17.56 **	-9.48 **	11.98**	6.61**
AG X AB	5.15**	8.51**	-2.54**	0.00	12.40 **	20.39 **	-11.32 **	-7.84 *	7.87**	8.76**
AG X YW	8.25**	11.70**	-2.11**	-3.12**	-2.95	-3.85	-17.58 **	-11.11 **	8.78**	13.18**
AG X CW	0.00	3.19**	13.37 **	10.42**	10.79**	15.94 **	-6.9	-11.76 **	-5.45**	-4.67**
AG X UHFBP4	0.96**	11.70**	-6.67**	-5.21**	4.21	10.16**	-8.39 *	-10.78 **	3.93**	4.79**
AG X CW308	7.22**	10.64**	1.53**	3.65**	4.40	8.70**	-8.01 *	-6.21	2.70**	3.55**
AB X YW	14.13**	11.70**	7.61**	10.42**	5.08	12.55**	-9.70 **	-2.61	-1.03	2.98**
AB X CW	10.87**	8.51**	-3.55**	-1.04**	11.72**	19.65 **	1.26	5.23	27.57 **	28.10 **
AB X UHFBP4	-2.88**	7.45**	-0.51	2.08**	0.93	8.10*	-1.89	1.96	1.52*	1.94*
AB X CW308	-5.15**	-2.13**	-7.11**	-4.69**	1.30	8.50**	-14.15 **	-10.78 **	51.23 **	51.86 **
YW X CW	17.78 **	12.77**	11.05**	9.90**	1.52	6.24	-11.82 **	-4.9	-5.96**	-2.15**
YW X UHFBP4	-6.73**	3.19**	5.13**	6.77**	0.25	5.98	-7.58 *	-0.33	43.96 **	49.79 **
YW X CW308	-7.22**	-4.26**	-4.08**	-2.08**	3.44	7.70*	-14.24 **	-7.52 *	-7.07**	-3.31**
CW X UHFBP4	-3.85**	6.38**	11.79 **	13.54 **	19.72 **	26.56 **	-9.40 **	-11.76 **	5.10**	-11.40**
CW X CW308	-1.03**	2.13**	1.02**	3.12**	-3.68	0.80	-10.90 **	-9.15 *	13.51**	2.07**
UHFBP4 X CW308	0.00	10.64**	-6.12**	-4.17**	0.75	6.51*	-11.22 **	-9.48 **	34.65 **	21.07 **
SEm±	0.16	0.16	0.24	0.24	2.20	2.20	2.44	2.44	0.53	0.53
CD @ 5%	0.46	0.46	0.70	0.70	6.25	6.25	6.90	6.90	1.51	1.51
CD@ 1%	0.61	0.61	0.93	0.93	8.32	8.32	9.18	9.18	2.02	2.02

*and**: significant at p= 0.05, p= 0.01, respectively
BP: heterosis per cent over better parent; CC: heterosis over commercial check (Indra)
AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder

Table 2b : Better parent and commercial heterosis of growth and yield traits

Crosses	Number of lopes per fruit		Pericarp thickness		Number of fruits per plant		Average fruit weight		Total yield per plant	
	BP	CC	BP	CC	BP	CC	BP	CC	BP	CC
AM X AG	5.88**	14.89**	46.72**	14.29**	8.18**	-0.83*	5.51*	5.51*	14.83 *	-2.3
AM X AB	0.00	6.38**	-12.79**	-11.90**	25.61 **	28.75 **	14.71 **	14.71 **	58.31 **	34.69 **
AM x YW	1.00**	7.45**	33.05**	8.16**	12.70 **	14.58 **	17.56 **	17.56 **	47.69 **	25.65 **
AM X CW	-1.92**	8.51**	5.24**	-18.03**	1.35**	-6.25**	0.54	0.54	1.23	-13.87
AM X UHFBP4	9.00**	15.96**	24.48**	2.04**	26.84 **	22.08 **	12.22 **	12.22 **	50.93 **	28.41 **
AM X CW308	2.00**	8.51**	4.53**	-13.61**	34.09 **	22.92 **	17.94 **	17.94 **	61.58 **	37.47 **
AG X AB	0.98**	9.57**	10.44**	11.56**	3.25**	5.83**	-15.24 **	-23.99 **	-15.22	-29.92 **
AG X YW	13.73**	23.40**	8.79**	-11.56**	-0.41	1.25**	9.76 **	4.85*	11.53	-6.05
AG X CW	4.81**	15.96**	51.54**	17.01**	14.41 **	5.83**	5.64*	-5.26*	43.96 **	19.00 *
AG X UHFBP4	15.69**	25.53**	6.64**	-12.59**	7.79**	3.75**	23.74 **	10.98 **	46.91 **	21.44 *
AG X CW308	0.98**	9.57**	36.63**	12.93**	21.76 **	9.58 **	-5.77*	-15.49 **	32.71 **	9.71
AB X YW	-4.26**	-4.26**	13.80**	14.97**	0	2.5**	-10.88 **	-14.87 **	-1.96	-17.41
AB X CW	-5.77**	4.26**	-30.64**	-29.93**	28.05 **	31.25 **	21.48 **	-6.30 *	46.43 **	20.81 *
AB X UHFBP4	15.96**	15.96**	-36.36**	-35.17**	3.66**	6.25**	19.33 **	-7.95 **	25.59 **	-12.82
AB X CW308	-4.00**	2.13**	17.17**	18.37**	40.24 **	43.75 **	45.23 **	18.64 **	97.35 **	37.00 **
YW X CW	6.73**	18.09**	28.87**	4.76**	29.51 **	31.67 **	22.98 **	17.48 **	63.07 **	37.37 **
YW X UHFBP4	15.22**	12.77**	39.42**	14.29**	-4.1**	-2.5**	-18.30 **	-21.96 **	42.32 **	19.89 *
YW X CW308	-2.00**	4.26**	23.87**	2.38**	2.87**	4.58**	14.44 **	9.32 **	15.47 **	-2.73
CW X UHFBP4	-2.88**	7.45**	8.30**	-11.22**	17.32 **	12.92 **	38.23 **	6.34 **	51.42 **	24.93 **
CW X CW308	4.81**	5.96**	7.41**	-11.22**	8.11**	0	23.12 **	0.58	36.38 **	12.52
UHFBP4 X CW308	7.00**	13.83**	33.33**	10.20**	2.16**	-1.67**	-1.17	-19.26 **	31.00 **	-9.80
SEm±	0.16	0.16	0.02	0.02	0.23	0.23	1.68	1.68	6.52	6.52
CD @ 5%	0.45	0.45	0.06	0.06	0.66	0.66	4.77	4.77	18.49	18.49
CD @ 1%	0.60	0.60	0.08	0.08	0.88	0.88	6.34	6.34	24.62	24.62

*and**: Significance at p= 0.05, p= 0.01respectively

BP: Heterosis percent over better parent. CC: heterosis over commercial check (Indra). AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder

Table 3 : Mean performance of parents

Parent	NPB	NSB	PH (cm)	DFF	DFH	FL (cm)	FW (cm)	NLF	PT (cm)	NFP	AFW (g)	YP (g)
AM	3.23	6.63	46.00	87.00	112.00	7.68	5.33	3.33	0.38	7.33	80.47	612.97
AG	3.23	6.23	49.73	73.00	96.67	8.13	5.46	3.40	0.33	6.60	72.17	595.57
AB	3.07	6.57	53.77	71.67	106.00	8.10	4.96	3.13	0.49	8.20	62.07	500.13
YW	2.90	6.33	49.27	77.00	110.00	8.39	5.20	3.06	0.39	8.13	76.87	606.90
CW	3.00	5.50	52.53	72.33	93.00	6.80	5.33	3.46	0.37	7.40	61.37	594.40
UHFBP4	3.47	6.50	53.07	75.00	99.33	6.73	5.13	3.06	0.40	7.70	61.90	481.77
CW308	3.23	6.53	52.27	72.33	104.00	7.25	5.56	3.33	0.40	7.20	65.73	496.07
Range	2.90-3.47	5.5-6.63	46-53.77	71.67-87	93-112	6.8-8.39	4.96-5.56	3.06-3.46	0.33-0.4	6.6-8.2	61.37-80.47	481.77-612.97
CD @ 5%	0.46	0.71	6.25	7.96	6.90	1.51	0.52	0.45	0.06	0.66	4.77	18.49

AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder
NPB-number of primary branches, NSB-number of secondary branches, PH- plant height, DFF-days to 50% flowering, DFH-days to first harvest, FL-fruit length, FW-fruit weight, NLF- number of lobes per fruit, PT-pericarp thickness, NFP-fruits/plant, AFW-average fruit weight, YP-total yield per plant

Table 4 : Mean performance of crosses

Crosses	NPB	NSB	PH (cm)	DFF	DFH	FL (cm)	FW (cm)	NLF	PT (cm)	NFP	AFW (g)	YP (g)
AM x AG	3.33	7.07	45.50	69.00	92.00	8.58	5.88	3.60	0.56	7.93	84.90	703.87
AM x AB	3.60	7.23	44.53	87.00	105.00	8.39	5.57	3.33	0.43	10.30	92.30	970.37
AM x YW	3.77	7.47	45.53	69.33	97.67	8.42	5.71	3.36	0.53	9.17	94.60	905.27
AM x CW	3.10	6.20	47.53	81.00	101.00	7.96	5.62	3.40	0.40	7.50	80.90	620.53
AM x UHFBP4	3.77	7.73	44.77	75.00	95.67	8.43	5.67	3.63	0.50	9.77	90.30	925.17
AM x CW308	3.50	6.50	58.53	70.00	92.33	8.60	6.28	3.40	0.42	9.83	94.90	990.43
AG x AB	3.40	6.40	60.43	70.67	94.00	8.77	5.95	3.43	0.54	8.47	61.17	504.93
AG x YW	3.50	6.20	48.27	69.00	90.67	9.13	5.64	3.86	0.43	8.10	84.37	676.90
AG x CW	3.23	7.07	58.20	75.00	90.00	7.69	5.66	3.63	0.57	8.47	76.23	857.36
AG x UHFBP4	3.50	6.07	55.30	69.00	91.00	8.45	5.67	3.93	0.42	8.30	89.30	874.97
AG x CW308	3.47	6.63	54.57	72.33	95.67	8.35	6.10	3.43	0.55	8.77	68.00	790.40
AB x YW	3.50	7.07	56.50	72.67	99.33	8.30	5.45	3.00	0.56	8.20	68.50	595.00
AB x CW	3.40	6.33	60.07	84.33	107.33	10.33	5.51	3.26	0.34	10.50	75.40	870.36
AB x UHFBP4	3.37	6.53	54.27	85.67	104.00	8.22	5.20	3.63	0.31	8.50	74.07	628.10
AB x CW308	3.07	6.10	54.47	69.00	91.00	12.25	5.73	3.20	0.58	11.50	95.47	987.03
YW x CW	3.53	7.03	53.33	69.67	97.00	7.89	5.89	3.70	0.51	10.53	94.53	989.70
YW x UHFBP4	3.23	6.83	53.20	76.33	101.67	12.08	5.70	3.53	0.56	7.80	62.80	863.77
YW x CW308	3.00	6.27	54.07	72.33	94.33	7.80	5.91	3.26	0.50	8.37	87.97	700.80
CW x UHFBP4	3.33	7.27	63.53	70.00	90.00	7.14	5.48	3.36	0.43	9.03	85.57	900.07
CW x CW308	3.20	6.60	50.60	70.67	92.67	8.23	5.81	3.63	0.43	8.00	80.93	810.64
UHFBP4 x CW308	3.47	6.13	53.47	70.67	92.33	9.76	5.80	3.56	0.54	7.86	64.97	649.87
Range	3-3.77	6.13-7.47	44.53-63.53	69-87	90-107.33	7.14-10.33	5.2-5.91	3.2-3.93	0.4-0.58	7.8-11.5	68-94.9	504.93-990.43
CD @ 5%	0.46	0.71	6.25	7.96	6.90	1.51	0.52	0.45	0.06	0.66	4.77	18.49

AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder
NPB-number of primary branches, NSB-number of secondary branches, PH- plant height, DFF-days to 50% flowering, DFH-days to first harvest, FL-fruit length, FW-fruit weight, NLF-Number of lobes per fruit, PT-pericarp thickness, NFP-fruits/plant, AFW-average fruit weight, YP-total yield per plant

Table 5a : Range of heterosis with top two parents and hybrids

Range of heterosis % over	Number of primary branches	Number of secondary branches	Plant height	Days to 50% flowering	Days to first harvest	Fruit length
BP	-7.22-17.78	-7.11-16.58	-17.17-19.72	-20.69-16.59	-17.86-1.26	-7.07-51.23
CC	-4.26-20.21	-5.21-20.83	-11.29-26.56	-11.54-8.12	-11.76-5.23	-11.40-49.79
			No. of heterotic crosses over			
BP	5	11	12	6	16	16
CC	18	13	12	11	11	16
Top two parents with their mean values	UHFBP4 (3.47) AM, AG, CW308 (3.23)	AM (6.63) AB (6.57)	AB (53.77) UHFBP4 (53.07)	AB (71.67) CW308, CW (72.33)	CW (93) AG (96.67)	YW (8.39) AG (8.13)
Top two hybrids over BP	YW x CW AM x YW	AM x UHFBP4 AG x CW	CW x UHFBP4 AG x AB	AM x AG AM x YW	AM x AG AM x CW308	AB x CW308 YW x UHFBP4
Top two hybrids over CC	AM x YW AM x UHFBP4	AM x UHFBP4 AM x YW	CW x UHFBP4 AG x AB	AM x AG AG x YW AG x UHFBP4 AB x CW308 AM x YW	AG x CW CW x UHFBP4 AG x YW	AB x CW308 YW x UHFBP4

Table 5b : Range of heterosis with top two parents and hybrids

Range of heterosis % over	Fruit weight	Number of lobes per fruit	Pericarp thickness	Number of fruits per plant	Average fruit weight	Total yield per plant
BP	1.43-12.81	-4.26-15.69	-36.36-51.54	-4.1-40.24	-18.30-45.23	-15.22-97.35
CC	-0.36-14.18	-4.26-25.53	-35.17-18.37	-6.25-43.75	-23.99-18.64	-17.41-37.37
			No. of heterotic crosses over			
BP	21	15	18	18	15	17
CC	16	20	12	16	10	11
Top two parents with their mean values	CW308 (5.56) AG (5.46)	CW (3.46) AG (3.40)	AB (0.49) UHFBP4, CW308 (0.40)	AB (8.2) YW (8.13)	AM (80.47) AG (72.17)	AM (612.97) AB (595.57)
Top two hybrids over BP	AM x CW308 YW x CW	AB x UHFBP4 AG x UHFBP4	AG x CW AM x AG	AB x CW308 AM x CW308	AB x CW308 CW x UHFBP4	AB x CW308 AM x AB
Top two hybrids over CC	AM x CW308 AG x CW308	AG x UHFBP4 AM x YW	AB x CW308 AG x CW	AB x CW308 YW x CW	AB x CW308 AM x CW308	AM x CW308 YW x CW

AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder

and Aditika (2018) who have reported positive heterosis for this trait.

Earliness is indicated by negative heterosis which leads to early access to market fetching good price. For days to 50 per cent flowering, similar performance of parents and hybrids were recorded, however, with respect to days to first harvest, heterobeltiosis ranged from -17.86 to 1.26 and the hybrids recording earliest harvest were AM x AG (-17.86) and AM x CW308 (-17.56). Similarly range of heterosis over standard parent was -11.76 to 5.23 and hybrids, AG x CW (-11.76), CW x UHFBP4 (-11.76), AG x YW (-11.11) recorded highest negative heterosis. Sharma et al. (2013) and Hegde (2016) also reported similar magnitude of heterosis.

High yield is the most desirable character and major goal of all breeding programmes. This is a complex trait influenced by other traits like number of fruits per plant, fruit weight, fruit size, yield per plant etc. For fruit length, standard heterosis ranged from -7.07 to 51.23 and the hybrids with top performance were AB x CW308 (51.23) and YW x UHFBP4 (43.96). Similarly, heterobeltiosis ranged from -11.40 to 9.79 and hybrids AB x CW308 (51.86), YW x UHFBP4 (49.79) recorded maximum value. Similar performance of parents and hybrids for fruit width was observed. For number of lobes per fruit, heterobeltiosis ranged from -4.26 to 15.96 and the maximum was showed by AB x UHFBP4 (15.96) and AG x UHFBP4 (15.69). Similarly, range of heterosis over standard

check was -4.26 to 25.53 and the hybrids AG x UHFBP4 (25.53) and AG x YW (23.40) recorded maximum value.

More pericarp thickness contributes to higher fruit weight and also post-harvest quality of fruits. For this trait, heterobeltiosis ranged from -36.36 to 51.54, the hybrids AG x CW (51.54) and AM x AG (46.72) showed maximum heterobeltiosis, whereas, heterosis over standard check ranged from -35.17 to 18.37, the hybrids, AB x CW308 (18.37) and AG x CW (17.01) showed maximum for pericarp thickness. For number of fruits per plant, heterobeltiosis ranged from -4.1 to 40.24 and the hybrids with maximum heterosis were AB x CW308 (40.24) and AM x CW308 (34.09). Similarly, heterosis over standard check ranged from -6.25 to 43.75, AB x CW308 (43.75) and YW x CW (31.67) recorded maximum for pericarp thickness. The range of heterobeltiosis for average fruit weight was -18.30 to 45.23, the hybrids AB x CW308 (45.23) and CW x UHFBP4 (38.23) recorded maximum. Range of heterosis over standard check ranged from -23.99 to 18.64 and the hybrids which showed maximum were AB x CW308 (18.64) and AM x CW308 (17.94). For YP, heterobeltiosis ranged from -15.22 to 97.35 and the hybrids AB x CW308 (97.35) and AM x AB (58.31) showed maximum. Range of heterosis over standard check ranged from -17.41 to 37.37, AMCW308 (37.47) and YW x CW (37.37) recorded maximum for yield per plant. This was in line with findings of Aditika (2018) and Nalwa (2019) in capsicum. The result indicates that maximum yield per plant in the hybrids mentioned was attributed by maximum number of fruits per plant. Present experiment showed high degree of heterosis for yield in most of crosses (Table 2). The range of mean values of hybrids were more than the parents for all the studied growth and yield characters (Table 3 and 4). Aditika (2018) also have reported increase in mean values for growth and yield traits in hybrids in capsicum.

CONCLUSION

It can be concluded that Arka Mohini x CW308 has the best heterotic cross combination and had significantly higher heterosis and high *per se* value over commercial check with respect to number of primary branches, number of secondary branches, plant height, days to 50% flowering, days to first harvest, fruit length, fruit width, number of lobes per fruit, number of fruits per plant and average fruit weight. The cross Arka Mohini x CW308 also showed high heterobeltiosis for traits such as fruit length, number of fruits per plant, fruit width and average yield per plant. Hence, this hybrid can be utilized to obtain desirable segregates for improvement of horticultural traits in bell pepper.

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Original Research Paper

Assessment of growth and yield parameters in recombinant inbred line populations of tomato (*Solanum lycopersicum* L.) through correlation and path analysis

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is high value crop, also called as protective food due to its high nutritional and biochemical compounds. Correlation and path analysis was carried out for 147 tomato recombinant inbred line population. Correlation studies suggested that the association of fruit yield per plant was positive and significant with plant height (0.595), branches per plant (0.657), fruits per cluster (0.500), clusters per plant (0.717), average fruit weight (0.244) and fruits per plant (0.891). Path analysis revealed that among eleven characters studied only two characters *viz.*, average fruit weight (0.415) and fruits per plant (0.817) showed very high positive and direct effect on yield per plant. This study helps to understand the mutual relationship among various traits thereby assist in selecting the character contributing to the yield.

Keywords: Correlation, path analysis, tomato, yield

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important vegetable grown all over the world due to its economic significance and prospective health benefits as a good source of antioxidants, vitamins and mineral. It belongs to the family Solanaceae with the diploid chromosome number 2n=24 (Jenkins, 1948). All the species of tomato are native to Western South America (Rick, 1976), except the cultivated species *Solanum lycopersicum* (L.), which is native to the Peru-Ecuador region (Rick, 1969). It is grown as an annual or short-lived perennial herbaceous plant with a taproot system and determinate, semi-determinate and indeterminate growth habits.

Tomato cultivation is spread on a global surface of 5.05 million hectares with a production of 186.82 million tons and productivity of 37.10 metric tons. Globally, the main producers included China, which alone produces about 63 million tons, ≈ 33 %, of the total production, followed by India (19.00 million tons), Turkey (12.80 million tons), the USA (10.90 million tons) and Egypt (6.90 million tons) (Anon., 2020). In India, tomato occupies an area of 0.84 million hectares with a production of 20.33 million tons and productivity of 24.18 metric tons per hectare. Karnataka, occupies second place in the country with an area of 64.25 thousand hectares and production of 2081 thousand tons and productivity 32.40 metric tons per hectare (Anon., 2022).

The natural genetic variation for most of the yield contributing characters is considerable in this crop in the region and there is a need for the breeders to restructure the materials for increasing the production and productivity. Correlation study in yield and yield attributing characters will be of value in selection of traits during improvement. Path analysis provides an effective means of finding out direct and indirect causes of association and permits a critical examination of given correlation and measures the relative importance of each factor. It gives more accurate pattern of trait association through direct and indirect effects.

MATERIALS AND METHOD

The experiment was conducted at Kittur Rani Chennamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkote, Karnataka, from November 2019 to December 2021. Two genetically diverse parents were used *viz.*, 'Anagha' (resistant to bacterial wilt disease and

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average fruit weight 50-55 g) and 'FBT-41' (semi-determinate, small red, flat-surfaced fruits, carries the *Ty-1* and *Ty-3* genes) providing resistance to ToLCV disease. The parent 'FBT-41' was procured from the Center for Biotechnological Research, College of Horticulture, Bengaluru. These parents were employed to develop a total of 147 recombinant inbred lines through crossing and the development of F_1 hybrids, followed by selfing up to the F_6 generation using the single-seed descent method of generation advancement.

In each line, 20 plants were planted, and the recommended agronomic practices were followed throughout the growing season. Five plants were randomly tagged and selected for observations. All the lines were field evaluated using an augmented randomized block design. 'Sankranti' and 'PKM-1' varieties of tomato were used as checks.

Growth parameters, such as plant height (cm) and the branches per plant were recorded 90 days after transplanting. Flowering parameters, including days to first and 50% flowering and clusters per plant, were recorded as soon as the first flower appeared. Remaining yield and quality parameters, such as fruits per cluster, average fruit weight (g), locules per fruit, fruit length (cm), fruit diameter (cm), fruits per plant, total yield per plant (kg), total soluble solids (ºBrix), and pH, were recorded at the final harvest for all 147 lines, including parents and checks. The recorded data were subjected to Fischer's method of analysis of variance, as described by Federer & Raghavrao (1975). Mean data were used for correlation and path coefficient analysis, as suggested by Miller et al. (1958) and Dewey & Lu (1959), respectively.

RESULT AND DISCUSSION

The analysis of variance indicated significant differences among the recombinant inbred lines for all the characters studied (Table 1). The extent of variability present in the germplasm offers opportunities for crop improvement programmes and is also dependent on the level of heritability for each trait.

The correlation analysis helps in examining the possibility of improving yield and its attributing traits through an indirect selection of their highly correlated component traits. In this investigation, correlation coefficients were worked out on 147 developed recombinant inbred lines of tomato (Table 2). The study of the association of component characters with a complex traits like yield is very helpful for ease of gainful selection in any breeding programme. It has been established that the structure of yield must be probed through its components rather than yield.

The association of fruit yield per plant was positive and significant with plant height (0.595), branches per plant (0.657), fruits per cluster (0.500), cluster per plant (0.717), average fruit weight (0.244), fruits per plant (0.891). Since these associated characters were in the desirable direction, it indicated that simultaneous selection for these characters would be rewarding in improving the fruit yield. The characters such as days to first flowering (-0.679) and 50% flowering (-0.246) showed negative significant correlation, indicating that these attributes are highly influence fruit yield in tomato and therefore, important for bringing improvement in fruit yield. The relationship between fruit yield and fruits per plant and average fruit weight was also reported (Yadav et al., 2020; Sharma et al., 2021). The remaining characters are positive but non-significant *viz.,* number of locules per fruit, fruit length, and fruit diameter doesn't have effect on fruit yield.

The coefficient of correlation does not give the true picture under complex situations. Under such situations, path coefficient analysis provides a mean to determine the direct influence of one variable (cause) upon another variable (effect). For the establishment of cause-and-effect relationship, path coefficient analysis offers an opportunity for partition of correlation coefficient into component of direct and indirect effects (Wright, 1921). Path coefficient analysis is the effective measure of direct and indirect causes of association and also depicts the relative importance of each factor involved in contributing to the final product that is yield (Dewey & Lu, 1959). Path coefficient analysis was carried out by taking fruit yield per plant as dependent variable. Positive and negative, direct and indirect effect of yield components on fruit yield per plant is presented in Table 3.

Path analysis revealed that out of eleven characters studied, six characters showed positive direct effect, among them average fruit weight (0.415) and fruits per plant (0.817) showed very high direct effect on yield per plant. Therefore, these characters can be considered for direct selection criteria for the improvement of yield in tomato, which indicates that

Table 1 : Analysis of variance for yield component and quality traits in Anagha × FBT-41 cross

Source of variation	DF	Mean sum of squares													
		PHT	PB	NOFPC	NOCPP	FLO	DFF	D50F	FL	FD	AFW	NOF	YPP	TSS	PH
Block	2	1	0.3	0.11	0.08	0.17	0.11	1.33	0.0033	0.03	7.48*	0.88	0.0017	0.02**	0.00053
Entries	153	196.09**	2.49**	1.54**	22.88**	0.63**	6.32**	10.33**	0.64**	0.72**	70.14**	769.35**	1.33**	0.06**	0.05**
Checks	6	817.6**	2.01**	1.37**	19.05**	0.89**	18.55**	15.08**	0.61**	2.94**	89.11**	691.83**	2.05**	0.49**	0.06**
Lines	146	171.59**	2.47**	1.4**	22.69**	0.61**	5.84**	8.09**	0.63**	0.56**	69.76**	771.36**	1.28**	0.04**	0.05**
Checks vs. Lines	1	45.1**	7.91**	22.87**	72.95**	2.41**	2.76	304.09**	3.49**	11.51**	11.58*	940.75**	4.33**	0.69**	0.27**
Error	12	0.89	0.28	0.08	0.49	0.05	0.87	1.56	0.02	0.03	1.46	1.9	0.00082	0.002	0.0048

*=significant at 5%, **= significant at 1% probability level

PHT-plant height (cm), PB-number of branches per plant, NOFPC-number of fruits per cluster, NOCPP-number of clusters per plant, FLO-number of locules per fruit, DFF-days to first flowering, D50F-days to 50 percent flowering, FL-fruit length (cm), FD-fruit diameter (cm), AFW-average fruit weight (g), NOF-number of fruits per plant, YPP-total yield per plant (kg), TSS- total soluble solids (ºBrix) and PH-pH

Table 2 : Correlation co-efficient for yield and component traits in Anagha × FBT-41 cross

Traits	PB	NOCPP	NOFPC	FLO	DFF	D50F	FL	FD	AFW	NOF	YPP
PHT	0.923**	0.888**	-0.075	-0.051	-0.443**	-0.070	-0.091	0.015	-0.008	0.598**	0.595**
PB	1	0.936**	-0.023	-0.058	-0.474**	-0.074	-0.089	0.016	-0.035	0.671**	0.657**
NOCPP		1	-0.004	-0.065	-0.507**	-0.101	-0.086	0.029	-0.035	0.734**	0.717**
NOFPC			1	0.072	-0.303**	-0.178*	-0.231**	-0.145	-0.267**	0.644**	0.500**
FLO				1	0.173*	-0.006	-0.079	-0.127	-0.051	-0.018	-0.049
DFF					1	0.451**	-0.093	-0.047	-0.254**	-0.573**	-0.679**
D50F						1	-0.108	0.008	-0.145	-0.186*	-0.246**
FL							1	0.385**	0.534**	-0.218**	0.019
FD								1	0.298**	-0.074	0.075
AFW									1	-0.188*	0.244**
NOF										1	0.891**

Critical rp value at 5% = 0.159, * Significant at p =0.05, Critical value at 1% = 0.208, ** Significant at p=0.01

PHT-plant height (cm), PB-number of branches per plant, NOCPP-number of clusters per plant, NOFPC-number of fruits per cluster, FLO-number of locules per fruit, DFF-days to first flowering, D50F-days to 50 percent flowering, FL-fruit length (cm), FD-fruit diameter (cm), AFW-average fruit weight (g), NOF-number of fruits per plant and YPP-total yield per plant (kg)

Table 3 : Phenotypic path coefficient analysis for yield and component traits in Anagha × FBT-41 cross

Traits	PHT	PB	NOCPP	NOFPC	FLO	DFF	D50F	FL	FD	AFW	NOF	YPP (rg)
PHT	**-0.0154**	0.0337	0.0886	-0.0061	0.0003	0.0079	0.0000	0.0005	0.0003	-0.0032	0.4888	0.5954
PB	-0.0142	**0.0365**	0.0934	-0.0019	0.0004	0.0084	0.0000	0.0005	0.0004	-0.0146	0.5485	0.6573
NOCPP	-0.0137	0.0342	**0.0998**	-0.0004	0.0004	0.0090	0.0001	0.0005	0.0006	-0.0144	0.6006	0.7167
NOFPC	0.0012	-0.0009	-0.0004	**0.0822**	-0.0005	0.0054	0.0001	0.0014	-0.0031	-0.1110	0.5262	0.5005
FLO	0.0008	-0.0021	-0.0065	0.0059	**-0.0064**	-0.0031	0.0001	0.0005	-0.0027	-0.0210	-0.0146	-0.0492
DFF	0.0068	-0.0173	-0.0506	-0.0249	-0.0011	**-0.0177**	-0.0002	0.0006	-0.0010	-0.1055	-0.4681	-0.6791
D50F	0.0011	-0.0027	-0.0100	-0.0146	0.0000	-0.0080	**-0.0005**	0.0006	0.0002	-0.0603	-0.1523	-0.2465
FL	0.0014	-0.0033	-0.0086	-0.0190	0.0005	0.0017	0.0001	**-0.0059**	0.0081	0.2221	-0.1785	0.0187
FD	-0.0002	0.0006	0.0029	-0.0119	0.0008	0.0008	0.0001	-0.0023	**0.0211**	0.1239	-0.0603	0.0755
AFW	0.0001	-0.0013	-0.0035	-0.0219	0.0003	0.0045	0.0001	-0.0031	0.0063	**0.4158**	-0.1534	0.2440
NOF	-0.0092	0.0245	0.0733	0.0529	0.0001	0.0102	0.0001	0.0013	-0.0016	-0.0780	**0.8177**	0.8912

rg: correlation coefficient with total yield per plant, diagonal values indicate direct effects, residual effect=0.028

PHT-plant height (cm), PB-number of branches per plant, NOCPP-number of clusters per plant, NOFPC-number of fruits per cluster, FLO-number of locules per fruit, DFF-days to first flowering, D50F-days to 50 percent flowering, FL-fruit length (cm), FD-fruit diameter (cm), AFW-average fruit weight (g), NOF-number of fruits per plant and YPP-total yield per plant (kg)

Table 4 : Yield and yield related traits of the better performing RILs from the cross Anagha × FBT-41

Parents/Lines/checks	PHT	PB	NOCPP	NOFPC	FLO	DFF	D50F	FL	FD	AFW	NOF	YPP
Anagha	68	7	15	5	3.4	26	30	3.6	3.5	52.35	75	3.93
FBT 41	71	5.8	13.8	6.1	2.6	29	34	4.1	4.5	39.5	84.18	3.33
TRIP2-17	91	8.2	22.3	5.9	4	24	29	4.1	5.2	38.62	131.57	5.08
TRIP2-21	98	8	19.6	5.6	3	24	28	4.1	4.2	36.25	109.76	3.98
TRIP2-22	100	8.4	24.6	6.2	2	23	28	4.1	5.4	38.56	152.52	5.88
TRIP2-24	94	8.1	21.5	5.9	4	25	30	4.2	6.2	35.65	126.85	4.52
TRIP2-29	95	8.1	20.6	4.5	4	24	30	4.1	6.2	52.35	92.7	4.85
TRIP2-35	94	8.6	26.3	6.2	2	24	28	3.9	4.9	36.1	163.06	5.89
TRIP2-37	86	7.3	15.6	7.5	2	23	29	3.5	6.8	39.54	117	4.63
TRIP2-40	93	8.6	22.3	6.1	2	24	30	4.2	5.2	35.65	136.03	4.85
TRIP2-42	102	8.4	24.9	4.5	2	23	27	4.5	5.6	40.25	112.05	4.51
TRIP2-51	82	6.6	15.4	5.1	2	23	28	5.4	4.1	55.6	78.54	4.37
TRIP2-52	96.85	8.5	21.6	6.2	4	24	30	3.9	6.2	40.25	133.92	5.39
TRIP2-57	75.9	6	15.3	5.9	2	23	28	5.3	6.5	52.35	90.27	4.73
TRIP2-74	102.8	8.1	21.9	5.1	4	23	27	4.1	5.6	35.25	111.69	3.94
TRIP2-8	86	7.6	19.1	5.9	3	25	29	4.1	5.3	53.65	112.69	6.05
TRIP2-95	100	8	21.2	4.2	3	23	27	4.1	5.2	46.32	89.04	4.12
TRIP2-96	98.1	7.8	19.2	5.1	2	24	31	4.1	5.6	44.54	97.92	4.36
TRIP2-102	103	7.6	21.5	4.8	3	23	27	4.1	5.6	41.25	103.2	4.26
TRIP2-138	78	6.1	14.2	5.4	2	24	29	5.5	6.9	55.65	76.68	4.27
Sankranti (check)	101	8	12	6	2	26	31	4.2	5.5	38.65	74	2.86
PKM-1 (check)	62	7.2	16.2	5	3	23	29	4.5	6.2	45.2	81	3.66

PHT-plant height (cm), PB-number of branches per plant, NOCPP-number of clusters per plant, NOFPC-number of fruits per cluster, FLO-number of locules per fruit, DFF-days to fir st flowering, D50F-days to 50 percent flowering, FL-fruit length (cm), FD-fruit diameter (cm), AFW-average fruit weight (g), NOF-number of fruits per plant and YPP-total yield per plant (kg)

emphasis should be laid on fruits per plant while applying selection strategies in this population as the findings are supported by Behera et al. (2020), Basavaraj et al. (2021) & Kumar et al. (2021). The residual effect (0.028) obtained was less than 0.5, suggesting that some of the characters have not been included, which may be responsible to enhance the fruit yield of tomato (Table 3).

Out of 147 RILs developed and evaluated for growth and yield traits only 20 RILs were performed better than the standard checks used *i.e.* Sankranti and PKM-1 (Table 4). Therefore, these stabilized F_6 generation RILs can be used to develop F_1 hybrids or can be released as variety.

CONCLUSION

The association of fruit yield per plant was positively significant with most of the morphological characters under study. Path analysis revealed that number of fruits per plant and average fruit weight (g) showed highest positive direct effect on fruit yield per plant. Therefore, these characters may be considered in selection criteria for the improvement of yield in tomato. The lines which were showing high yield than standard checks can be used in future breeding programme.

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Original Research Paper

Floral biology studies in wild melon [*Cucumis melo* L. ssp. *agrestis* (Naudin) Pangalo var. *agrestis* Naudin]

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ABSTRACT

Studies on floral morphology, phenology and biology of wild melon revealed that the ratio of staminate and pistillate flowers was 3.40:1. The longevity of the male flowers were between 5 and 6 days, whereas, female flowers between 6 and 7 days. Anthesis was observed from 4.00 am to 10.00 am, while, the anther dehiscence started from 5.00 am which was continued to 7.00 am. The peak anthesis was observed from 8.00 am to 9.00 am and anther dehiscence from 6.00 am to 6.30 am. Freshly opened flowers showed pollen viability up to 98.35%, decreased upon closure and crashed to 17.48% in 3 days. Pollen germination was occurred after 15 minutes of incubation and continued up to 24 h of incubation. The stigma receptivity lasts from one to two days of anthesis. Major pollinator of wild melons observed was honey bee, mostly visited between 9:00 am to 6:00 pm.

Keywords: Anthesis, floral biology, phenology, pollen viability, wild melon

INTRODUCTION

India is considered to be secondary centre of origin for melons. The genus *Cucumis* contains cultivated cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) and other wild species *viz.*, *C. prophetarum*, *C. callosus*, *C. hystrix*, *C. setosus* and *C. sativus* var. *hardwickii* (Chakravarty, 1982). Pitrat (2017) has classified wild melon traits (subsp. Agrestis) into two groups *viz.*, agresitis and *badi kachri*, the former is known as *Mekke kayi* (wild melon) in northern Karnataka and the later as semi domesticated. Botanically, it is an annual trailing vine plant with monoecious flower and has five sepals gamosepalous, petals are polypetalous, yellow colour, stamens are connective appendiculate, contain three anthers which are fused, ovary is fusiform with dense hairs, lobed and wet (Pandey et al., 2021). The wild species of melons possess various morphological and agronomic characters as well as resistance to pests and diseases. Therefore, emphasis has been laid on the utilization of these wild species of melons for the improvement of cucumber and muskmelon (Deakin *et al*., 1971).

Wild melon belongs to Cucurbitaceae family, with chromosome number 2n=24. It is an annual, trailing vine plant with monoecious flowers. The flowering period is from July to November. In Northern Karnataka, it is being cultivated as intercrop with sorghum on marginal lands with least crop husbandry. Due to its pleasant flavour, vivid hues (green, yellow, saffron, red, *etc*.) and nutritional profile, this underexploited cucurbit has gained a status of high value and taken pride of place in rural traditional cuisine (Kouonon et al., 2009). It is essential to conserve rare and threatened plant species like wild melons and the successful conservation programme relays on information of reproductive biology. The domain of reproductive biology includes floral biology, pollination dynamics, fertilization and embryogeny, seed development and germination (Marbaniang et al., 2018). Knowledge on floral biology is important for breeders for crop improvement and to frame hybridization work (Dhall et al., 2011).

Considering the scope of varietal improvement and conservation of endangered plants, an experiment was carried out to study the floral biology such as time of

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anthesis, time of anther dehiscence, duration of stigma receptivity, pollen viability, longevity of flower and floral morphology to determine the effective hybridization time in wild melon.

MATERIALS AND METHODS

The experiment was conducted in the fields of the Department of Vegetable Science, College of Horticulture, Bagalkot during 2022. The experiment site was located at 16.18º N latitude and 75.07º E longitude with an altitude of 533 meters above mean sea level, in the northern dry zone of Karnataka. The promising wild melon genotypes HUB-9 suited for summer were studied using completely randomised design with 4 replications and 20 plants per replication. All the agronomical practices were adopted as per the recommendations of package of practice followed for pumpkin (Anon., 2019).

Observations on floral phenology, flower morphology and floral biology were recorded as per standard descriptors for melon (Srivastava et al., 2001; IPGRI, 2003). Other observations recorded were time of anthesis, anthesis (%), anther dehiscence (%), longevity of flowers, pollen viability (%), *in vitro* pollen germination (%) and stigma receptivity. Floral phenology of the wild melon was studied on five randomly selected plants (overall 30 male and female flowers) and female: male flower ratio and flowering season. The flowers from middle rows were selected at the time of anthesis (8 to 9 am). The floral morphology, flower structure, pollen grain shape were studied by dissecting flowers and images were captured using the Stereozoom Microscope (LMI, England, model SZM167).

Anthesis time, anthesis (%) and anther dehiscence (%) were recorded on ten flower buds in each replication. Anthesis time was recorded at different times of the day (early morning, morning, afternoon, evening and late in the evening) and anther dehiscence (%) was observed before and after flower opening using magnifying lens. Longevity of male and female flowers was recorded by observing 20 flowers of male and female flowers from bud stage up to either fruit set or drop off. The pollinators of wild melon were observed by visual counts and species were identified.

In vitro pollen germination was observed on germinating media containing 10% sucrose solution + 10 ppm boric acid (Brewbaker & Kwack, 1963). Stigma receptivity was studied by using the starving method, starting one day before anthesis, on the day of anthesis and one, two and three days after anthesis. For each genotype, sixty flower buds in total were studied. The selected flower buds were hand-pollinated with fresh pollen collected from male flowers at different intervals and expressed in percentage. By keeping an eye on the ovary as it grows in the pollinated flowers, fruit set was confirmed and the percentage was calculated.

RESULTS AND DISCUSSION

Floral phenology

The flowering in wild melon started 30 days after sowing, male flowers emerged first and female flowers appeared 8 days after male flowers. The total blooming period of male flowers was 40 to 45 days and female flowers, about 30 to 35 days, similar to the observations of Tschoeke et al. (2015). The extended blooming period in wild melon may be attributed to local weather conditions and genetic variations of the crop. The number of male and female flowers per vine was 109 and 33, respectively. Mean daily production of male flowers was consistently higher than female flowers. The production of a greater number of male flowers over female flowers would have favoured effective pollination and ensured sufficient amount of pollen deposits on female flowers, thus helping in effective pollination (Deyto & Cervancia, 2009). The male: female sex ratio per vine throughout the blooming period observed was 3.40: 1 as against earlier reported 6:1 ratio of male: hermaphrodite flowers in melons (Tschoeke et al., 2015). The variation in number of days taken to first flower bud appearance among cultivars was due to environmental conditions (Delaplane & Mayer, 2000).

Flower morphology

Wild melon (*Cucumis melo* ssp. *agrestis*) exhibited monoecious vine character (Fig. 1a) by producing unisexual flowers of globular shape at bud stage and showed actinomorphic symmetry. The average length and diameter of male flowers was 4.08 cm and 3.88 cm, respectively, born solitary or sometimes in a cluster of 2- 3 on thin pedicel having length of 1.55 cm. The staminate flowers contained 5 sepals which are fused, 1.10 cm long, with dense hairs and 5 petals which were free, yellow in colour with length of 1.78 cm and inserted into the calyx-tube. Stamens were

observed to be connective appendiculate, capitate and had 4 filaments and 3 anthers (Fig. 1b), where filaments were free in nature and anthers were fused together (Fig. 1c). The average length and diameter of female flower was 4.45 cm and 3.45 cm, respectively, always borne solitarily on leaf axile with a short pedicel of 0.75 cm. The pistillate flowers contained 5 fused sepals (that were fused, hairy, deeply lobed, 0.89 cm long) and 5 petals which were polypetalous, yellow in colour having length of 1.58 cm and inserted into the calyx-tube. The ovary length was 1.83 cm and diameter 5.59 mm. The ovary was inferior, fusiform (spindle shaped) without hairs and showed free central placentation. The style was short with lobed stigma and showed wet surface (Fig. 1d). Similar results were reported by Pandey et al. (2021).

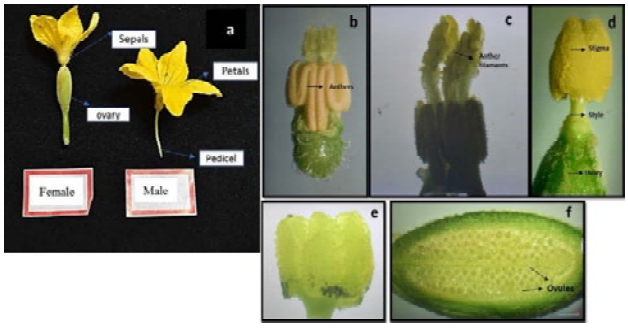

Fig. 1 : Flower characters of wild melon, (a) female and male flowers, (b) anthers of staminate flower, (c) anther filaments, (d) style, stigma and ovary, (e) lobed stigma with wet surface, (f) L.S of ovary

The flowers of wild melon were borne on the leaf axils. Exposed flowers were easy to access by pollinators and the flat structure was convenient for landing and the yellow colour could attract the pollinators (Goulson, 1999). The flowers remained open only for a day and the pollination had enhanced fruit set (Deyto & Cervancia, 2009). The morphology of flower was designed in such a way that both the plant and the pollinator mutually benefited.

Anthesis time, anthesis (%) and anther dehiscence (%)

The flower opening began at 4.00 am and it continued till 10.00 am and remained open till evening. Flowers opening was observed in both male and female at 04:00 to 05:00 am, at 06:00 am apex of petals separated, at 07:00 am flower half opened, by 08:00 to 09:00 am flowers were completely opened (Fig. 2), similar to the observations of Tschoeke et al. (2015). The variation of 1- 2 h for anthesis may be due to the local weather conditions. Since, on cloudy days, anthesis was slower than the sunny days (Kiill et al., 2016). Anthesis was totally ceased after 10.00 am. Peak period of anthesis was observed between 8.00 am to 9.00 am (72.00%) as maximum number of flowers opened during this period (Table 1) because of favourable temperature and relative humidity.

Fig. 2 : Anthesis in wild melons: male flower (a, b, c, d) and female flower (e, f, g, h)

Dehiscence of anthers in wild melon started at 5.00 am and continued till 7.00 am with a peak period from 6.00 to 6.30 am (68.50%) (Table 1). Deyto & Cervancia (2009) also reported similar findings. The temperature range of 30º to 35ºC is most favorable for anther dehiscence, which may vary from species to species and location to location with environmental conditions.

Table 1 : Flower opened, duration and anther dehiscence in wild melon

Duration	Flowers opened (Nos.)	Duration	Anther dehiscence (%)
6.00- 7.00 am	10.80	5.00- 5.30 am	8.30
7.00- 8.00 am	11.80	5.30- 6.00 am	15.30
8.00- 9.00 am	72.00	6.00- 6.30 am	68.50
9.00-10.00 am	5.50	6.30- 7.00 am	8.00

Pollen viability (%) and *in vitro* pollen germination (%)

The maximum pollen viability (98.35%) was recorded at the time of anthesis, while, it decreases at advance stage. Pollen viability after 48 h of anthesis was 51.65% and minimum was observed at 72 h after anthesis (17.48%) (Fig. 3 & 4). Revanasidda & Belavadi (2019) observed pollen viability of one day in muskmelon, whereas, in wild melon it was up to 3 days. The decrease in pollen viability after anthesis may be due to dehydration of pollen, drying and wilting of male flowers in *Cucurbita* (Agbagwa et al., 2007). In wild melon, both flowers are open from 9 am and close at 6 pm, at this time, flowers are accessible to pollinators resulting maximum viability as plants with insects have longer pollen viability than those with wind pollination (Bassani et al., 1994).

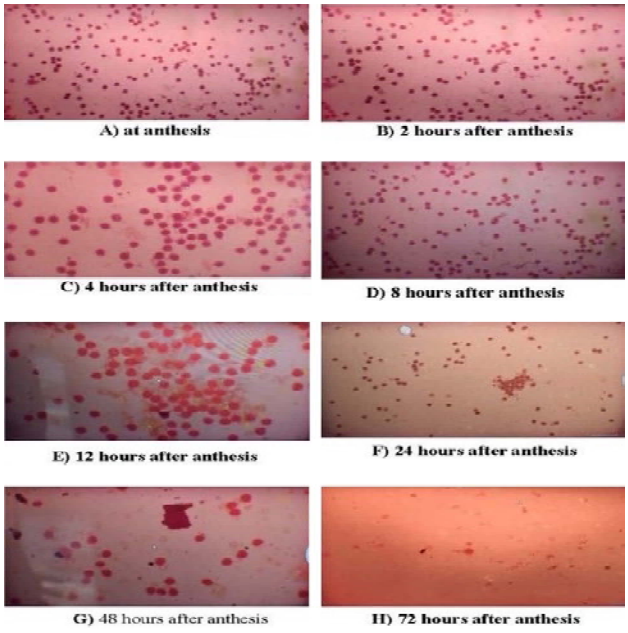

Fig. 3 : Viability of pollen at different time intervals

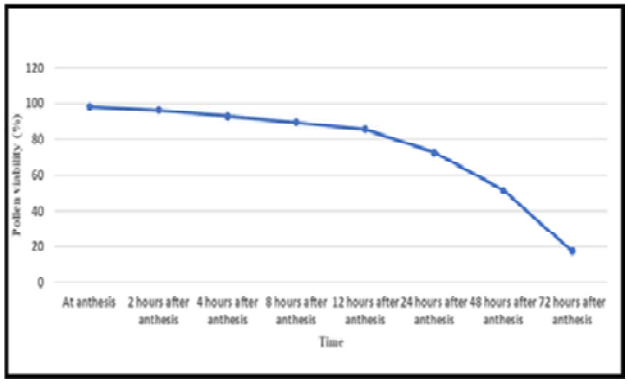

Fig. 4 : Pollen viability (%)

Pollen germination was observed to start after 15 minutes of incubation and continued up to 24 h. The minimum (52.08 %) pollen germination was recorded at 15 minutes of incubation, it was increased to 96.93% at 24 h (Table 2). Higher pollen germination could be due to availability of nutrients for a longer period to pollinators (Zaman, 2006).

Table 2 : *In vitro* pollen germination (%) in wild melon

Incubation period	Pollen germination (%)
15 minutes after incubation	52.08
30 minutes after incubation	67.23
45 minutes after incubation	76.38
1 hour after incubation	83.38
2 hours after incubation	88.18
4 hours after incubation	93.88
12 hours after incubation	95.25
24 hours after incubation	96.93

Stigma receptivity

The stigma became receptive one day prior to anthesis and remained receptive for two days after anthesis. The fruit set at one day before anthesis, on the day of anthesis, one day and two days after anthesis was noticed to be 14.75%, 80.75%, 23.00% and 16.95%, respectively (Table 3). Stigma became fully receptive on the day of anthesis (80.75) as surface of the stigma appeared to be stickier and more bulged due to which a greater number of pollen grains would have settled and germinated, leading to higher degree of fruit set. After 3 days of anthesis, stigma was found to be non-receptive due to advancement of time, the surface of the stigma dried and lead to less pollen accumulation (Naik et al., 2013).

Table 3 : Duration of stigma receptivity on artificial crossing (%) in wild melon

Time of pollination	Fruit set (%)
One day before anthesis	14.75
On the day of anthesis	80.75
One day after anthesis	23.00
Two days after anthesis	16.95

The longevity of the male and female flowers was 5 to 6 and 6 to 7 days, respectively. Staminate flower took 3 days from bud initiation to opening

(Fig. 5 & 6). Pistillate flowers took 4 days from bud stage to opening and for fruit set. Similar findings were also reported by Deyto & Cervancia (2009) and Ekeke et al. (2018).

Fig. 5 : Longevity of male flower, (a) 1st day- bud stage, (b) 2nd day-bud stage, (c) 3rd day-anthesis, flower remained open till evening, (d-e) 4th day-petals closed started to fade, (f-g) 5th day-flower closed fully, bent slightly and pointed upward, (h) 6th day-flower dried up completely

Fig. 6 : Longevity of female flower: (A) 1st day- bud stage, (B) 2nd day-bud elongation, (C) 3rd day-bud elongation, (D-E) 4th day-anthesis, flower remained open till evening, (F) 5th day-petals closed and started to fade, (G) 6th day-petals dry and hang down at tip, (H) 7th day-fruit set

Flower visitors and pollinators

Wild melon flowers were mainly visited by *Apis dorsata* and *Apis cerana*, between 09:00 am to 6:00 pm with two peak visiting from 9:00 am to 11:00 am and 3:00 pm to 5:00 pm. They spend 5-10 seconds on male flower in search of nectar or pollen. These pollen carrying bees pollinate nearby female flower stigma. The results are in accordance with the finding of Revanasidda & Belavadi (2019) in muskmelon and Ekeke et al. (2018) in cucumber.

CONCLUSION

In wild melon, peak anthesis was recorded between 08:00 to 09:00 am and anther dehiscence 06:00 to 06:30 am. Maximum pollen viability was recorded at the time of anthesis, while, stigma receptivity and pollen germination was recorded maximum on the day of anthesis. Hence, for hybridisation, manual pollination to be carried out on the day of anthesis which gives maximum fruit set and seed yield.

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Original Research Paper

Comparative analysis of BLUP and GCA for parental selection in marigold (*Tagetes erecta* L.) for hybrid development

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ABSTRACT

The area under marigold cultivation is increasing over the years and so is the demand for marigold seeds. To meet the increasing demand, hybrid varieties are preferred as they produce higher yields, for which the right parental selection is of major concern. Male sterility being the prerequisite for economical hybrid seed production of marigold, we have attempted to strategize the selection of male sterile seed parent and fertile pollen parent for yield and yield-related traits. The study was undertaken across multiple forms of male sterile lines morphologically varying in apetaloid and petaloid types, therefore use of BLUP and GCA was evaluated as a criterion to select the parents for the hybridization program. Results suggested apetaloid male sterile lines as better seed parents for days to bud initiation, while, petaloid male sterile lines can be selected for the improvement of shelf life and flower diameter. Results from BLUP and GCA were in agreement with each other for the traits studied. However, BLUP-based comparison of different lines is less tedious as it eliminates the laborious procedure of developing multiple hybrids and evaluating them to study the combining ability effects.

Keywords: BLUP, flower yield, GCA, hybrid seed production, marigold

INTRODUCTION

Marigold (*Tagetes erecta* L.), is the hardiest annual crop, cultivated in various agroclimatic conditions (Raghava, 2000). It is in great demand in the Indian market as a traditional loose flower used in various religious and cultural festivals, and extraction of carotenoids as a nutraceutical (Shao & Ren, 2016 and Ren & Reilly, 2018). It is widely cultivated around fruit orchards and commercial vegetable fields as a trap crop against root-knot nematode, aphids and whiteflies (Stavridou & Biezla, 2017; El- Naggar et al., 2017).

Marigold belongs to the family Asteraceae with its characteristic composite flowers consisting of ray and disc florets. Functional anthers in marigold are hidden within disc florets in the center of the flower, making emasculation a difficult process. The use of male sterile lines dramatically decreases the hybrid seed production cost by eliminating the tedious manual emasculation step (Huang et al., 2014; Wu et al., 2016). In marigold, apetaloid and petaloid types are the major morphological male sterile forms reported. In apetaloid male sterility, petals and androecium are modified into filament like structure (Gupta et al., 1999; He et al., 2009), while, flowers with only ray florets and absence of androecium are associated with petaloid male sterility (Tejaswini et al., 2016). Pre-breeding plays a pivotal role in developing parents for hybrids, providing a source of male sterility and fertility controlling genes, allowing efficient hybrid seed production. By incorporating male sterility, breeders can streamline the production of hybrid seeds, ensuring a consistent supply of high-quality planting materials for commercial flower cultivation.

Selection of breeding lines and the development of hybrids that show better and stable performance is a long-term strategy to cater immediate, medium and long-term needs of the farmers. Best Linear Unbiased Prediction (BLUPs) allows the comparison of lines over time (generation, year) and space (location, block) by minimizing their effects (Tajalifar & Rasooli, 2022). Also, BLUP can help in the selection of stable and better-performing genotypes/lines in a breeding program (Asfaw et al., 2020; Acharya et al., 2020). Furthermore, general combining ability (GCA) also suggests the suitability of genotypes/lines for the

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hybridization program. Estimated GCA by analyzing the average performance of genotypes in a series of crosses, provides the strength of the genotypes/lines to be used as a parent in the hybrid development program (Sprague & Tatum, 1942). The present study was attempted to identify reliable strategy for the selection of parents for yield and yield-related traits to be used in the hybridization program.

MATERIALS AND METHODS

Fifteen marigold breeding lines representing different categories of sterility and fertility (Table 1 & Fig. 1) were selected from pre-breeding population maintained at ICAR- Indian Institute of Horticultural Research, Bengaluru, India which is situated at 13° 08' 26.6" N latitude and 77° 30' 2.2" E longitude and 890 m above mean sea level.

Stabilisation and maintenance of breeding lines used in the study is presented in Table 1. Male sterility of vegetatively propagated petaloid lines are cytoplasmically inherited, while, apetaloid male sterility is controlled by nuclear genes and are seed propagated (Tejaswini et al., 2016). In the present study, petaloid male sterile lines were also used that are seed propagated and controlled by nuclear genes (unpublished data). Breeding lines were evaluated for morphological traits viz., days to bud initiation, flower diameter, shelf life, number and yield of flowers per plant, for three consequent years 2020 (summer), 2021 (winter) and 2022 (rainy).

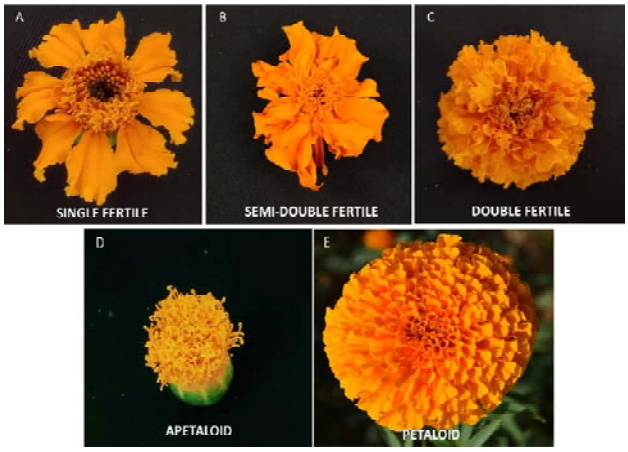

Fig. 1 : Male fertile flower types in marigold, A. single fertile, B. semi-double fertile, C. double fertile; Male sterile flower types, D. apetaloid, E. petaloid

Table 1 : Description of breeding lines used as pollen and seed parents for derivation of hybrids of marigold

Breeding Line	Flower form	Male Sterile: fertile plants	Propagation	Maintenance of breeding lines	Flower color
IIHRM 4-4	Single fertile	0:1	Seed	Selfing	Orange
IIHRM 5-2	Single fertile	0:1	Seed	Selfing	Yellow
IIHRM 4-13	Semi-double fertile	0:1	Seed	Selfing	Orange
IIHRM 1-11	Semi-double fertile	0:1	Seed	Selfing	Yellow
IIHRM 1-11-2	Semi-double fertile	0:1	Seed	Selfing	Yellow
IIHRM 5-4	Double fertile	0:1	Seed	Selfing	Orange
IIHRM 1-12	Double fertile	0:1	Seed	Selfing	Yellow
IIHRM 7-4	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 7-5	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 3-2	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRM 3-5	Apetaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRM 6-5	Petaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Orange
IIHRM 2-9	Petaloid	1:1	Seed	Intercrossing between sterile and fertile plants within the line	Yellow
IIHRMOs-1	Petaloid	1:0	Vegetative	Vegetative	Orange
IIHRMYs-1	Petaloid	1:0	Vegetative	Vegetative	Yellow

Hybrids were developed within the yellow and orange color group, resulting in a total of 34 hybrid combinations. Lines studied comprised petaloid and apetaloid male sterile lines as seed parents and single, double and semi-double fertile as pollen parents (Table 5). As seed propagated male sterile lines segregates into sterile and fertile lines; such lines were used both as seed and pollen parents. Hybrids developed were evaluated in randomized complete block design with three replications along with the parents to analyze the combining ability and contribution of parents to hybrids.

The phenotypic data recorded were analyzed using R software version 4.2.0. Levene's test (Levene, 1960) was carried out to confirm the homogeneity of error variances. Within and across the seasons, analysis of variance (ANOVA) was carried out in the 'metan' R package. For all the traits, best linear unbiased predictors (BLUPs) were calculated using the META-R software version 6.0 (Alvardo et al., 2020).

RESULTS AND DISCUSSION

The significance of mean sum of squares for seasons and genotypes x season in pooled ANOVA revealed the influence of the environment on the expression of these traits (Table 2). BLUPs were calculated within and across seasons for all the parental lines for five traits, as it minimizes the seasonal and space (location, block) effects allowing to compare the parents across five seasons.

Performance of different male sterile and fertile lines as parents for hybridization based on BLUP values

The use of BLUP generates more accurate estimation of genetic parameters and unbalanced experimental designs can also be used to predict the genotypic values, which also helps in evaluating the performance of the same genotypes/lines in different conditions by estimating the genetic correlations (Abu-Ellail et al., 2018).

Based on BLUP values, apetaloid male sterile lines were early to flower and took shorter time for bud initiation. Flower diameter and shelf life were recorded maximum in petaloid male sterile group except IIHRMOs-1 (Table 3). Petaloid lines have no functional pollen in turn exhibited longer life than other lines, as increase in ethylene production following pollination and fertilization regulates flower senescence (Serek et al., 1995). Semi-double fertile and apetaloid sterile lines produced a relatively more number of flowers and yield per plant than other groups as their flower diameter and per flower weight were higher compared to apetaloid lines (Table 3). Pedigree-based BLUP procedure was reported to enhance selection efficiency for production-related traits in *P. zonale* and shelf-life-related traits in *D. caryophyllus* L. (Molenaar et al., 2018), while, Acharya et al. (2020) and Ashwini et al. (2021) used the BLUP method to select genotypes with preferable agronomic traits in alfalfa and horse gram, respectively.

Performances of breeding lines and crosses based on combining ability effects

Among the breeding lines, apetaloid male sterile lines were good general combiners for days to bud initiation but were poor combiners for other traits. Seed propagated petaloid male sterile line (IIHRM 3-2) as female parent was found to be good general combiner for days to bud initiation and flower diameter.

Table 2 : Pooled analysis of variance of marigold breeding lines for yield and quality-related quantitative traits

Source of variation	df	Mean sum of squares				
		Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant (g)
Seasons	4	2073.93**	1.19*	6.51*	1185396**	71672**
Treatment	14	778.69**	6.07*	15.50**	399603**	2965494**
Replication within seasons	10	4.51	0.01	0.02	3630	12456
Season x Treatment	56	37.67**	0.40*	0.43*	257027**	211468**
Error	140	1.08	0.02	0.03	411	1495

Table 3 : Parental lines BLUP values and their ranges for five quantitative traits

Breeding line	Days to bud initiation	Range	Flower diameter (cm)	Range	Shelf life (days)	Range	Flowers per plant (Nos.)	Range	Flower yield per plant (g)	Range
Single fertile										
IIHRM 4-4	53.26	36.00 - 64.00	4.04	3.00 - 4.50	2.84	2.00 - 4.00	101.38	59.00 - 190.00	270.45	118.00 - 380.00
IIHRM 5-2	65.00	56.00 - 74.00	4.06	4.00 - 4.40	2.61	2.00 - 3.00	63.82	51.00 – 95.00	209.25	153.00 - 285.00
Semi-double fertile										
IIHRM 4-13	47.88	38.00 - 56.00	3.94	3.00 - 4.00	2.84	2.00 - 4.00	286.60	236.00 - 338.00	1116.37	708.00 - 2690.00
IIHRM 1-11	50.00	42.00 - 57.00	4.72	4.30 - 5.00	2.68	2.00 - 3.00	222.97	200.00 - 260.00	1003.70	700.00 - 2088.00
IIHRM 1-11-2	69.33	61.00 - 77.00	4.05	4.00 - 4.30	3.53	2.00 - 5.00	51.96	40.00 - 75.00	192.61	120.00 - 192.50
Double fertile										
IIHRM 5-4	52.46	34.00 - 64.00	4.07	4.00 - 4.50	3.48	3.00 - 4.00	144.06	236.00 - 338.00	628.68	448.00 - 804.00
IIHRM 1-12	54.00	45.00 - 63.00	5.07	5.00 - 5.30	4.52	4.00 - 5.00	120.02	101.00 - 143.00	526.46	408.00 - 758.00
Apetaloid										
IIHRM 7-4	43.07	35.00 - 49.00	2.87	2.00 - 3.00	2.53	2.00 - 4.00	196.25	162.00 - 245.00	740.20	567.00 - 1040.00
IIHRM 7-5	46.35	38.00 - 53.00	3.20	2.00 - 3.50	2.76	2.00 - 4.00	200.27	174.00 - 240.00	716.31	556.80 - 1217.00
IIHRM 3-2	47.67	38.00 – 56.00	3.78	3.50 – 4.00	2.81	2.00 - 4.00	188.81	166.00 - 211.00	597.49	498.00 – 750.00
IIHRM 3-5	51.67	43.00 - 60.00	3.98	3.70 - 4.00	2.68	2.00 - 4.00	134.34	103.00 - 160.00	411.67	309.00 - 480.00
Petaloid										
IIHRM 6-5	53.82	47.00 - 62.00	4.99	4.30 - 5.50	4.19	3.00 - 5.00	146.71	116.00 - 222.00	765.32	575.00 - 1110.00
IIHRM 2-9	54.33	45.00 - 64.00	4.39	4.00 - 5.00	5.32	4.00 - 6.00	197.26	170.00 - 224.00	1917.58	1530.00 - 2835.00
IIHRMOs-1	57.22	30.00 - 72.00	3.50	1.50 - 4.00	2.61	2.00 - 3.00	73.60	12.00 - 120.00	263.50	12.00 - 333.00
IIHRMYs-1	59.00	50.00 - 67.00	4.02	4.00 - 4.20	4.39	3.00 - 5.00	113.83	102.00 – 130.00	638.08	408.00 - 1440.00

Similarly, vegetatively propagated petaloid male sterile line IIHRMYs-1 was a good general combiner for shelf life. For the number of flowers per plant and yield per plant, IIHRM 6-5, a seed propagated petaloid male sterile line showed good general combining ability. Based on these results, seed propagated petaloid male sterile lines were good general combiners for days to bud initiation, flower diameter, number of flowers and yield per plant. Among pollen parents, semi-double fertile and double fertile lines were good general combiners for days to bud initiation. In addition, semi-double fertile lines were good combiners for flower diameter, shelf life, number and yield of flowers per plant. IIHRM 4-13, a semi-double fertile line showed an excellent general combining ability as a tester for all the traits under study (Table 4).

The performance of parental lines in combination with all other lines is reflected by GCA effects, parents with the highest GCA effects have a greater impact on the trait improvement. Singh & Misra (2008) identified good combiners for earliness in flowering, yield and yield attributes in African marigold.

Table 4 : General combining ability effects of marigold hybrids

Breeding line	Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant (g)
Male sterile seed parents					
Apetaloid					
IIHRM 3-2	-8.13	-1.15	-1.17	100.15	78.84
IIHRM 3-5	0.78	-0.80	-1.33	23.77	-381.25
IIHRM 7-4	-2.51	-0.11	-0.27	-17.31	-236.60
IIHRM 7-5	-5.73	0.28	-0.27	7.02	-127.71
Petaloid					
IIHRM 2-9	-6.79	1.34	0.67	28.23	247.67
IIHRM 6-5	-6.73	0.25	-0.27	103.13	387.73
IIHRMOs-1	7.49	-0.21	0.40	-46.42	-11.71
IIHRMYs-1	8.08	0.35	1.05	-59.78	31.28
Pollen parent					
Single fertile					
IIHRM 5-2	3.70	-0.09	-0.25	-18.60	-163.83
IIHRM 4-4	-1.46	0.22	-0.60	-10.87	-256.43
Semi double fertile					
IIHRM 1-11	-5.97	0.12	-0.25	69.07	186.42
IIHRM 1-11-2	0.20	0.26	-0.25	-39.18	-401.83
IIHRM 4-13	-3.96	0.22	0.40	23.88	201.73
Double fertile					
IIHRM 5-4	-1.21	-0.06	-0.10	24.80	57.73
IIHRM 1-12	-3.88	-0.19	0.00	23.23	169.00

Comparison of BLUP and GCA as a criterion for selection of parents

Performance of hybrids indicated that BLUP and GCA results were in agreement with each other for the traits studied. Both BLUP and GCA showed that apetaloid lines for days to bud initiation; petaloid lines for shelf life and flower diameter when used as parents to develop hybrids could give desirable results. For traits like number of flowers and yield per plant, petaloid line IIHRM 6-5 and semi-double fertile IIHRM 1-11 manifested relatively high BLUP and GCA.

CONCLUSION

Selection of the right parents to develop desirable hybrids is an important decision-making step in a hybrid breeding program. The ability of parents to produce superior hybrids is confirmed by assessing their progeny performance to study the combining abilities of parents which is a tedious and time-consuming procedure. BLUP-based comparison of different lines is a much easier way as it eliminates the laborious procedure of developing hybrids and evaluating them to study the combining ability effects. Based on the results, BLUPs could be used as a criterion to select parents for hybridization.

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Table 5 : Mean performance of parents and their hybrids for yield and yield contributing characters

Breeding line	Days to bud initiation	Flower diameter (cm)	Shelf life (days)	Flowers per plant (Nos.)	Flower yield per plant
Orange hybrids					
IIHRM 6-5 x IIHRM 4-4	49.33	6.07	2.67	208.33	637.67
IIHRM 6-5 x IIHRM 4-13	45.67	6.07	3.67	293.67	1546.33
IIHRM 6-5 x IIHRM 5-4	45.67	6.33	3.33	333.00	1199.00
IIHRM 7-4 x IIHRM 4-4	50.00	6.30	2.67	182.33	496.00
IIHRM 7-4 x IIHRM 4-13	45.00	6.17	3.67	192.33	818.00
IIHRM 7-4 x IIHRM 5-4	58.33	4.93	3.00	99.00	196.00
IIHRM 7-5 x IIHRM 4-4	48.33	5.17	2.00	149.33	331.00
IIHRM 7-5 x IIHRM 4-13	49.00	7.27	4.00	193.33	934.33
IIHRM 7-5 x IIHRM 5-4	46.33	6.13	4.33	204.00	571.33
IIHRMOs-1 x IIHRM 4-4	61.00	7.00	3.67	117.33	469.33
IIHRMOs-1 x IIHRM 4-13	59.00	5.00	4.33	117.00	468.00
IIHRMOs-1 x IIHRM 5-4	59.33	6.00	3.67	164.00	1224.33
IIHRMOs-1 x IIHRM 6-5	65.33	6.00	3.33	129.67	933.67
IIHRMOs-1 x IIHRM 7-4	61.00	5.17	4.00	132.67	676.67
IIHRMOs-1 x IIHRM 7-5	61.00	5.00	4.33	112.00	597.33
Yellow hybrids					
IIHRM 2-9 x IIHRM 5-2	57.00	5.83	3.67	157.67	1324.33
IIHRM 2-9 x IIHRM 1-11	45.00	6.70	4.00	280.00	1890.00
IIHRM 2-9 x IIHRM 1-11-2	46.67	8.77	4.33	189.33	1111.00
IIHRM 2-9 x IIHRM 1-12	46.67	7.00	4.00	168.67	899.67
IIHRM 3-2 x IIHRM 5-2	56.00	4.00	2.00	178.33	610.00
IIHRM 3-2 x IIHRM 1-11	42.67	4.97	2.33	387.33	1975.67
IIHRM 3-2 x IIHRM 1-11-2	48.67	4.20	2.00	190.00	428.33
IIHRM 3-2 x IIHRM 1-12	42.67	5.20	2.33	327.67	1535.67
IIHRM 3-5 x IIHRM 5-2	59.00	5.73	2.00	175.67	871.33
IIHRM 3-5 x IIHRM 1-11	45.33	5.00	2.00	205.33	492.67
IIHRM 3-5 x IIHRM 1-11-2	65.33	5.00	2.00	70.00	525.33
IIHRM 3-5 x IIHRM 1-12	56.00	4.00	2.00	136.67	820.00
IIHRMYs-1 x IIHRM 5-2	65.33	7.00	4.67	96.67	773.33
IIHRMYs-1 x IIHRM 1-11	65.67	6.77	4.00	86.33	621.67
IIHRMYs-1 x IIHRM 1-11-2	62.67	6.00	4.00	76.67	562.33
IIHRMYs-1 x IIHRM 1-12	61.67	6.00	5.00	142.67	1655.00
IIHRMYs-1 x IIHRM 2-9	60.00	6.00	5.00	170.67	1979.67
IIHRMYs-1 x IIHRM 3-2	63.00	5.83	4.00	140.67	1577.33
IIHRMYs-1 x IIHRM 3-5	67.67	5.00	4.00	62.67	459.67
CV	3.07	3.13	10.31	6.85	7.73
CD at 5%	2.73	0.30	0.59	19.35	115.70

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Original Research Paper

Genetic divergence in Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev) based on morphological traits

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ABSTRACT

Genetic diversity of thirty-one genotypes of Chrysanthemum were analysed for various growth and flowering related traits. Analysis of variance revealed significant differences among the genotypes for all the morphological traits studied. The clustering pattern based on Mahalanobis D^2 statistics categorised genotypes into six distinct clusters. The largest cluster *i.e.* cluster III composed of eleven genotypes followed by cluster II with nine genotypes, cluster I having eight genotypes and cluster IV, V, and VI with one genotype each. The maximum inter-cluster distance was recorded between clusters IV and cluster V (376.87) followed by clusters IV and cluster VI (344.96) and, cluster II and cluster IV (196.81). The maximum intra-cluster distance was observed for cluster III (56.57), followed by cluster II (46.87) and cluster I (29.52). Among all the clusters, genotypes in cluster II recorded highest cluster mean values for number of branches per plant (7.15), number of leaves (119.72) and flowers (91.69) per plant. Among nine characters, number of flowers per plant contributed maximum to divergence (32.26%). Therefore, for chrysanthemum improvement, highly diverse genotypes can be used as parents for crossing to generate high variability.

Keywords: Chrysanthemum, genetic diversity, Mahalanobis D^2 statistics, morphological traits

INTRODUCTION

Chrysanthemum is a high-profit floricultural crop belonging to the family Asteraceae and ranked second to rose in market trade (Nguyen et al., 2020), mainly grown for cut flower, loose, pot plant and landscaping. There are approximately 200 species in the genus Chrysanthemum, but most of them are sub-divided into 38 satellite genera of the chrysanthemum complex (Dalda-Sekerci, 2023). More recently, only 41 species have been attributed to the genus Chrysanthemum (Hadizadeh et al., 2022). The cultivated chrysanthemum is originally native of Asia (China and Japan) and northeastern Europe (Baliyan et al., 2014).

Morphological trait measurement is a commonly used index, since it provides a simple technique of quantifying genetic variation, while, simultaneously assessing genotype performance under normal growing environments (Fu et al., 2008). The hybridization of florist chrysanthemum began after 1850 when the practice of culturing chrysanthemum in greenhouses began. Several hundred cultivars have been commercialized, indicating that there are substantial genetic variations that can be manipulated under cultivations to produce a wide array of phenotypic variation (Jo et al., 2015).

Germplasm collection and evaluation have received a lot of attention in India. However, the information for higher flower yield and yield contributing characters is limited. In order to choose diverse parents to complement various breeding programmes, it is necessary to estimate the genetic variation and method of inheritance of various plant traits in chrysanthemum.

In chrysanthemum, the investigation, classification, identification, and diversity analysis of the cultivars became highly challenging due to the large number of cultivars, abundant morphological variation, wide distribution and complex genetic background. Therefore, the present study was carried out to select suitable genotypes for breeding programmes based on the genetic diversity estimation conducted on a group of chrysanthemum genotypes obtained from various locations in India.

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Table 1 : List of thirty-one chrysanthemum genotypes used for diversity study

Genotype	Source	Flower form	Growth habit
A1 Collection	Dooblabylekere, Bengaluru	Semi-double	Semi-erect
Appu	CSIR-NBRI, Lucknow	Semi-double	Erect
Arka Chandrakant	ICAR-IIHR, Bengaluru	Decorative	Spreading
Arka Chankdrika	ICAR-IIHR, Bengaluru	Decorative	Erect
Arka Kirti	ICAR-IIHR, Bengaluru	Double Korean	Erect
Arka Pink Star	ICAR-IIHR, Bengaluru	Semi-double	Spreading
Arka Usha Kiran	ICAR-IIHR, Bengaluru	Semi- double	Erect
Arka Yellow Gold	ICAR-IIHR, Bengaluru	Decorative	Erect
Autumn Joy	PAU, Ludhiana	Decorative	Spreading
Coffee	CSIR-NBRI, Lucknow	Semi- double	Spreading
Fitonia	CSIR-NBRI, Lucknow	Single	Semi-erect
Flirt	CSIR-NBRI, Lucknow	Decorative	Erect
Garden Beauty	PAU, Ludhiana	Spoon	Spreading
Gulmohar	CSIR-NBRI, Lucknow	Double Korean	Erect
Heritage	CSIR-NBRI, Lucknow	Semi-double	Semi-erect
Jubilee	CSIR-NBRI, Lucknow	Semi-double	Erect
Marigold	Local Collection	Pompon	Erect
Mayur	CSIR-NBRI, Lucknow	Semi-double	Erect
NBRI Little Kusum	CRIS-NBRI, Lucknow	Pompon	Spreading
Pachai Local	Local Collection	Semi-double	Spreading
Pink Cloud	CSIR-NBRI, Lucknow	Semi- double	Erect
Ratlam Selection	CSIR-NBRI, Lucknow	Decorative	Erect
Rekha	CSIR-NBRI, Lucknow	Single	Spreading
Shukla	CSIR-NBRI, Lucknow	Semi-double	Semi-erect
Statesman	PAU, Ludhiana	Semi-double	Erect
Sunil	CSIR-NBRI, Lucknow	Spoon	Erect
Vasanthika	CSIR-NBRI, Lucknow	Single	Semi-erect
White Dolley	CSIR-NBRI, Lucknow	Pompon	Spreading
White Local	Local Collection	Semi-double	Erect
White Prolific	CSIR-NBRI, Lucknow	Semi-double	Erect
Winter Queen	PAU, Ludhiana	Spoon	Spreading

MATERIALS AND METHODS

Plant material

The present study was carried out in the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, during 2019-20 and 2020-21. The experimental site was geographically located at 13°58' N Latitude, 78°E Longitude and at an elevation of 890 meter above mean sea level. In total, thirty-one chrysanthemum genotypes obtained from different locations of India (Table 1) were evaluated for various growth and flowering traits under naturally ventilated polyhouse in completely randomized design with three replications.

The plants of all genotypes were raised through terminal cuttings taken from healthy stock plants. After transplanting, plants were imposed with photoperiod of 15/9 hours for 30 days and 'black in' (dark conditions) until flower bud initiation. Uniform package of practices was followed throughout the experiment to ensure good growth. Five uniformly

Table 2 : Combined analysis of variance of nine characters for 31 genotypes of chrysanthemum

Source of variation	df	Plant height (cm)	No. of branches plant^{-1}	No. of leaves plant^{-1}	Days to bud initiation	Days to first flower opening	Days to optimum flowering	Flower diameter (cm)	No. of flowers plant^{-1}	Flowering duration (days)
Treatment	30	8,230.95 **	58.67 **	13,872.46 **	471.52 **	3,867.80 **	3,852.28 **	22.18 **	9,507.31 **	902.86 **
Error	62	0.25	12.81	10.19	1.00	4.74	6.02	4.22	0.08	5.29

grown plants per replication were tagged for recording observations of various growth and flowering traits, *viz*., plant height (cm), number of branches and leaves per plant, days to bud initiation and first flower opening, number of flowers per plant, optimum flowering (the number of days taken to 50% flowering from the date of 'black in' treatment), flower diameter (cm) and flowering duration (days) to check superiority of genotypes. The data recorded for both the years were pooled and analyzed statistically.

Quantitative traits data analysis

The mean values of the genotypes in each replication for quantitative characters were used for statistical analysis. The data were processed with the help of the software programme SPAR-1 (Doshi & Gupta, 1991) utilizing various standard statistical procedures. The data recorded on nine different quantitative traits were subjected to the D^2statistic of Mahalanobis, and grouping of the genotypes into different clusters was done by using Ward's minimum variance (Rao, 1952) and average intra-and-inter cluster distances were calculated following Tocher's method.

RESULTS AND DISCUSSION

Analysis of variance indicated highly significant differences among the genotypes for all the characters studied indicating the presence of sufficient amount of variability (Table 2).

Cluster constellation

Thirty-one genotypes were classified into six clusters on the basis of D^2 values calculated from the analysis of nine morphological traits (Fig. 1). Among all the clusters, cluster III being the largest consisted of eleven genotypes, followed by cluster II with nine genotypes, cluster I having eight genotypes and cluster IV, V, and VI with one genotype each (Table 4). The variable number of accessions in different clusters pointed toward the presence of wide range of genetic diversity within as well as between the clusters. Similarly, Bhargav et al. (2023) recorded two clusters in China aster, while, Baliyan et al. (2014) reported four clusters in chrysanthemum, and Kavitha & Anburani (2009) recorded eight clusters in African marigold.

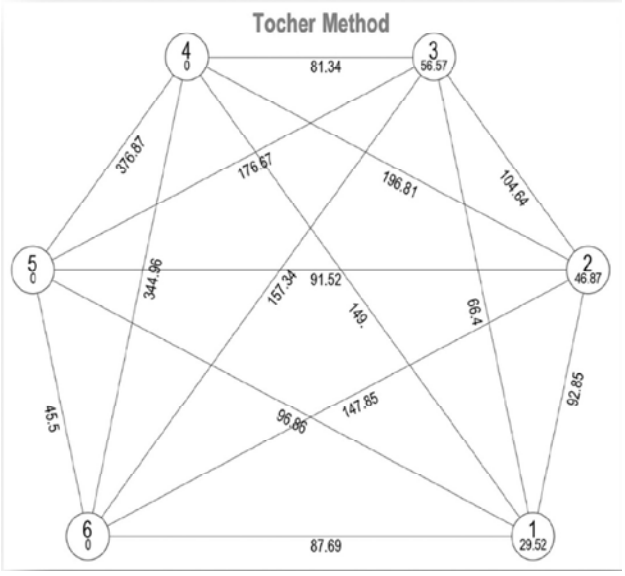

Fig. 1 : Inter and intra cluster distances for thirty-one genotypes in chrysanthemum

The group constellation of thirty-one chrysanthemum genotypes was also plotted in the form of a dendrogram (Fig. 2), where, Euclidian distances in dendrogram highlighted the genetic divergence between and within the clusters. The pattern of distribution of genotypes from various eco-geographical regions into various clusters with different divergence values was random, supporting that genetic diversity and geographical diversity are unrelated. Natural or artificial selection, breeding material exchange, genetic drift, and environmental variation may be the primary factors influencing this genetic diversity in addition to geographic origin. The results of present study are substantiated with the similar conclusions reported on diversity (Kumar et al., 2011; Baliyan et al., 2014) indicated that geographic diversity cannot always be used as an index of genetic diversity. It is quite

possible that many of the genotypes obtained from different geographical regions could share a common ancestor. Moreover, progenies obtained through breeding incorporate genes from varied sources, thus losing the basic geographical identity of the genotype (Bharathi & Jawaharlal, 2014).

Average inter as well as intra-cluster distances in all the genotypes were computed through Mahalanobis (D^2) analysis (Table 3). On the basis of D^2 values between two genotypes or two clusters, choice of divergent parents can be made for hybridization purpose. The inter-cluster distance ranged from

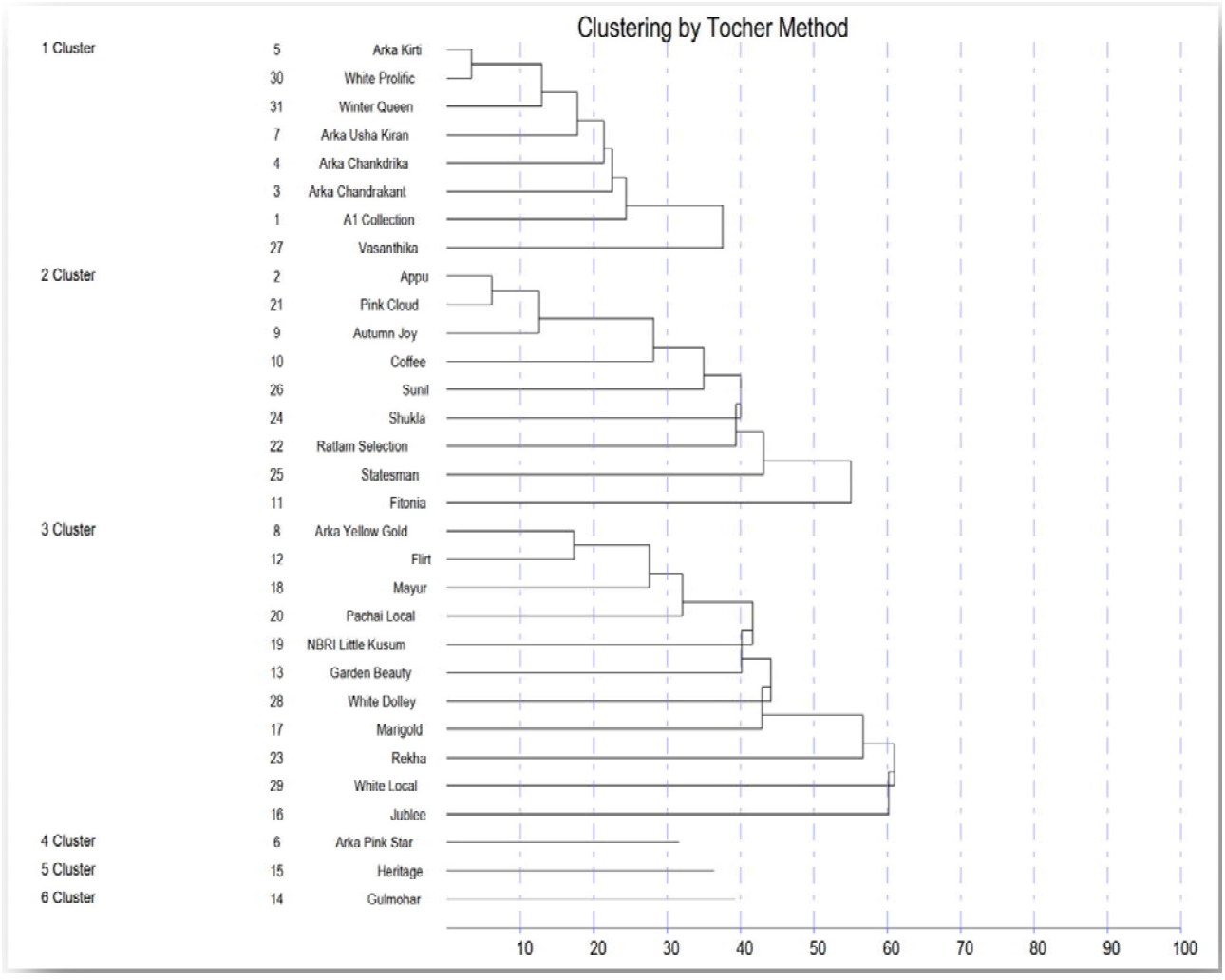

Fig. 2 : Dendrogram showing Euclidean distances based on nine quantitative traits of thirty-one chrysanthemum genotypes

Table 3 : Average intra and inter-cluster distances computed through D^2 analysis

Clusters	Cluster distances					
	I	II	III	IV	V	VI
I	**29.52**	-	-	-	-	-
II	92.85	**46.87**	-	-	-	-
III	66.40	104.64	**56.57**		-	-
IV	149.00	196.81	81.34	**0.00**	-	-
V	96.86	91.52	176.67	376.87	**0.00**	-
VI	87.69	147.85	157.34	344.96	45.50	**0.00**

45.5 (cluster V and VI) to 376.87 (cluster IV and V). The maximum (376.87) inter-cluster distance was recorded between clusters IV (Arka Pink Star) and cluster V (Heritage) followed by clusters IV (Arka Pink Star) and cluster VI (Gulmohar) (344.96) and, cluster II (Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia) and cluster IV (Arka Pink Star) (196.81). This clearly indicated that the genotypes present in these clusters contain a wide range of genetic diversity which could be exploited through hybridization program to get better recombinants in the segregating generations. The results suggested that cross combination of genotypes from most divergent clusters IV and V, and IV and IV, respectively would be more vigorous when used as parent in hybridization programme for obtaining wide spectrum of variation among the segregates.

The least intercluster distance was observed between clusters V (Heritage) and cluster VI (Gulmohar) followed by cluster I (Arka Kirti, White Prolific, Winter Queen, Arka Usha Kiran, Arka Chankdrika, Arka Chandrakant, A1 Collection and Vasanthika) and cluster II (Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia). The less inter-cluster distances indicated lower degree of divergence and close genetic makeup of these genotypes (Kaur et al., 2021), making them less suited for use in a hybridization programme between the genotypes in the cluster.

In the present investigation, cluster III (56.57) showed the highest intra-cluster distance followed by cluster II (46.87) and cluster I (29.52). Genotypes belonging to cluster III had the maximum heterogeneity among the assembled genotypes that is also clear from the assembling pattern of the genotypes in the dendrogram (Fig. 2 and Table 4). It means that the genotypes in this cluster were highly diverse for the traits under study. However, null intra cluster distances were recorded in cluster IV, V and VI because of the inclusion of single genotype in each. The lower intra-cluster distances indicated homogenous nature of genotypes within the clusters, while, higher values displayed heterogeneous nature of clusters (Kaur et al., 2021). Lower level of intra- cluster distances explained narrow genetic variation within the cluster. Therefore, the members of same cluster are not expected to yield desirable recombinants. These results for genetic variation within and between the clusters are substantiated with the previous reports (Kavitha & Anburani, 2009; Baliyan et al., 2014). The genetic diversity observed within and between the clusters can be utilized in selection of genetically diverse genotypes which is important for exploitation of heterosis and development of desirable recombinants (Panwar et al., 2014).

Cluster-wise performance

The considerable morphological diversity of different clusters could also be explained from the average performance of the genotypes for nine morphological traits (Table 5).

Among all the clusters, genotypes in cluster II had highest cluster mean values for number of branches per plant (7.15), number of leaves per plant (119.72), and number of flowers per plant (91.69). The high yield potential in chrysanthemum is associated with

Table 4 : Distribution of chrysanthemum genotypes in different clusters through D^2 analysis based on quantitative traits

Clusters	Number of Genotype(s)	Genotype(s)
I	8	Arka Kirti, White Prolific, Winter Queen, Arka Usha Kiran, Arka Chankdrika, Arka Chandrakant, A1 Collection and Vasanthika
II	9	Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia
III	11	Arka Yellow Gold, Flirt, Mayur, Pachai Local, NBRI Little Kusum, Garden Beauty, White Dolley, Marigold, Rekha, White Local and Jublee
IV	1	Arka Pink Star
V	1	Heritage
VI	1	Gulmohar

Table 5 : Cluster means for nine quantitative traits in thirty-one chrysanthemum genotypes

Clusters	Plant height (cm)	Branches per plant (Nos.)	Leaves per plant (Nos.)	Days to flower initiation	Days to first flower opening	Days to optimum flowering	Flowers per plant (Nos.)	Flower diameter (cm)	Flowering duration (days)
I	58.02	4.35	85.54	23.61	60.55	78.29	50.11	5.65	43.75
II	65.74	7.15	119.72	20.61	63.82	79.74	91.69	5.87	47.17
III	50.31	7.14	93.47	19.15	52.93	69.59	54.56	4.73	41.91
IV	26.92	7.00	110.33	11.16	31.00	51.33	56.67	3.93	37.33
V	92.50	5.83	101.67	28.84	88.33	104.50	67.83	5.70	47.50
VI	103.27	6.33	69.50	27.83	80.83	97.83	31.67	6.03	57.00

the number of branches per plant, number of leaves and flowers per plant. However, cluster IV recorded least plant height (26.92), days to flower initiation (11.16), days to first flower opening (31.00), days to optimum flowering (51.33), flower diameter (3.93) and flowering duration (37.33). The clustering pattern could be used to select parents for cross combinations that are expected to generate the highest possible variability for various economic characters. Similar type of observations for morphological variability in chrysanthemum has been reported (Kameswari et al., 2014; Kumar et al., 2016).

In the present study, higher mean for plant height was recorded in cluster VI followed by cluster V, while, number of branches per plant was recorded maximum in cluster II, followed by cluster III. The maximum number of leaves per plant was recorded in cluster II, followed by IV.

Minimum days to flower initiation, first flower opening and optimum flowering was in cluster IV, followed by cluster III. Maximum flower diameter and flowering duration was recorded in cluster VI, followed by cluster II and cluster V, respectively, while, maximum number of flowers per plant was recorded in cluster II, followed by cluster V.

Highly varied genotypes from these clusters may be exploited in a hybridization programme to develop highly desired recombinants for the improvement of yield. Among various groups, the genotype belonging to cluster VI (Gulmohar) and cluster V (Heritage) can be used to develop variety suitable for cut flower purpose. The genotypes were more distinct between cluster IV and III, therefore, hybridization of Arka Pink Star from cluster IV and Arka Yellow Gold, Flirt, Mayur, Pachai Local, NBRI Little Kusum, Garden Beauty, White Dolley, Marigold, Rekha, White Local and Jublee from cluster III may result in earliness to flowering. Similarly, the high yielding genotypes of cluster II can also be utilized in breeding programmes. Additionally, the precise selections for specific characteristics may help in the development of trait-specific high-yielding inbreds. To generate a wide range of variability or segregation for the target traits, the parents for hybridization should be selected from clusters expressing moderate to high D^2 values (Kaur et al., 2021).

Trait-wise contribution to diversity

The relative contribution of individual characters towards genetic divergence has been computed (Table 6) and revealed that among all the characters,

Table 6 : Contribution (%) of the traits towards genetic divergence in chrysanthemum genotypes

Source	Contribution (%)	Times ranked 1st
Plant height (cm)	11.40	53
Number of branches per plant	15.91	74
Number of leaves per plant	10.32	48
Days to flower initiation	2.80	13
Days to first flower opening	13.98	65
Days to optimum flowering	0.43	2
Number of flowers per plant	32.26	150
Flower diameter (cm)	10.11	47
Flower duration (days)	2.80	13

number of flowers per plant contributed maximum to divergence (32.26%), followed by number of branches per plant (15.91%), days to first flower opening (13.98%) and plant height (11.40%). These characters can be given more emphasis during selection of at least one parent for hybridization program.

Number of leaves per plant, flower diameter, flower duration and days to optimum flowering showed contribution of 10.32%, 10.11%, 2.80% and 0.43%, respectively. The importance of morphological traits in genetic diversity has been substantiated with the findings of Kameswari et al. (2014) and Kumar et al. (2016).

CONCLUSION

Genetic divergence has been considered as an important factor in selecting genetically diverse parents for efficient and successful hybridization programme. Clustering pattern based on Mahalanobis D^2 statistic revealed that diverse geographic origins of the genotypes could not necessarily be an index of variation and the factors such as genetic drift, selection pressure and environment may be responsible for discrepancy of genotypes. Among nine characters, number of flowers per plant contributed maximum to divergence. Therefore, for chrysanthemum improvement, the clustering pattern could be used to select parents for cross combinations that are expected to generate the highest possible variability.

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Original Research Paper

Evaluation of *Heliconia* for growth, flowering and flower yield

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ABSTRACT

Heliconia is utilized as ornamental plants, usually being grown both as landscaping plants and as cut flowers, owing to colour and the longer durability of its floral bracts. Forty-one genotypes of Heliconia were evaluated for growth, flowering and flower yield. Significant variation was observed among genotypes for vegetative and floral characteristics. The results revealed that the maximum number of leaves per sucker was recorded in *H. hirsuta* followed by *H.* 'Golden Torch Adrian' and *H.* 'GT Sunshine', while, maximum plant height was recorded in varieties *viz.*, *H. caribea* (459.33 cm) followed by *H.* 'She' (337.67 cm) and *H. rauliniana* upright (305.00 cm). However, maximum leaf length was observed in *H. caribea* (314.33 cm) followed by *H.* 'She' (203.67 cm) and *Heliconia metallica* (175.00 cm). Maximum sucker production was recorded in *H.* 'Tropics' followed by *H. psittacorum* 'Petra' and *H.* 'Guyana'. Among the flowering traits, early flower initiation was recorded in Golden Torch (136.00 days) followed by Lady Di (152.00 days). The rachis length ranged from 72.67 cm (*H.* 'Golden Torch') to 10.00 cm (*H.* 'Hirsuta'), however, longest spike was recorded in *H.* '*rauliniana* upright' (131.33 cm) followed by *H. rostrata* 'Parrots Beak' (115.67 cm) and *H. rostrata* Ten Days (97.00 cm), whereas, shortest spike was recorded in *H. bihai* (9.33 cm).

Keywords: Bracts, Heliconia, speciality flower, spike, sucker

INTRODUCTION

Heliconia is a popular specialty flower with varied forms and rich colour, which is in high demand in global as well as domestic markets. It belongs to the family Heliconiaceae and are native to South and Central America and Islands of South Pacific. The genus has about 250-300 species distributed primarily in Neotropical areas from North of Mexico to South of Brazil (Malakar & Biswas, 2022). Heliconia derive their beauty from highly modified leaves or bracts and colour varies from pink, red, orange, yellow, green, with different combinations of sizes and shapes. Flower spikes as well as the foliage of Heliconia are used for flower arrangements, landscaping, potted plants, cut foliage etc. Selection of promising genotypes of Heliconia for production of cut flowers with quality is vital for expanding floral industry. Hence, there is a need to identify suitable varieties of Heliconia for production of quality flowers and high yield. This will bring a vast scope for expansion of the crop on a commercial scale to meet the domestic as well as export demand. Considering this, the present study was undertaken to evaluate 41 Heliconia genotypes for growth, flowering and flower yield under open grown conditions.

MATERIALS AND METHODS

The experiment was undertaken at Hadapsar Research farm, ICAR-Directorate of Floricultural Research, Pune during 2017-2020 with two replications in randomized block design. The treatment consisted of 41 genotypes of *Heliconia viz.*, *H. psittacorum* 'Kathy', *H. psittacorum* 'Petra', *H. stricta* 'Jamaican Dwarf', H-23, *H.* spp. 'Prince of Darkness', *H. psittacorum* 'Lady di', *H. bihai* 'Schaefer', *H.* 'Kenya Red', *H. rostrata* 'Parrots beak', *H. bihai* 'Dwarf', *H. psittacorum* x *H. spathocircinata* 'Golden Torch', *H. psittacorum* 'Sassy', *H. psittacorum* 'Strawberries and Cream', *H. metallica, H. psittacorum* x *H. spathocircinata* 'Alan Carle', *H. latispatha,* H-13 'Silver Dust', *H. bihai* 'Firebird', *H.orthotricha* 'She', H-25, *H. latispatha* 'Distans', *H. psittacorum* x *H. spathocircinata* 'Guyana', *H. bihai* 'yellow', *H.* 'Red', 'Pedro Ortiz', *H. stricta* 'Iris', *H. psittacorum* x *H. spathocircinata* 'Adrian', *H. psittacorum* x *H. spathocircinata* 'Tropics', *H. psittacorum* x *H. spathocircinata* 'Yellow Parrot', 'Golden Torch

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Sunshine', *H. chartaceae* 'Temptress', *H. stricta* 'Lobster Claw', *H.* Jacquinii, *H.* 'Fireflash', *H. hirsuta, H.* 'Meccas Pink', *H. rostrata* 'Ten Days', *H. rauliniana* upright, *H. caribea, H. angusta* 'Red Christmas' and *H. accuminata* 'Guyana'. Suckers of all the genotypes were planted at a spacing of 1.5 x 1.5 m. Uniform cultural operations were carried out throughout the experiment. Various vegetative traits *viz.*, plant height, number of leaves and suckers per plant, number of leaves per sucker, leaf length, leaf width, leaf petiole length and floral characters *viz.*, days to first flowering, rachis length, spike length, number of bracts per floret per spike, internodal length, width and length of bract, number of flowering shoots per plant were recorded. Data recorded were subjected to statistical analysis and analysis of variance (ANOVA) was used to test the significance of genotypic differences (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Vegetative traits

Heliconia genotypes under the study exhibited wide variation for vegetative characters (Table 1). Vegetative characters are significantly important as they play a vital role in deciding the good crop yield. Maximum plant height was recorded in varieties *viz.*, *H. caribea* (459.33 cm) followed by *H.* 'She' (337.67 cm) and *H. rauliniana* 'upright' (305.00 cm). The genotypes *H. psittacorum* 'Strawberries and Cream', 'Pedro Ortiz', *H. angusta* 'Red Christmas', *H. psittacorum* 'Lady di', *H.* spp. 'Prince of Darkness', *H. hirsuta, H. psittacorum* 'Kathy', *H. bihai* 'Dwarf' and *H. stricta* 'Jamaican Dwarf' recorded less than one meter height, which can be best suited as potted plants. The genotypes *H. stricta* 'Lobster Claw', *H. psittacorum* x *H. spathocircinata* 'Guyana', *H. psittacorum* x *H. spathocircinata* 'Alan Carle', *H.* 'Fireflash', *H. latispatha* 'Distans', *H. bihai* 'Firebird', *H. psittacorum* x *H. spathocircinata* 'Yellow Parrot', *H. psittacorum* x *H. spathocircinata* 'Golden Torch', *H.* 'Meccas Pink', *H.* 'Kenya Red', *H. bihai* 'Schaefer', 'Golden Torch Sunshine', *H. psittacorum* 'Petra' and *H. psittacorum* x *H. spathocircinata* 'Adrian' were intermediate with 1 to 2 meter plant height and could be used as border plants in landscaping. However, the genotypes *H. caribea, H. orthotricha* 'She', *H. rauliniana* 'upright', *H. rostrata* 'Ten Days', *H. metallica, H. latispatha, H. jacquinii*, *H. rostrata* 'Parrots beak', *H. chartaceae* 'Temptress', *H. psittacorum* x *H. spathocircinata* 'Tropics', *H. psittacorum* 'Sassy' and *H. stricta* 'Iris' were recorded more than 2 meter high and could be used for hedging/screening purpose in landscaping. Wider variation for plant height in different Heliconia have been reported (Sheela et al., 2007). Such variations in plant height among the Heliconia genotypes could be attributed mainly due to genetic makeup of the genotype.

Productivity of Heliconia is dependent on suckering habit and number of flowering suckers per clump in a year, while, suckering habit determines its commercial viability. Total number of suckers produced is a critical factor in determining yield potential of a cultivar. Maximum sucker production was recorded in *H.* 'Tropics' followed by *H. psittacorum* 'Petra' and *H.* 'Guyana'. Ramachandrudu & Thangam (2012) and Thangam et al. (2014) also reported variability in number of suckers per plant in Heliconia. High variability for the number of suckers per clump, may be due to ploidy levels, genomic constitution, more aeration and light due to suckering nature of genotypes (Dalawai et al., 2017; Nihad et al., 2019).

Cut foliage of Heliconia are used as backdrop material in flower arrangements, bouquet preparation as well as stage decorations. In the present study, wide variation was observed for various leaf traits. A leaf of lanceolate shape with medium width is highly preferred for floral decorations. Maximum number of leaves per sucker was recorded maximum in *H. hirsuta* followed by *H.* 'Golden Torch', 'Adrian' and *H.* 'GT Sunshine', however, it was recorded maximum per clump in *H.* 'Tropics' followed by *H.* 'Guyana' and *H.* 'Alan Carle'. Number of leaves on stem during inflorescence emergence can serve as a useful indicator for Heliconia growers to quantify the plants expected to bloom for market planning.

Maximum leaf length was observed in *H. caribea* (314.33 cm) followed by *H.* 'She' (203.67 cm) and *Heliconia metallica* (175.00 cm), it was recorded least in Jamaican dwarf (31.67 cm). Leaf width ranged from 6.33 cm (*H.* 'Strawberries and Cream') to 39.00 cm (*H. caribea*). Petiole length was recorded maximum in *H.* 'She' followed by *H. caribea* and *H. latispatha,* whereas, least observed in *H.* 'Hirsuta'. Qualitative descriptors related to petiole have been reported (Malakar & Biswas, 2022). Heliconia genotypes *viz.*,

Table 1 : Evaluation of Heliconia genotypes for vegetative growth

Genotype	Plant height (cm)	Suckers/ plant (Nos.)	Leaves/ sucker (Nos.)	Leaves/plant/ clump (Nos.)	Leaf length (cm)	Leaf width (cm)	Leaf petiole length (cm)
H. psittacorum 'Kathy'	79.67	64.33	4.67	134.33	51.33	9.50	14.33
H. psittacorum 'Petra'	104.66	79.67	4.66	153.00	56.00	7.00	17.67
H. stricta 'Jamaican Dwarf'	39.33	28.00	3.33	82.00	31.67	8.00	9.00
H-23	103.33	13.66	4.33	32.67	62.66	11.33	16.33
H. spp. 'Prince of Darkness'	89.00	15.33	3.67	43.33	53.67	13.00	17.00
H. psittacorum 'Lady di'	91.33	64.67	4.33	150.33	47.33	8.33	10.00
H. bihai 'Schaefer'	111.33	49.0	3.66	137.00	48.67	10.33	9.67
H. 'Kenya Red'	113.00	76.000	4.66	177.00	58.00	11.00	15.66
H. rostrata 'Parrots beak'	229.67	59.33	5.33	178.33	73.66	16.33	20.33
H. bihai 'Dwarf'	50.66	61.33	5.00	107.33	44.33	10.34	15.67
H. psittacorum x *H. spathocircinata* 'Golden Torch'	128.67	53.00	4.33	110.67	81.33	13.67	27.33
H. psittacorum 'Sassy'	220.33	59.00	4.00	134.00	104.00	20.68	41.33
H. psittacorum 'Strawberries and Cream'	93.67	39.00	4.33	98.00	55.33	6.33	18.67
H. metallica	271.00	55.33	4.00	198.67	175.00	26.00	64.33
H. psittacorum x *H. spathocircinata* 'Alan Carle'	186.67	63.67	4.33	277.00	123.00	14.07	49.67
H. latispatha	256.33	56.66	4.00	217.67	167.67	24.00	66.00
H-13 'Silver Dust'	136.00	9.00	4.67	33.67	54.00	16.00	15.00
H. bihai 'Firebird'	149.67	46.00	4.33	181.67	92.33	17.33	35.00
H. orthotricha. 'She'	337.66	45.99	3.67	164.00	203.67	26.33	87.00
H-25	220.00	25.00	4.33	96.00	128.00	13.00	55.33
H. latispatha Distans	167.33	17.67	3.67	58.67	82.67	14.67	29.00
H. psittacorum x *H. spathocircinata* 'Guyana'	189.00	61.67	4.00	245.67	115.00	13.66	51.33
H. bihai 'yellow'	123.00	26.00	4.67	126.00	84.00	11.00	38.00
H. 'Red'	217.00	12.00	4.33	44.00	148.00	28.50	58.00
H. 'Pedro Ortiz'	93.00	10.67	4.66	43.33	60.00	25.00	14.33
H. stricta 'Iris'	212.33	47.33	4.00	185.33	150.66	21.00	57.67
H. psittacorum x *H. spathocircinata* 'Adrian'	102.00	13.00	5.33	55.67	58.67	11.50	22.00
H. psittacorum x *H. spathocircinata* 'Tropics'	221.00	99.00	2.67	394.33	139.33	16.33	54.67
H. psittacorum x *H. spathocircinata* 'Yellow Parrot'	146.00	65.33	3.33	194.00	87.33	12.33	34.33
H. 'Golden Torch Sunshine'	109.00	27.00	5.33	142.00	64.33	11.67	18.33
H. chartaceae 'Temptress'	228.33	10.33	4.33	38.33	141.33	28.00	54.00
H. stricta 'Lobster Claw'	194.00	46.00	4.33	179.00	84.00	12.50	34.00
H. 'Jacquinii'	234.67	33.33	3.67	122.00	158.67	27.17	53.67
H. 'Fireflash'	168.33	32.99	4.00	131.33	93.00	15.33	34.33
H. hirsuta	88.00	8.00	6.00	45.00	46.00	10.00	5.00
H. 'Meccas Pink'	123.00	41.00	5.33	198.00	79.00	13.00	32.00
H. rostrata 'Ten Days'	290.00	24.33	3.33	78.33	115.00	20.67	13.67
H. rauliniana 'upright'	305.00	46.00	4.33	178.67	140.33	25.17	52.33
H. caribea	459.33	30.67	3.99	117.00	314.33	39.00	80.00
H. angusta 'Red Christmas'	91.67	44.33	5.33	223.00	48.33	13.33	23.67
H. accuminata 'Guyana'	165.66	77.00	4.00	302.67	97.00	13.32	41.33
S.E.m+	10.09	7.64	0.41	21.99	5.91	0.92	3.50
CD (P=0.05)	30.26	22.92	1.24	65.98	17.73	2.77	10.51

Guyana, Tropics, Alan Carle, *H.* 'Red and *H. latispatha* produced desirable quality leaves for decoration purpose.

Flowering characters

Significant differences were observed among Heliconia genotypes for days taken for first flowering (Fig. 1). *H. psittacorum* varieties recorded early flowering, which is ideal to catch early market. Data revealed that the commercial Heliconia genotype Golden Torch also recorded early flower initiation (136.00 days) followed by Lady di (152.00 days). The genotypes *H. psittacorum* varieties *viz.*, Golden Torch, Lady Di, Kathy, Sassy, Petra, Strawberries and Cream, Kenya Red and Alan Carle were ready for market at earlier dates compared to robust varieties like H. 'Red', *H. wagneriana* and *H. stricta* 'Iris' which recorded delayed flowering. Majority of Heliconia genotypes under the study recorded first flowering within 300 days from the date of planting of suckers. Catley and Brooking (1996) also reported that *H. psittacorum* cv. Golden Torch initiated flowering in 140-146 days after planting. Dalawai et al., (2017) reported that *H. latispatha* 'Orange' shown its first visible flower in 110.88 days, whereas, *H. wagneriana* 'Red' was late to initiate flower (316.50 days).

Length of rachis and spike are important characters which decides aesthetic appeal, display value and usefulness for various floral decorations. The data pertaining to various floral traits is furnished in Table 2. The rachis length ranged from 72.67 cm (*H.* 'Golden Torch') to 10.00 cm (*H.* 'Hirsuta'), however, longest spike was recorded in *H.* '*rauliniana* upright' (131.33 cm) followed by *H. rostrata* 'Parrots Beak' (115.67 cm) and *H. rostrata* Ten Days (97.00 cm), while, shortest spike was recorded in *H. bihai* (9.33 cm). Higher spike length enables easy handling of the inflorescence, making it suitable for flower arrangements and bouquet making. The commercial genotypes *viz.*, Kathy, Petra, Prince of Darkness, Lady Di, Kenya Red, Guyana, Adrian, Tropics produced spike length of more than 50 cm, which are ideal for flower decoration. Auclar et al. (2022) opined that very short stems limit their use in arrangements, with a minimum stem length of 80 cm being required. On the other hand, large stems greater than 1.51 m, require careful handling to avoid tip-over or unwanted breakage.

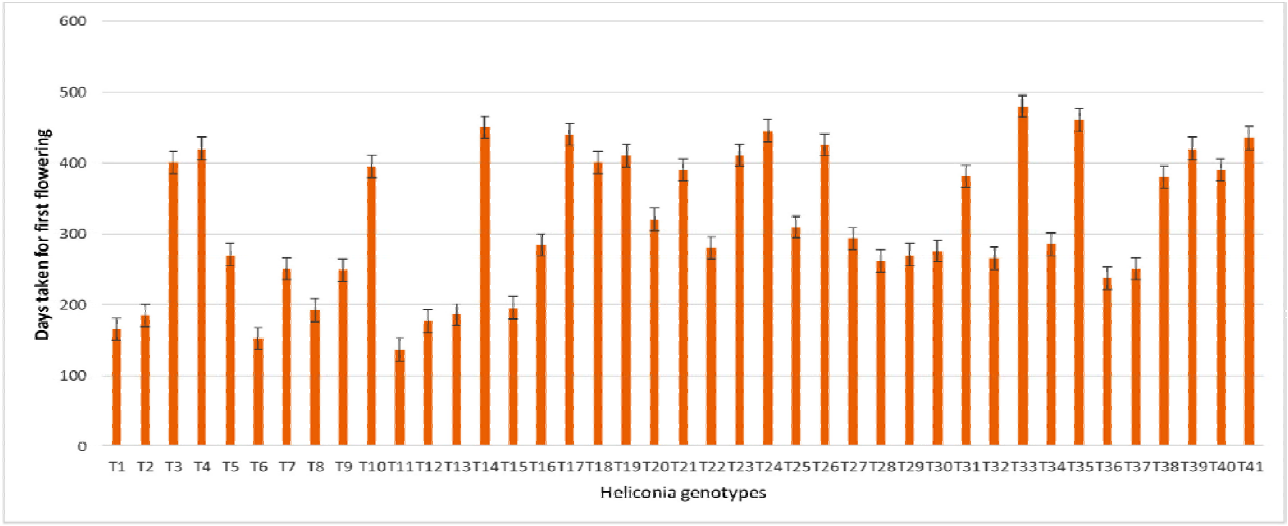

Fig. 1 : Days taken for first flowering among different Heliconia genotypes under the study

T_1: *H. psittacorum* 'Kathy',T_2: *H. psittacorum* 'Petra', T_3: *H. stricta* 'Jamaican Dwarf',T_4: H-23,T_5: *H.* spp. 'Prince of Darkness', T_6: *H. psittacorum* 'Lady di', T_7: *H. bihai* Schaefer, T_8: *H.* Kenya Red, T_9: *H. rostrata* 'Parrots beak', T_{10}: *H. bihai* Dwarf, T_{11}: *H. psittacorum* x *H.spathocircinata* 'Golden Torch', T_{12}: *H. psittacorum* 'Sassy', T_{13}: *H. psittacorum* 'Strawberries and Cream', T_{14}: *H. metallica*, T_{15}: *H. psittacorum* x *H. spathocircinata* 'Alan Carle', T_{16}: *H. latispatha,* T_{17}: H-13 Silver Dust, T_{18}: *H. bihai* Firebird, T_{19}: *H. orthotricha* 'She', T_{20}: H-25, T_{21}: *H. latispatha* 'Distans', T_{22}: *H. psittacorum* x *H. spathocircinata* 'Guyana', T_{23}: *H. bihai* 'Yellow', T_{24}: *H.* Red, T_{25}: *H.* 'Pedro Ortiz', T_{26}: *H.stricta* 'Iris', T_{27}: *H. psittacorum* x *H. spathocircinata* 'Adrian', T_{28}: *H. psittacorum* x *H. spathocircinata* 'Tropics', T_{29}: *H. psittacorum* x *H. spathocircinata* 'Yellow Parrot', T_{30}: Golden Torch Sunshine, T_{31}: *H. chartaceae* 'Temptress', T_{32}: *H. stricta* 'Lobster Claw', T_{33}: *H.* Jacquinii, T_{34}: *H.* Fireflash, T_{35}: *H. hirsuta,* T_{36}: *H.* Meccas Pink, T_{37}: *H. rostrata* 'Ten Days', T_{38}: *H. rauliniana* 'Upright', T_{39}: *H. caribea,* T_{40}: *H. angusta* 'Red Christmas' and T_{41}: *H. accuminata* 'Guyana'

Table 2 : Evaluation of Heliconia genotypes for flowering traits

Genotype	Rachis length (cm)	Spike length (cm)	Bacts/floret/ spike (Nos.)	Internodal length between florets/bract (cm)	Width of bract (cm)	Length of bract (cm)	Flowering shoots/plant (Nos.)
H. psittacorum 'Kathy'	12.17	61.67	3.33	3.77	1.93	9.83	32.33
H. psittacorum 'Petra'	10.67	64.33	3.67	2.67	2.00	9.67	29.67
H. stricta 'Jamaican Dwarf"	13.00	20.33	3.33	0.93	2.03	7.70	18.33
H-23	20.00	60.00	4.33	2.00	2.00	14.70	5.00
H. spp. 'Prince of Darkness'	15.00	51.67	4.67	2.57	2.00	13.00	6.67
H. psittacorum 'Lady di'	10.33	73.00	4.00	2.66	2.00	9.67	43.33
H. bihai 'Schaefer'	12.00	76.33	3.33	2.67	2.33	11.67	12.33
H. 'Kenya Red'	14.00	71.33	4.33	2.00	2.00	9.67	9.67
H. rostrata 'Parrots beak'	36.00	115.67	15.67	1.33	3.00	6.33	1.33
H. bihai 'Dwarf'	39.00	9.33	3.67	2.67	2.00	15.67	11.00
H. psittacorum x *H. spathocircinata* 'Golden Torch'	72.67	17.00	4.67	2.00	2.00	16.67	33.00
H. psittacorum 'Strawberries and Cream'	69.00	10.33	3.00	1.33	2.00	9.00	24.00
H. psittacorum x *H. spathocircinata* 'Alan Carle'	23.33	80.00	6.00	4.17	2.27	20.67	14.00
H. bihai 'Firebird'	24.00	51.00	3.33	5.67	2.03	15.67	1.33
H. orthotricha 'She'	33.00	82.00	5.33	3.50	2.13	19.33	4.33
H-25	21.67	96.67	5.00	4.00	2.10	16.00	16.00
*H. latispatha I'*Distans'	21.33	72.33	4.00	2.83	0.57	20.00	27.33
H. psittacorum x *H. spathocircinata* 'Guyana'	22.00	86.00	4.00	2.23	2.00	13.00	1.67
H. bihai 'yellow'	14.00	60.00	3.33	3.00	2.03	10.00	1.33
H. 'Red'	35.00	60.33	5.33	3.10	4.90	17.23	2.33
H. stricta 'Iris'	35.01	76.67	5.33	4.50	2.50	22.00	1.33
H. psittacorum x *H. spathocircinata* 'Adrian'	13.00	57.00	3.33	2.17	1.33	12.50	4.33
H. psittacorum x *H. spathocircinata* 'Tropics'	16.00	78.00	3.33	2.67	2.50	11.00	8.33
H. psittacorum x *H. spathocircinata* 'Yellow Parrot'	16.33	63.33	4.33	2.70	1.70	15.00	29.00
'Golden Torch Sunshine'	15.33	59.33	4.67	2.53	2.07	11.33	17.00
H. 'Fireflash'	13.00	66.00	3.67	2.00	0.53	15.00	19.00
H. hirsuta	10.00	47.00	3.33	2.33	1.33	10.00	1.33
H. Meccas 'Pink'	16.00	46.00	5.00	3.00	1.33	15.00	8.00
H. rostrata 'Ten Days'	25.00	97.00	10.00	3.00	2.33	10.50	1.33
H. rauliniana 'upright'	40.00	131.33	10.67	5.33	1.17	27.00	21.33
H. caribea	40.67	95.33	8.33	5.17	3.00	19.33	1.67
H. angusta 'Red Christmas'	12.00	59.67	3.67	2.67	2.33	9.17	11.00
H. accuminata 'Guyana'	13.02	54.08	4.52	2.35	1.99	11.21	18.51
S.E.m+	1.74	4.51	0.55	0.32	0.17	0.68	2.51
CD (P=0.05)	5.22	13.54	1.66	0.97	0.51	2.03	7.53

The display value of Heliconia increases with increase in number of bracts. In the present study, highest number of bracts/florets per spike was recorded in *H. rostrata* 'Parrots Beak' (15.67 cm) followed by *H. rauliniana* upright (10.67 cm) and *H. rostrata* Ten Days (10.00 days). The genotype with longer inflorescence in Heliconia has a greater number of bracts per spike. *H.* '*rauliniana* upright' (131.33cm), *H.rostrata* 'Parrots beak' (115.67 cm) and *H. rostrata* Ten Days (97.0 cm) recorded longer inflorescence with more bract count per spike. Variation in number of bracts per plant might be due to its intrinsic factor (Kannan et al., 2019; Auclar et al., 2022).

Internodal length between florets/bract ranged from 0.93 cm (Jamaican dwarf) to 5.67 cm (Firebird). Higher bract size contributes to greater attractiveness. Highest bract width was observed in *H.* Red (4.90 cm), while, *H. psittacorum* varieties yielded smaller bracts. Light flower stem is a desirable characteristic for cut Heliconia. Thangam et al. (2014) also reported similar findings on varying width and length of bract and internodal length between florets/ bract among Heliconia genotypes.

Maximum number of flowering shoots per plant was recorded in the genotype *H.* 'Lady di' (43.33) followed by *H.* 'Golden torch' (33.00) and *H. psittacorum* 'Kathy' (32.33). The increase in spike yield might be attributed to the early flower initiation, greater production of suckers with more clumping might have resulted the production of greater number of spikes. Variations in flowering parameters in the present study might be attributed to the flowering cycle, probably related to the genetic makeup of individual genotypes and seasonality of flower production. The results of variations in spike yield are in conformity with the findings of Santhosh et al. (2018) and Meenakshi et al. (2012).

CONCLUSION

In the present investigation, the Heliconia genotypes Guyana, Tropics, Alan Carle, *H.* 'Red and *H. latispatha* produced desirable quality leaves for cut foliage purpose, while, genotypes with longer flowering duration *viz.*, Golden Torch, Lady Di, Kathy, Sassy, Petra and Strawberries and Cream were found suitable for landscaping. The genotypes *viz.*, Jamaican dwarf, Lady Di, Kathy, Strawberries and Cream, Sassy and *H. bihai* were found suitable as potted plants owing to their short and compact dense nature.

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Original Research Paper

Multivariate analysis for various agro-morphological traits of turmeric (*Curcuma longa* L.)

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ABSTRACT

Turmeric is one of the potential spice crops having importance in culinary, colouring in textiles and therapeutic in pharmaceutical industries. The present investigation was carried out to estimate the genetic diversity of 21 turmeric genotypes representing different geographical locations of India. The principal component (PC) analysis indicated that the most of the variation among the genotypes was contributed by the first two principal components (61.38%), which were largely governed by plant height, number of leaves per plant, leaf lamina length, leaf area, total leaf area, collar girth and weight of the mother rhizomes per clump. These traits showed high positive correlation with first two PCs and influenced significantly for grouping. Based on PC correlation analysis, it is evident that morphological and yield attributing traits of PC1 and PC2 are influenced and contributed for most of the variation among the genotypes. The cluster analysis revealed that the 21 genotypes fall into five clusters, and among them most divergent with distinct genotypes were cluster I, III and cluster IV. However, IISR Pragati, Rajendra Sonali and NDH 8 were found superior for fresh rhizome yield and Acc. 849 was found unique with robust mother rhizome. The present study contributes to the knowledge of genetic diversity and defining strategies for yield improvement in turmeric.

Keywords: Clustering, multivariate analysis, principal component analysis, turmeric

INTRODUCTION

Turmeric (*Curcuma longa* L.) is one of the most important species which is used as a spice, colouring agent, medicinal purposes and vital part of Asian culinary. It is a rhizomatous perennial herb from the ginger family, Zingiberaceae originated from South East Asia and distributed almost all Asian countries. India ranks first in terms of production, consumption, and export (Spices Board, 2022). Turmeric possess multiple pharmacological properties including antimicrobial, antioxidant, chemopreventive, antimutagenic, bioprotectant, anticancer, hepatoprotective, anti inflammatory, antithrombotic, hypo glycaemic properties and immune modulatory potential (Iweala et al., 2023; Jyotirmayee & Mahalik, 2022; Razavi et al., 2021).

Turmeric is mainly regarded as triploid (2n = 63) which have speculated origins by dibasic amphiploidy or secondary polyploidy. Although, turmeric is mainly propagated through rhizomes, seed set and germination was also reported (Nair & Sasikumar, 2009). The crop improvement efforts in turmeric mainly depended on identifying superior genotypes through selection, polyploidy, and mutation breeding. The genetic variability and diversity in turmeric is largely depended on collection of germplasm from different geographical locations (Chandra et al., 1997). Wide genetic diversity has been reported in turmeric (Gupta et al., 2015; Mishra et al., 2015; Prasath et al., 2016 and Aarthi et al., 2022). The turmeric yield was the most complex trait influenced by multiple factors such as genetic and environment (Prasath et al., 2014). Utilisation of conventional breeding in turmeric is seldom because the variability is mostly based on selection of germplasm and exploitation of variability. The objective of the present study was to estimate genetic diversity of traits and clustering of genotypes through multivariate analysis in turmeric.

MATERIALS AND METHODS

A total of 21 turmeric genotypes *viz.*, IISR Pragati, IISR Prathiba, IISR Alleppey Supreme, Rajendra Sonali, Megha Turmeric 1, Waigon Turmeric, Roma, CIM Pitambar, Uttar Rangini, Chhattisgarh Haldi 2, NDH 8, Co 3, Acc. 1545, Erode Local, Mydukur

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Local, Acc. 849, Acc. 379, Acc. 14, Acc. 179, Acc. 214 and Acc. 69/5/22/I_3 were collected from different geographical locations across India were used for the present study. The experiment was conducted at the ICAR-Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi (11° 36' 34" N and 75° 49' 12" E), Kozhikode, Kerala, over two seasons (2021-2022 and 2022-2023) using a randomised block design (RBD) with two replicates. Standard cultural practises were followed to grow the crop (Prasath *et al.,* 2022). Morphological and yield characters were recorded as per distinctness, uniformity and stability (DUS) guidelines of Protection of Plant Variety & Farmers Rights Authority, 2009 (PPV & FRA 2009). The observations recorded on plant height, number of leaves and tillers per plant, petiole length, leaf lamina length, leaf lamina width, leaf area, total leaf area, collar girth, number of primaries per clump, weight of primaries rhizomes per clump, number of mother rhizomes per clump, weight of mother rhizomes per clump, and fresh rhizome yield per plant (g). The data was standardised using mean and standard deviation and subjected to principal component analysis (PCA), Eigen values, biplot between PC1 and PC2, and clustering was done based on 'K means' by using R 4.3.0 software (R Core Team, 2023).

RESULTS AND DISCUSSION

The analysis of variance revealed significant difference for all the traits except number of tillers per plant. The principal component analysis indicated first four components contributed over 85% of variation for traits under study (Table 1). The PC1, PC2, PC3 and PC4 displayed 41.46%, 19.92%, 16.03% and 8.23%,

Table 1 : Loading Eigen values and variation of turmeric genotypes for different principal components

Source	Loading Eigen values				Correlation coefficients of PCs with traits		
	PC1	PC2	PC3	PC4	PC1	PC2	PC3
PH	0.326	0.189	-0.280	-0.139	0.785	0.317	-0.419
NL	0.328	0.001	0.300	-0.059	0.791	0.002	0.448
NT	-0.108	0.317	-0.240	0.422	-0.260	0.529	-0.361
PL	0.049	0.513	-0.112	0.084	0.120	0.857	-0.167
LLL	0.360	0.123	-0.169	0.079	0.868	0.206	-0.253
LLW	0.310	-0.072	-0.012	0.398	0.747	-0.120	-0.018
LA	0.388	0.040	-0.084	0.259	0.935	0.067	-0.126
TLA	0.402	0.017	0.101	0.102	0.969	0.028	0.151
CG	0.336	-0.094	0.244	-0.146	0.811	-0.158	0.366
PN	-0.050	0.365	-0.236	-0.538	-0.121	0.611	-0.353
PW	-0.117	0.303	0.518	0.096	-0.283	0.504	0.776
MN	-0.048	0.498	-0.031	0.085	-0.116	0.832	-0.045
MW	0.313	0.100	0.104	-0.469	0.753	0.166	0.156
FRY	-0.067	0.293	0.566	0.051	-0.160	0.487	0.849
Eigen values	5.81	2.79	2.24	1.15	-	-	-
S«^{2}- test P value	3.33e-16	2.11e-05	9.04e-04	2.83e-01	-	-	-
Variation (%)	41.46	19.92	16.03	8.23	-	-	-
Cumulative variation (%)	41.46	61.38	77.41	85.64	-	-	-

PH=plant height, NL=number of leaves, NT=number of tillers, PL=petiole length, LLL=leaf lamina length, LLW=leaf lamina width, LA=leaf area, TLA=total leaf area, CG=collar girth, PN=number of primaries clump^{-1}, PW=weight of primaries rhizomes clump^{-1}, MN=number of mother rhizomes clump^{-1}, MW=weight of mother rhizomes clump^{-1}, FRY=fresh rhizome yield plant^{-1}, PC=principal component

Table 2 : Rotated matrix results of agro-morphological traits of turmeric

PC1	PC2	PC3	PC4
Plant height Number of leaves per plant Leaf lamina length Leaf area Total leaf area Collar girth Weight of mother rhizomes per clump	Petiole length Number of primary rhizomes per clump Number of mother rhizomes per clump	Weight of primary rhizomes per clump Fresh rhizome yield	Number of tillers per plant Leaf lamina width

respectively. In the present study, PCA described by the first two axes suggested that the observed traits inside the axes had a significant impact among the genotypes. The first principal component accounts the most variability of 41.46% with substantial loadings which was contributed positively by plant height, number of leaves per plant, leaf lamina length, leaf area, total leaf area, collar girth and weight of the mother rhizomes per clump. It was clear that most of the morphological traits along with weight of mother rhizome per clump influenced the PC1. The PC2 was dominated by petiole length, number of primary rhizomes and mother rhizomes per clump (Table 1 & 2).

Correlation analysis was also performed to understand the relationship of each trait with principal components (Table 1). Total leaf area followed by leaf area, leaf lamina length, collar girth, number of leave, plant height, weight of the mother rhizomes, and leaf lamina width showed highest positive correlation with the PC1. On the other hand, PC2 showed maximum positive correlation with petiole length, number of mother rhizomes, number of primary rhizomes, number of tillers, weight of primary rhizomes and fresh rhizome yield per plant. While Fresh rhizome yield and weight of primary rhizomes per clump was positively correlated with PC3. Based on PC analysis, it is evident that morphological and yield attributing traits of PC1 and PC2 are influenced and contributed for most of the variation among the genotypes. Roy et al. (2011) also found that PC1 and PC2 had the most diverse components with highest share of traits.

Contribution of PC scores for all the 21 turmeric genotypes are represented in the Table 3. The maximum positive PC score was observed for the Acc. 849, followed by Roma, Acc. 1545, Waigon turmeric, Mydukur Local, CIM Pitambar and IISR Prathiba in the PC1, whereas, in PC2 it was observed maximum for genotypes Uttar Rangini followed by Acc. 69/5/22/I_3, Acc. 14, Acc. 179, Chhattisgarh Haldi 2, Acc. 379 and NDH 8. The genotypes, Megha turmeric 1, Erode Local, Co 3 and IISR Alleppey supreme recorded maximum scores for PC3. IISR Pragati and Acc. 214 recorded maximum PC score in PC4. Estimation of principal component score helps to identifying genotypes which are most influenced by a particular trait. The emphasis on these traits will pave way for precise selection of genotypes from pool of turmeric germplasm for future breeding programs.

The extent of variation correlated with each principal component is presented through Scree plot (Fig. 1), which visualizes the relation between Eigen values and principal components, amount of variation contributed by the principal components. As presented in the scree plot, the first three principal components exhibited maximum variation. The elbow curve explained the Eigen values for PC1, PC2, PC3 and PC4 which gradually decreased (5.81, 2.79, 2.24 and 1.15, respectively). Apparently, the PC1 recorded the highest variance, followed by others, as also reported in principal component analysis studies (Roy et al., 2011; Bahadur et al., 2016; Bahadur & Meena, 2016). Promising genotypes were categorized based on the highest PC scores respectively under first four principal components are presented in Table 4.

The Eigen vectors exhibits influence of each trait on the principal component axes. The length of the vector indicates the impact of each trait on the other traits, and the angle between the vectors indicates their relationship. An obtuse angle between vectors means that there is a positive relationship between the traits, while a perpendicular angle indicates a negative or no relationship. It was evident from the Fig. 2, that all the traits were divided into two

Table 3 : PCA scores of 21 turmeric genotypes

Genotype	PC1	PC2	PC3	PC4
IISR Pragati	-3.384	-1.722	-2.454	0.893
IISR Prathiba	0.248	-2.007	-0.124	-0.099
IISR Alleppey Supreme	-0.099	-0.774	1.609	0.547
Rajendra Sonali	-4.797	-1.040	-1.106	-0.510
Megha Turmeric 1	-1.795	0.009	2.350	-2.535
Waigon Turmeric	1.677	1.643	-3.296	-0.319
Roma	3.220	-2.327	0.969	1.607
CIM Pitambar	0.347	-2.506	-1.083	0.105
Uttar Rangini	0.242	2.066	1.124	1.329
Chhattisgarh Haldi 2	-0.085	1.342	0.953	0.888
NDH 8	-4.101	0.408	-1.361	-0.678
Co 3	-1.432	0.979	1.694	-0.999
Acc. 1545	1.715	-2.915	0.081	-0.331
Erode local	1.736	-0.758	1.881	-1.413
Mydukur Local	1.454	-1.400	0.165	0.271
Acc. 849	5.986	1.447	-2.169	-1.775
Acc. 379	-0.421	1.309	0.345	0.324
Acc. 14	-0.782	1.953	0.782	1.077
Acc. 179	-0.483	1.361	0.206	0.454
Acc. 214	1.049	0.893	0.192	1.312
Acc. 69/5/22/I3	-0.294	2.041	-0.758	-0.148

Table 4 : Selected genotypes based on highest positive PC scores in each of 4 components

PC1	**PC2**	**PC3**	**PC4**
IISR Prathiba	Uttar Rangini	IISR Alleppey Supreme	IISR Pragati
Waigon Turmeric	Chhattisgarh Haldi 2	Megha Turmeric 1	Acc. 214
Roma	NDH 8	Co 3	
CIM Pitambar	Acc. 379	Erode local	
Acc. 1545	Acc. 14		
Mydukur Local	Acc. 179		
Acc. 849	Acc. 69/5/22/I_3		

groups where total leaf area, leaf area, leaf lamina length, number of leaves per plant, mother weight, plant height and collar girth had significant effect on first two principal components. According to the loading plot (Table 1), total leaf area showed a significant level of variance, followed by leaf area on positive side, whereas on negative side weight of primary rhizomes per clump followed by number of tillers per plant for PC1. The present results are in line with the reports of Roy et al. (2011) and Bahadur et al. (2016). The results suggest that the focus on traits which positively influence fresh rhizome yield will be a key factor for future crop improvement in turmeric.

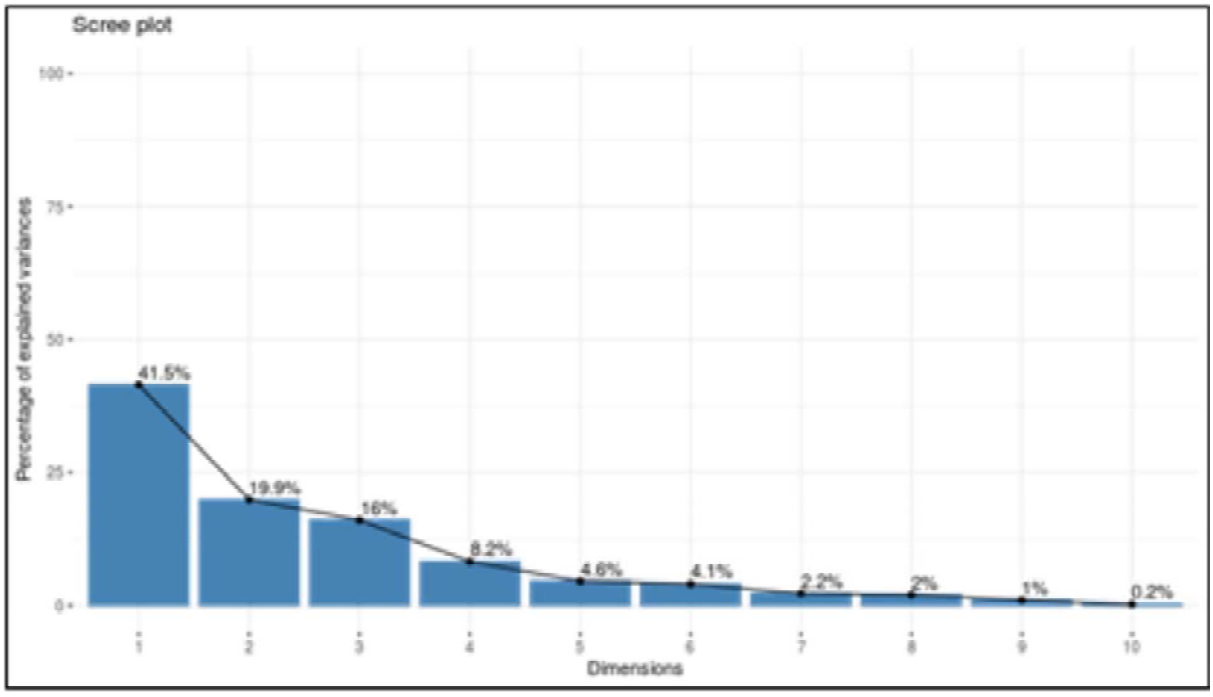
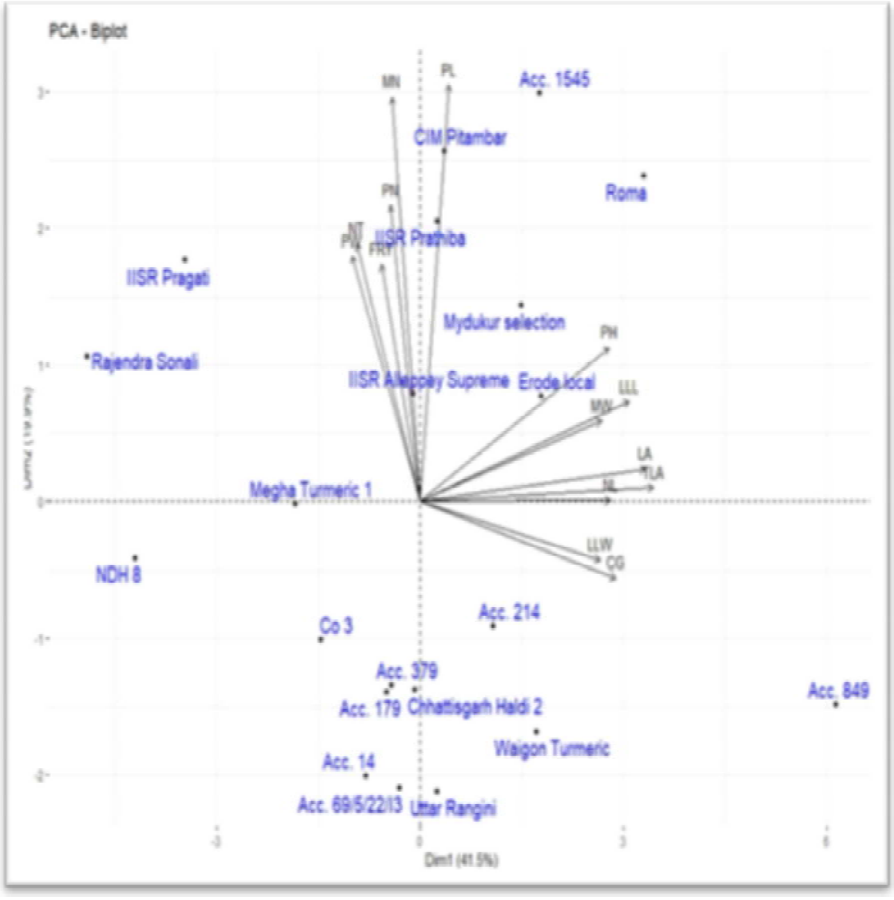


Fig. 1 : Scree plot and threshold Eigen values for agro-morphological traits 21 turmeric genotypes in principal component analysis



PH=plant height, NL=number of leaves, NT=number of tillers, PL=petiole length, LLL=leaf lamina length, LLW=leaf lamina width, LA=leaf area, TLA=total leaf area, CG=collar girth, PN=number of primaries clump^{-1}, PW=weight of primaries rhizomes clump^{-1}, MN=number of mother rhizomes clump^{-1}, MW=weight of mother rhizomes clump^{-1}, FRY=fresh rhizome yield plant^{-1}

Fig. 2 : Distribution and grouping of genotypes and variables across first two principal components

The clustering of genotypes was done based on k means and 21 genotypes were grouped into five clusters (Fig. 3) based on the similarity of genotypes for morphological and yield traits. The following are the details of the five clusters:

Cluster I: Acc 849

Cluster II: Waigon turmeric, Uttar Rangini, Chhattisgarh Haldi-2, Acc. 379, Acc. 14 Acc. 179, Acc. 214 and Acc. 69/5/22/I_3

Cluster III: IISR Pragati, Rajendra Sonali and NDH 8

Cluster IV: Megha Turmeric 1, IISR Alleppey Supreme and Co 3

Cluster V: IISR Prathiba, Roma, CIM Pitambar, Acc 1545, Erode local and Mydukur Local.

The cluster analysis results showed that the genotypes in cluster I was primarily categorised by the weight of mother rhizome per clump, while, cluster II was characterized by leaf lamina length. Cluster III was mainly contributed by fresh rhizome yield, however, cluster IV distinguished by the number of tillers, collar girth, and leaf lamina width and cluster V by the number of primaries and weight of mother rhizomes. Our morphological data also supports the clustering well. The most divergent clusters are, Cluster I, III and IV, whereas, cluster I have only one genotype and other two clusters having three genotypes each that differing significantly for morphological and yield

Fig. 3 : Grouping of 21 turmeric accessions using clustering based on K means

Each number 1-21 denotes respective genotype *viz.,* 1-IISR Pragati, 2-IISR Prathiba, 3-IISR Alleppey Supreme, 4-Rajendra Sonali, 5-Megha Turmeric 1, 6-Waigon Turmeric, 7-Roma, 8-CIM Pitambar, 9-Uttar Rangini, 10-Chhattisgarh Haldi 2, 11-NDH 8, 12-Co 3, 13-Acc. 1545, 14-Erode Local, 15-Mydukur Local, 16-Acc. 849, 17-Acc. 379, 18-Acc. 14, 19-Acc.179, 20-Acc. 214 and 21-Acc.69/5/22/I_3.

traits compared to the other clusters. The present findings are consistent with previous studies (Bahadur et al., 2016; Bahadur & Meena, 2016; Aswathi et al., 2022; Vithya et al., 2022), where, similarly by homogeneity of genotypes based on similarity of traits.

CONCLUSION

Considerable amount of genetic diversity is present among the turmeric genotypes which will be useful for future crop improvement programs in turmeric. Principal component analysis and cluster analysis suggests that most of the variation is contributed from the first two principal components. Traits such as total leaf area, leaf area, number of mother rhizomes and petiole length had significant effect on first principal component. Among the five clusters, most divergent clusters with distinct genotypes such as IISR Pragati, Rajendra Sonali and NDH 8 which are yielding superior fresh rhizome yield and Acc. 849, later was unique one with distinct and robust mother rhizomes. The genotypes from the diverse clusters having superior performance for desirable traits could be selected for as parents in hybridization.

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Original Research Paper

Isolation and characterization of microsatellite markers from *Garcinia morella* using next generation sequencing technology and cross-species amplification

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ABSTRACT

The fruit-bearing medicinal tree, *Garcinia morella*, grows in the tropical rain forests of India's Western Ghats, Indo-Chinese Himalayan regions and Sri Lanka. Its fruit rinds are used as a garnish and in seasoning during food preparation. In order to genetically exploit it and assess diversity, development of microsatellite markers was attempted. We partially sequenced genomic DNA using the Illumina Hiseq 2000 platform and examined sequence data for microsatellite loci. We obtained high-quality 10653 Mbp data and was assembled into 1613263 contigs. A total of 121199 SSRs were discovered, Di nucleotide repeats were predominant (42.5%), followed by mono and tri nucleotide repeats (30.4 and 7.9%, respectively). We were able to design primers for 52901 microsatellites. Genetic analysis of 48 SSR loci, showed PIC values ranging from 0.067 to 0.939 with a mean value of 0.7547. The allele per locus ranged from 2 to 24 with a mean of 13. These microsatellite markers can be employed for genetic diversity analysis, molecular characterization and mapping different traits.

INTRODUCTION

Garcinia morella (Gaertn.) Desr., family Clusiaceae, is commonly recognized as 'Indian gamboges' or 'Ceylon gamboges.' This tree is found in the tropical rain forests of the Western Ghats in India, Sri Lanka, and the Indo-China Himalayan regions. Its habitat spans various regions, including the forests of Eastern Bengal, Khasi Mountains, and both Western and Eastern Peninsular India. It particularly thrives in the rain forests of Karnataka state within the Western Ghats (Parthasarathy et al., 2013a). *G. morella* is a multipurpose tree grown as a plantation crop along with *Garcinia indica* (Kokum) and *Garcinia cambogia* (Malabar tamarind). The fruit rinds are used as a condiment and a garnish. From *G. morella,* many bioactive compounds are isolated from the fruits and the bark such as moreollin (Subba Rao et al., 1978) and gambogic acid (Tang et al., 2011), respectively, which are evaluated for their antibiotic and anticancer properties. The yellow color from the gamboge is used as dye to draw paintings on traditional house walls.

To ensure the efficient utilization and conservation of germplasm, it is essential to gain precise understandings of genetic relationships and diversity. In light of this, the development of markers, especially microsatellite markers, emerges as a valuable technique to evaluate genetic diversity, interpopulation relationships, and molecular characterization. The development of molecular markers would help in the construction of genetic linkage map, enable the study of trait inheritance, and subsequently enable the pinpointing of markers closely linked to vital agronomic traits. (Bohra et al., 2011). Till date, there are no reports on development of microsatellite markers for *G. morella*. Here, we report the partial sequencing of *G. morella* genome, *de novo* assembly and identification of SSR markers.

MATERIALS AND METHODS

Plant material

The total genomic DNA from *G. morella* was used for genome sequencing. The plant material was obtained from the germplasm collection of the College of Forestry, Sirsi, Karnataka, India.

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Genome sequencing and assembly

High quality genomic DNA was isolated from the leaves of selected *G. morella* genotypes using modified CTAB method (Ravishankar et al., 2000). Total genomic DNA was sequenced using next generation sequencing Illumina HiSeq2000 platform at M/s Genotypic Pvt. Ltd, Bengaluru facility. Raw data obtained was processed for quality control. High quality data were used for assembly into contigs. *De novo* assembly of reads into contigs was performed using SOAPdenovo2-src-r240 software (Luo et al., 2012).

Survey, identification and design primers for genomic SSR markers

All assembled scaffolds were screened for the presence of SSRs using MISA software (http://pgrc.ipk-gatersleban.de/misa). MISA files were transferred to Microsoft Excel where SSRs were classified into mono-, di-, tri-, tetra-, penta- and hexa-nucleotide and compound repeats. Primer pairs flanking the repeats were designed using Primer 3 software (http://wwwgenome.wi.mit.edu/genomesoftware/other/Primer3.html) (Koressaar & Remm, 2007).

PCR standardization and Genotyping

Genomic DNA of thirty *G. morella* genotypes was adjusted to a final concentration of 25 ng/µl separately. A total of 50 SSR primers was selected randomly and was synthesized with M13 tail. These M13 tailed primers were first screened for amplification using pooled total genomic DNA from five randomly selected genotypes. Fluorescence based M13 tailing PCR method of Schuelke (2000) was used to amplify the microsatellites in a quick, accurate and efficient manner. PCR was carried out in the 20 µl reaction volume containing 2 µl of 10X reaction buffer, 2.0 µl of 1 mM dNTPs, 0.9 µl (5 pmol) of forward, 0.9 µl reverse primers (5 pmol), labeled M13 probe 1.2 µl (5 pmol), 5.0 µl (50-75 ng) of template genomic DNA, 0.8 µl (2 U) of Taq DNA polymerase and 7.2 µl of nuclease free water. The PCR cycling profile was: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec., 55°C for 30 sec., 72°C for 1 min and a final extension at 72°C for 5 min. The primers were employed for amplification of thirty genotypes and two related species *Garcinia indica* and *Garcinia gummigutta*. These PCR products were separated on the automatic 96 capillary automated DNA Sequencer (Applied Biosystems, ABI 3730 DNA Analyzer) at Eurofins facility, Bengaluru.

Genetic analysis of SSR markers

The raw data generated were analyzed and compiled using Peak Scanner V1.0 software (Applied Biosystems, USA) for detecting the allele size in bp**.** The results obtained were used for genetic analysis using Cervus 3.0 software (Kalinowski et al., 2007). The number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and Polymorphic information content (PIC) were estimated. The probability of identity (PI) was calculated using IDENTITY 3.0 software (Wagner & Sefc, 1999).

RESULTS AND DISCUSSION

Sequence analysis, assembly, SSR identification and primer design

The NGS technology platform IlluminaHiseq 2000 was used for sequencing genomic DNA from *G. morella*. The sequencing run yielded 311687120075 bases from 118.7 millinon reads. Low quality reads were filtered out. Finally, around 11254 Mbp paired end reads were obtained. Using SOAPdenovo2-src-r240 (Luo et al., 2012) software, assembly optimization was done. Assembly with Kmer-63 was selected, as it is having the optimal reading for N50. This has resulted in 16,13,263 contigs with total assembly size 632, 848,976 bases. The weighted mean assembly size in scaffold (N50) was 426 bp. The total assembled size of the contigs was 632.8Mbp (Table 1).

Table 1 : Sequence analysis of *G. morella* genome

Sequence details	1613263
Total number of contigs	161326
Total number of examined sequences (bp)	632848976
Total number of identified SSRs	121199
Number of SSR containing sequences	111109
Number of SSR containing more than 1 SSR	9112

An SSR survey of genomic sequences using MISA software (http://pgrc.ipk-gatersleban.de/misa), revealed that 1613263 contigs contained 121199 SSR markers. The 2777 SSRs were present on CDS. Among the identified SSR repeats, the direpeats were the most abundant, accounting for 42.5% of total SSRs, followed by mono-repeats (30.4%), tri-repeats (7.9%), tetra-repeats (2.02%), penta-repeats (0.9%), and hexa-repeats (0.2%) and compound-repeat (16.3%) nucleotide types (Table 2, Fig. 1). Among the

repeat motifs of the di-nucleotide, the AT and TA repeat was the most common, and the predominant motifs of tri-nucleotide AAT and TTA (Table 3). AT-rich repeats were also more common repeats in tetra-, Penta- and hexanucleotide SSRs. Primers were designed for 52901 SSRs. The results of mapping SSR markers to coding sequences showed that 2777 SSR markers mapped on to the coding sequences. Among the repeat types mapped, trinucleotide repeats form predominant SSRs (68.9 %) on coding sequences (Table 3).

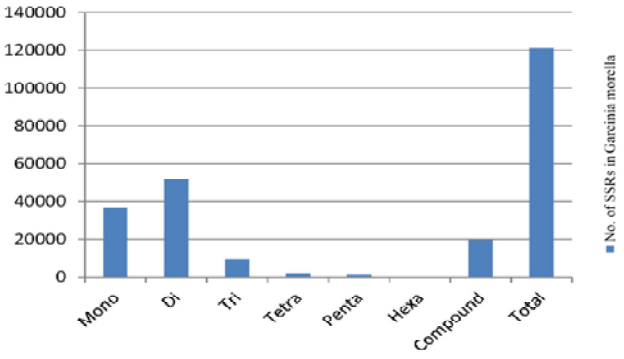

Fig.1 : Distribtuion of SSRs in *Garcinia morella* genome

Table 2 : Simple sequence repeat types in the *G. morella* contigs sequences

Motif length	Number of SSRs	Frequency (%)
Mononucleotide	36840	30.4
Dinucleotide	51569	42.5
Trinucleotide	9630	7.9
Tetra nucleotide	1893	1.6
Penta nucleotide	1110	0.9
Hexanucleotide	297	0.2
Complex/compound	19680	16.3
Total	121199	

Table 3 : Distribution of SSR mapped on coding sequences

Motif length	Number of SSRs	Frequency (%)
Mononucleotide	367	13.2
Dinucleotide	291	10.5
Trinucleotide	1916	68.9
Tetra nucleotide	2	0.007
Penta nucleotide	10	0.36
Hexanucleotide	26	0.93
Complex/compound	165	16.3
Total	2777	-

Genetic analysis and transferability of genomic SSRs

Genetic analysis of 48 SSR markers, using 30 accessions showed PIC values ranging from 0.067 to 0.939 with a mean value of 0.7547. The mean values of observed and expected heterozygosity were 0.2026 and 0.7852, respectively. The allele per locus ranged from 2 to 24 with a mean of 13. The probability of identity values ranged from 0.0058 to 0.7742 with a mean value of 0.1018 (Supplementary Table 4, and 5). The total probability of identity was 5.666690e-068. In cross species amplification, four out of 48 SSR primers amplified in *G. indica* and 39 amplified in *G. gummigutta* (Table 6).

Table 4 : Summary of genetic analysis

Parameter	Mean	Range
Polymorphic information content (PIC)	0.7547	0.067- 0.939
Observed heterozygosity (Ho)	0.2026	0.000- 0.760
Expected heterozygosity (He)	0.7852	0.07- 0.959
Allele per locus	13	2- 24
Probability of identity (PI)	0.1018	0.0058- 0.7742

Total number of alleles is 624; total probability of identity is 5.666690e-068

Table 5 : Cross species amplification of *G. morella* SSR primers with *G. indica* and *G. gummingutta*

Locus Name	Species	
	G. indica	*G. gummigutta*
GM_KVRw990	A	NA
GM_KVRx003	A	NA
GM_KVRx004	NA	A
GM_KVRx022	NA	A
GM_KVRx038	NA	A
GM_KVRx045	A	NA
GM_KVRx046	A	NA
GM_KVRx047	NA	A
GM_KVRx048	NA	NA
GM_KVRx049	NA	NA

GM_KVRx075	NA	NA
GM_KVRx076	NA	NA
GM_KVRx077	NA	NA
GM_KVRx090	NA	A
GM_KVRx091	NA	A
GM_KVRx092	NA	A
GM_KVRx093	NA	A
GM_KVRx094	NA	A
GM_KVRx095	NA	A
GM_KVRx133	NA	A
GM_KVRx134	NA	A
GM_KVRx135	NA	A
GM_KVRx432	NA	A
GM_KVRx433	NA	A
GM_KVRx434	NA	A
GM_KVRy205	NA	A
GM_KVRy206	NA	A
GM_KVRy207	NA	A
GM_IIHRb888	NA	A
GM_IIHRb889	NA	A
GM_IIHRg191	NA	A
GM_IIHRg192	NA	A
GM_IIHRt169	NA	A
GM_IIHRx419	NA	A
GM_IIHRx420	NA	A
GM_IIHRx421	NA	A
GM_IIHRx536	NA	A
GM_IIHRx537	NA	A
GM_IIHRz510	NA	A
GM_IIHRz511	NA	A
GM_IIHRz512	NA	A
GM_IIHRz572	NA	NA
GM_MRDa479	NA	A
GM_MRDa828	NA	A
GM_KVRa040	NA	A
GM_KVRa041	NA	A
GM_KVRa042	NA	A
GM_KVRa325	NA	A

A: amplified; NA: not amplified

Although *G. morella* holds economic significance as a tree species, studies regarding its genetic diversity are limited. Parthasarathy et al. (2013a) have compared the effectiveness of the inter-simple sequence repeats (ISSR) and RAPD profiling of 12 species of Indian *Garcinia* and showed that combined information of ISSR and RAPD could be a better tool to assess the diversity of Garcinia. Parthasarathy et al. (2013b) have reported that lack of awareness, coupled with habitat destruction, is leading to genetic erosion of *Garcinia morella* and other related species.

At present, the microsatellite markers are most widely used molecular marker for the analysis of diversity, genetic relatedness, mapping, crop breeding programs and population genetics (Varshney et al., 2005; Ravishankar et al., 2015a). SSR markers are highly reproducible, multi-allelic, PCR based, highly polymorphic, easy to use and amenable for automation (Ravishankar et al., 2015b & 2015c). However, the use of microsatellite markers for studying non-model species like wild *G. morella* has been impeded by a lack of available sequence information and genetic studies. Earlier, the detection of genomic SSRs and subsequent conversion to markers was expensive and time-consuming; involving the construction and screening of microsatellite enriched genomic DNA libraries (Glenn & Schable 2005; Ravishankar et al., 2011). Compared to this hybrid capture method using probes, the present NGS based method is fast, simple, and overcomes a number of technical difficulties. The advent of next-generation sequencing technologies, such as pyrosequencing, has made this process less complicated and easy (Zalapa et al., 2012; Ravishankar et al, 2018). As a result, a large number of SSR markers can be developed in short span of time and at a lower cost. This approach is especially useful for many tree crops where there are no available SSR markers.

In the present study high-throughput pyrosequencing Illimina platform Hiseq 2000 was used to develop genomic SSR markers in *G. morella*. The assembly of reads of the long sequences 16,13,263 contigs covering 632 Mb of the genome (Table 1). It was observed that di-repeats were predominant in nature accounting for 42.5%, followed by mono- (30.4%) and tri- (7.9%) repeats (Table 2). The mono, di and tri nucleotide repeats contributed to the major proportion of SSRs (80.8%) and very small share was contributed

by tetra, penta and hexa nucleotide repeats (Table 2). Among the repeat motifs of the di-nucleotide, the AT and TA repeat was the most common, and the predominant motifs of tri-nucleotide AAT and TTA. Among the SSRs mapped to coding sequences, the tri-nucleotide repeats were in very high frequency in *G. morella* (68.9%). This was also observed by Lawson et al. (2006) in both monocots (Rice- 64%) and dicots (Arabidopsis- 65.4%) in coding regions. The abundance of tri-nucleotides in coding regions is hypothesized to be the result of purifying selection which eliminates any SSRs causing frame shift mutations. However, it is unknown if selection is involved in the distribution of SSR types in genomic DNA (Celik et al., 2014). A similar trend was observed in other Garcinia species, *G. indica* (Ravishankar et al., 2021) and *G. gummigutta* (Ravishankar et al., 2017).

It was hypothesised that the dominant presence of a repeat motif with a specific sequence and length in the plant genome arises from the selection pressure exerted on that motif during its evolution. However, the molecular origins and development of microsatellites remain unclear. Replication slippage, considered the most common mutation mechanism, involves the addition or removal of motifs. Other processes, such as duplication events, uneven crossing over, and nucleotide substitution, may also contribute to microsatellite variation. However, these processes cannot explain the species-specific accumulation of certain motif repetitions (Sonah et al., 2011).

Genetic analysis and transferability of genomic SSR markers

In this study, 48 out of 50 SSR primers examined, amplified PCR products for *G. morella*. A high rate of successful amplification can be due to high-quality sequence data and the appropriate primer parameters, such as high GC content. In the present study, the genomic SSR markers exhibited a high degree of variation, with an average PIC value of 0.7547. Several of the genomic SSR markers amplified multiple alleles (averaging 13 per loci), as detailed in Supplementary Tables 4, and 5. This pattern could be attributed to the substantial heterozygosity within the species, which resulted in multiple number of alleles. Previous investigations employing RAPD markers revealed a substantial molecular diversity, with the heterogeneity index across species ranging from 0.81 to 0.82 in four specific species: *G. gummigutta, G. indica, G. cowa, and G. xanthochymus* (Parthasarathy et al., 2013b). In the present study, 35 SSR markers (72.9%) had more than 10 alleles per locus, indicating that high heterozygosity and diversity of accessions were used (Supplementary Table 4, and 5). The probability of identity (PI-the probability that two randomly selected diploid genotypes would be identical, assuming observed allele frequencies and random assortment) is very low for many loci (mean =0.1018). These low PI values confirm their applicability and use in DNA fingerprinting. Thus, these SSR markers can be easily employed for genotyping elite individuals. In cross species amplification using *G. indica* and *G. gummi-gutta* species, these SSR markers showed a relatively high percentage of amplification in *G. gummi-gutta* (81.25%) and low in *G. indica* (8.33%) (Table 6).

The present work describes the pyrosequencing-based approach for identifying and analysing microsatellite markers from *G. morella's* partial genome sequences. The *G. morella* genome sequence was thoroughly examined, and a large number of SSRs were found. The results from this study is an invaluable set of molecular markers that is essential for genetic research, genotyping, and the conservation studies on *Garcinia morella.*

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Original Research Paper

Yield sustainability through micronutrient management in guava

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ABSTRACT

A field study conducted with soil and foliar application of $ZnSO_4$ and borax at different phenological stages of guava cv. Shewta in sandy loam soil, revealed that highest sustainable yield index (0.80) was recorded with two sprays of 0.4% $ZnSO_4$ + 0.2% borax during fruit growth at one month interval, followed by 0.63 with three sprays of 0.4% $ZnSO_4$ + 0.2% borax before flowering, fruit set and during fruit growth. Highest TSS (13.13°Brix), acidity (0.652%) and ascorbic acid (262.21 mg/100 g) was recorded in two sprays of 0.4% $ZnSO_4$ + 0.2% borax at fruit growth in one month interval. Significant differences in Zn and B content in guava fruit pulp was recorded. It was noted that Guava fruit pulp had Zn content of 13.7 mg kg^{-1} in control tree fruits while 14.6 to 16.5 mg kg^{-1} in treated trees. Moreover, guava fruit pulp enriched with B (14.3 to 17.3 mg kg^{-1}) in treated tree fruits as compared to 12.4 mg kg^{-1} in control trees. Micronutrient contents in leaf tissues showed significant difference in Zn and B concentration, whereas, Fe, Mn and Cu contents were statistically non-significant. The index suggested for attaining the sustainability and to economize the nutrient application, technology package consisting of two sprays of 0.4% $ZnSO_4$ and 0.2% borax during fruit growth at one month interval should be adopted at growers' field.

Keywords: Fruit pulp, guava, micronutrient, sustainability index, yield

INTRODUCTION

Horticulture ecosystem not only provides the nutritional security but also equally important for providing ecosystem services. Fruit crops like guava provides ample amount of minerals and other nutrients for human health. However, its productivity, sustainability and nutrient content will depend on a number of edaphic, management and tree factors. In fact, the fruit productivity is a dependent factor and soil-tree-climate interactions act as independent factors. The guava productivity is also affected under different types of soil, management and resource conservation practices. Morales-Sillero et al. (2009) described the soil-tree response in olive productivity, while, Barne et al. (2011) observed positive effect of integrated nutrient management on guava yield and quality component by using NPK+Azotobacter+ PSB+FYM, and various crop regulation methods gives positive impacts on the guava sustainability (Das et al., 2007). However, Patel et al. (2013) reported significant difference in quality attributes during fruit growth and maturity.

In fact, the precise sustainability depends on the crucial factor of varietal response to the resource management. Adak et al. (2019a) recommended the need of developing soil nutrient index for precise nutrient management in orchards. The ecological significance is also acts as precursor for supporting the robust life cycling of trees considering micronutrient distribution pattern based on vast area (Adak et al., 2019c). Kumar et al. (2017) recommended green manuring and precision farming in mango, and soil & tree health management in guava for enhancing the sustainable yield index (Adak et al., 2020). The present investigation was, thus, laid out with the objective of assessing the sustainable yield index in guava along with nutrient dynamics in fruit pulp under the influence of micronutrient spray at different critical developmental stages of guava.

MATERIALS AND METHODS

Field experiment with soil and foliar application of micronutrients on guava cv. Shewta (10-11 years old) was conducted on sandy loam soils at experimental farm of ICAR-Central Institute of Sub-tropical Horticulture, Rehmankhera, Lucknow during 2015 to

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2017. Maintenance of crop field was carried out as per standard cultural practices. Third pairs of leaves as an index leaf during March were collected for assessing the leaf micronutrient status. Initial soil DTPA extractable Fe, Mn, Zn and Cu were estimated and found to be 3.56, 1.52, 0.18 and 0.24 mg kg^{-1}, respectively, while, leaf samples had 94, 51, 8.0 and 8.0 mg kg^{-1} of Fe, Mn, Zn and Cu, respectively. Leaf 'B' content was 22.4 mg kg^{-1}. Six treatments viz., T_1: 3 sprays of 0.4% $ZnSO_4$ + 0.2% borax before flowering, after fruit set and during fruit growth, T_2 : 2 sprays of 0.4% $ZnSO_4$ + 0.2% borax after fruit set and during fruit growth, T_3: 2 sprays of 0.4% $ZnSO_4$ + 0.2% borax during fruit growth at 1 month interval, T_4 : 2 sprays of 0.4% $ZnSO_4$ + 0.2% borax before flowering and during fruit growth, T_5 : soil application of 200 g $ZnSO_4$ + 50 g borax/tree just before flowering and T_6: control, were imposed every year as soil application in September-October and spraying at critical stage wise i.e. flowering, fruit set and development, in randomized block design with four replications. Fruit yield and fruit quality attributes *viz.*, acidity (%), TSS (°Brix), ascorbic acid (mg/100 g) and micronutrient content (Fe, Mn, Zn and Cu) recorded and analyzed during 2015-16 and 2016-17. Atomic absorption spectrophotometer (model 'Chemito'AA203D) was used to estimate the micronutrient contents. Quality attributes was estimated as per the standard procedures (Ranganna, 2001). Sustainable yield index (SYI) was calculated based on Singh et al. (1990) and also to identify the best treatment so that farmers get best option for enhancing yield in sandy loam soils of guava growing region.

$$SYI = (Y - \sigma_{n-1})/Y_m$$

Where, Y: average annual fruit yield and Y_m: maximum yield recorded in a given set of treatments from all years; σ_{n-1}: standard deviation.

Statistical significance of data and standard error of mean for yield and quality attributes, micronutrient concentration of guava fruit pulp and leaves was carried out using OPSTAT (Sheoran et al., 1998).

RESULTS AND DISCUSSION

Significant positive effect of soil and foliar applications of micronutrients at different phenological stages on guava fruit yield and fruit quality (Table 1) inferred improvement in fruit yield from 16.3 to 39.12 kg $tree^{-1}$ (control) and 26.28 to 73.57 kg $tree^{-1}$ (T_3), respectively during 2015-16 and 2016-17. The treatments and seasonal effects may be responsible for such yield enhancement. The role of Zn and B nutrition on TSS, acidity and ascorbic acid was also evidenced. In control fruit trees, lowest content of TSS (11.53, 11.78), acidity (0.548, 0.564), and ascorbic acid (239.81, 247.02) was recorded. Higher fruit quality attributes was observed in T_1 to T_3 treated guava trees where Zn and B was applied at different critical phenological stages. The SYI (sustainable yield index) in guava varied from 0.22 to 0.28. The highest SYI (0.80) was recorded in the treatment T_3 (Table 2). The index concluded that for maintaining the stability in guava yield, T_3 treatment should be practiced at farmers' field. Further the CV data also showed the yield variability across the treatments within the guava orchards. Standard deviations and associated univariate statistics of yield data practically suggested for wide spread variability.

In order to assess the effect of nutrient enrichment treatments on fruit pulp and leaf tissues of guava trees, statistical significance was observed in Zn and B concentrations (Table 3). Greater Zn concentrations (16.10 to 16.75 and 13.25 to 16.50 mg kg^{-1}) in fruit pulp of guava trees were recorded across T_1 to T_3 treatments, whereas, lower contents (13.00 to 14.43 mg kg^{-1}) under control plot (T_6) was recorded. Similarly, lowest B concentration (11.40 to 13.43 mg kg^{-1}) in fruit pulp of control plots and higher contents (15.38 to 16.45 and 15.95 to 18.15 mg kg^{-1}) in T_1 to T_3 treatments was recorded. The analysis inferred greater Zn and B content in guava fruit pulp.

Non-significant difference in foliar micronutrient concentration was evidenced in case of Fe, Mn and Cu (Table 4). The B content varied between 23.30 to 25.70 mg kg^{-1} in leaf tissues of T_6 treatment, while, 43.63 to 47.68 and 34.15 to 42.28 mg kg^{-1} in T_1 to T_3 treatments. Likewise, Zn concentration of 19.23 to 25.43 and 53.90 to 59.28 and 32.60 to 52.65 mg kg^{-1} was recorded in T_6 and T_1 to T_3 treated plots. Interestingly, T_4 and T_5 treatments showed lower yield, SYI, nutrient concentrations as compared to T_1, T_2 and T_3 treatments but greater than control plot (T_6). Greater nutrient build up (Zn and B) in the T_3 treatment was obtained because of two sprays during fruit growth times at one month interval. This may have facilitates nutrient penetration in the fruits. The economic calculations based on prices of $ZnSO_4$ and

Table 1 : Effect of phenological stage wise application of micronutrients on fruit yield and quality of guava

Treatment	Fruit yield (kg/tree)			TSS (°Brix)			Acidity (%)			Ascorbic acid (mg/100 g)		
	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean
T_1- Three sprays of 0.4% $ZnSO_4$+0.2 % borax before flowering, fruit set and during fruit growth	26.03	59.86	42.95	12.70	11.85	12.28	0.632	0.579	0.61	252.28	252.28	252.28
T_2- Two sprays of 0.4% $ZnSO_4$+0.2 % borax after fruit set and during fruit growth	24.82	62.04	43.43	12.83	12.10	12.47	0.628	0.615	0.62	252.87	256.37	254.62
T_3- Two sprays of 0.4% $ZnSO_4$+0.2 % borax during fruit growth at one month interval	26.28	73.57	49.93	13.13	12.40	12.77	0.616	0.652	0.63	259.88	262.21	261.05
T_4- Two sprays of 0.4% $ZnSO_4$+0.2 % borax before flowering and during fruit growth	20.58	49.39	34.98	12.63	12.13	12.38	0.648	0.584	0.62	244.85	254.04	249.45
T_5- Basal application of 200 g $ZnSO_4$+50 g borax/ tree just before flowering	19.28	46.26	32.77	12.73	11.95	12.34	0.632	0.584	0.61	247.44	252.87	250.16
T_6- Control	16.30	39.12	27.71	11.53	11.78	11.66	0.548	0.564	0.56	239.81	247.02	243.42
CD (P=0.05)	6.17	15.31		0.46	0.274		NS	0.037		12.37	3.85	
SEm±	2.00	5.00		0.15	0.09		0.03	0.01		4.10	1.30	

Table 2 : Sustainable yield index and other statistical parameters of yield of guava

Treatment	SYI		sd		Skewness		Kurtosis		CV (%)	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1- Three sprays of 0.4% $ZnSO_4$+0.2 % borax before flowering, fruit set and during fruit growth	0.28	0.63	5.7	13.2	0.32	0.32	-2.67	-2.67	22.1	22.1
T_2- Two sprays of 0.4% $ZnSO_4$+0.2 % borax after fruit set and during fruit growth	0.25	0.62	6.5	16.3	0.62	0.62	1.27	1.27	26.3	26.3
T_3- Two sprays of 0.4% $ZnSO_4$+0.2 % borax during fruit growth at one month interval	0.28	0.80	5.4	15.1	-0.28	-0.28	-3.82	-3.82	20.5	20.5
T_4- Two sprays of 0.4% $ZnSO_4$+0.2 % borax before flowering and during fruit growth	0.23	0.55	3.8	9.1	-0.05	-0.05	-2.06	-2.06	18.4	18.4
T_5- Basal application of 200g $ZnSO_4$+50 g borax/ tree just before flowering	0.23	0.54	2.7	6.5	-1.12	-1.12	1.98	1.98	14.1	14.1
T_6- Control	0.22	0.52	0.5	1.1	0.47	0.47	-3.23	-3.23	2.8	2.8

Table 3 : Effect of phenological stage wise application of Zn and B on micronutrient content of guava fruits

Treatment	Micronutrient concentration (mg kg^{-1}) in fruit pulp									
	Fe		Mn		Zn		Cu		B	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1- Three sprays of 0.4% $ZnSO_4$ + 0.2 % borax before flowering, fruit set and during fruit growth	117.4	117.50	8.25	7.50	16.75	13.25	6.43	6.25	15.53	17.40
T_2- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax after fruit set and during fruit growth	114.9	112.50	7.78	7.25	16.10	15.25	6.25	6.75	15.38	15.95
T_3- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax during fruit growth at one month interval	119.8	120.00	7.68	8.25	16.50	16.50	6.38	7.75	16.45	18.15
T_4- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax before flowering and during fruit growth	115.4	117.25	7.65	7.00	15.70	14.50	6.08	6.50	14.88	13.73
T_5- Basal application of 200g $ZnSO_4$ + 50 g borax/ tree just before flowering	113.3	115.75	7.40	6.50	15.38	13.75	5.95	6.00	17.23	13.13
T_6- Control	115.3	108.25	8.18	6.50	14.43	13.00	5.83	5.00	11.40	13.43
CD (P=0.05)	NS	5.6	NS	1.0	1.26	1.75	NS	1.4	2.86	2.41
SEm±	1.24	1.84	0.23	0.33	0.41	0.57	0.32	0.46	0.94	0.79

Table 4 : Effect of phenological stage wise application of Zn and B on micronutrient content of guava leaves

Treatment	Micronutrient concentration in leaves (mg kg^{-1})									
	Fe		Mn		Zn		Cu		B	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1- Three sprays of 0.4% $ZnSO_4$ + 0.2 % borax before flowering, fruit set and during fruit growth	247.3	358.8	124.4	209.5	53.90	32.60	6.38	11.00	44.93	37.83
T_2- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax after fruit set and during fruit growth	248.0	368.3	120.0	189.3	54.83	36.93	6.95	11.50	47.68	34.15
T_3- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax during fruit growth at one month interval	253.8	363.0	124.1	189.0	59.28	52.65	6.93	13.00	43.63	42.28
T_4- Two sprays of 0.4% $ZnSO_4$ + 0.2 % borax before flowering and during fruit growth	267.8	354.8	123.0	178.0	53.55	31.10	7.83	12.50	40.85	29.68
T_5- Basal application of 200g $ZnSO_4$ + 50 g borax/tree just before flowering	256.8	366.3	125.8	172.5	26.75	27.10	6.98	12.25	36.43	26.28
T_6- Control	261.8	340.0	120.7	170.5	19.23	25.43	6.05	13.00	23.30	25.70
CD (P=0.05)	NS	NS	NS	NS	6.57	16.35	NS	NS	9.6	11.2
SEm±	7.5	21.2	4.1	14.4	2.2	5.4	0.49	0.9	3.2	3.7

borax, laboures for treatments application along with guava selling process @ Rs. 20/- per kg of fruit was analyzed. In control plot, net profit of Rs. 524.2 per tree, while, in other spraying treatments (T_1 to T_4) was Rs. 649.3 to Rs. 948.3 and soil application (T_5) recorded Rs. 649.3 per tree. Therefore, in order to economize the micronutrient application and stage, T_3 treatment should be the best option for guava growers.

Assessment of yield sustainability in any agroecosystem is topmost priority as resources are getting scarce under the influence of climate change (Vittal et al., 2002). Wanjari et al. (2004) recommended the usefulness of SYI as an indicator for assessing the sustainability across systems. Actually, Neilsen et al. (2014) opined that tree performances is dependent on orchard floor management, and precise orchard managements were good enough for providing the ecosystem services (Montanaro et al., 2017) also. Adak et al. (2019b) reported the importance of adoption of advanced soil and water conservation practices in fruit orchards in order to sustain the soil and tree. The positive response of any nutrient doses and its split spray in single or in combination had significant positive effect (Ares et al., 2003). In the present field study, spraying of Zn and B during fruit growth and development was found to be beneficial for fruit growth and overall yield performance. Significant difference in pulp and leaf micronutrient content was the resultant for these types of nutrition application. Adak et al. (2018) also observed the variable content of B and K in major mango germplasm.

CONCLUSION

Robust guava cultivation needs resource allocation for the betterment of yields and quality fruit. Application of $ZnSO_4$ and borax before flowering, after fruit set and fruit growth stages enhanced the yield from 39.12 kg tree^{-1} (control) to 73.57 kg tree^{-1}. Likewise, improvement in TSS, acidity and ascorbic acid content was also observed. SYI suggested that for maintaining highest index, 2 sprays of 0.4% $ZnSO_4$ + 0.2% borax during fruit growth at monthly interval should be practiced. Farmers of Uttar Pradesh and other region should practice precise application of Zn and B for ensuring higher sustainability.

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Original Research Paper

Enhancement of tomato functional food value through nutrient supplementation with fish emulsion biostimulant

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ABSTRACT

Fish emulsion (FE) is a derivative of fish waste, commonly utilised within organic agricultural and horticultural applications, predominantly as a source of nitrogen within fertiliser regimens. However, as a biological derivative, FE is a complex of many bioactive compounds, and as such is also known to function as a biostimulant. Tomato (*Solanum lycopersicum*) is a functional food, commonly produced for consumption due to its desirable hedonic qualities and health-promoting properties associated with antioxidant phytochemicals. Accordingly, the work herein explored the potential for FE to alter the functional and hedonic measures of tomato. Results indicated that the supplementation of fertiliser regimens with FE during tomato growth significantly ($p = 0.001$) increased fruit total phenolic content by 1.25-fold, whilst not significantly impacting flavonoid content ($p = 0.418$) or fruit colour (assessed by image colour analyses). Additionally, the FE treatment did not substantially impact sensory perception of hedonic measures such as smell, taste, mouth feel, or visual appeal. Accordingly, the results herein indicate that FE is a desirable fertiliser supplement during tomato cultivation, to enhance the functional value of tomato fruits, and thereby provide enhanced health-promoting benefits from tomato consumption.

Keywords : Antioxidant, fertiliser, fish hydrolysate, organic, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most popular food crops in the world (PA Silva et al., 2019), with 189.1 Mt produced in 2021 (FAOSTAT, 2023). Whilst tomato fruits (and their derivatives) are widely consumed for their flavour, their consumption is also associated with improved health benefits such as reduced incidences of chronic diseases including cancer and cardiovascular disease (Borycka, 2017), resulting in the classification of tomatoes as a "functional food" (Viskelis et al., 2015). Key functional compounds within tomato fruits are carotenoids and phenolic compounds which contribute to a range of properties, including colour (Chaudhary et al., 2018), flavour (Viskelis et al., 2015), and antioxidant activity which is associated with promotion of health (Wise et al., 2020).

Fish processing produces large amounts of waste including heads, skin, and frames (bones), which are high in protein (nitrogen) that can be made plant bioavailable after further processing (hydrolysis) into agricultural products (Madende & Hayes, 2020). Therefore, fish-based agricultural products (emulsified fish waste, *i.e.* 'fish emulsion') have become a popular organic fertiliser N supplement (Ahuja et al, 2020). Fish emulsion (FE) is hypothesised to have biostimulant properties which promote plant growth and vigour via induction of plant endogenous molecular mechanisms including N-metabolism, phytohormone signalling, and defence mechanisms (du Jardin, 2015). Despite the common utilisation of FE during organic crop growth, the biostimulant impacts of FE to functional and hedonic measures of tomato fruits are not well characterised. Accordingly, the aim of this study was to explore the impacts of fertigation supplementation with a biostimulant FE to tomato fruit quality measures.

MATERIALS AND METHODS

Biostimulant solution

The biostimulant solution utilised in experiments was a FE produced from fish frames (~42% fish solid, derived from 25 kg/L fish, nutrient profile presented in Table S1, generously provided by Nutrifield Pty Ltd.

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Tomato growth and sample collection

Eight 2-week-old seedlings of tomato (*Solanum lycopersicum*) var. Money Maker were planted into eight individual plastic pots (14.5 L Deluxe Pot (300 mm), Garden City Plastics, Dandenong South, VIC), in Coco Perlite substrate (Nutrifield Pty Ltd., Melbourne, VIC) and grown in growth rooms with lighting and environmental conditions as per Wise et al. (2022). For the first 4 weeks the light: dark (L:D) ratio was 18:6 and for the remaining 9 weeks of growth the L:D ratio was 12:12. Each pot contained a 90 cm staked frame for the tomato vines to grow up (4-ring Flower Frame, Jack, Dandenong South, VIC). Seedlings were initially pruned at the apical bud, and four secondary branches were allowed to develop, with all other vegetative buds removed weekly throughout growth. Fertigation was provided to plants daily via a recirculating system wherein each pot contained a single 4 L/h dripper, which delivered the fertigation solution according to the timing schedule (Table 1).

Table 1 : Fertigation delivery schedule

Weeks	Fertigation program (split even throughout the day)	Volume (L) nutrients provided per day
1–5	1 × 15 min	1.0
6–8	1 × 25 min	1.7
9–13	2 × 25 min	3.3

The fertigation solution consisted of Coco A&B nutrients prepared weekly according to the schedule (Table 2) and corrected to pH 5.8 using pH Up (Nutrifield Pty Ltd., Melbourne, VIC) and pH Down (Nutrifield Pty Ltd., Melbourne, VIC). Four plants received an additional 3 mL/L of the biostimulant solution added into their fertigation solution prior to the pH adjustment. After 13 weeks of growth, ripe fruits were harvested for analyses.

Table 2 : Fertiliser dose regimen

Weeks	EC	Coco A&B concentration (mL/L)
1	1.1	1.75
2	1.3	2.25
3–4	1.5	2.75
5–6	1.8	3.00
7–8	2.0	3.50
9–10	2.2	4.00
11	2.0	3.50
12	1.8	3.00
13	1.5	2.75

Image analysis

Tomato fruits (n = 3 per treatment) were imaged and analysed as per Wise et al. (2022) with the following modifications; images were captured against a consistent white background and images were cropped manually (to the edge of the fruit) prior to analyses.

Determination of pH and Brix of crude fruit extract

Fruit crude extract was produced to measure Brix (ºBx) and pH as per Wise et al. (in press) with the modification of 20-fold dilution with water for pH measurement.

Determination of total phenolic and flavonoid content

Whole fruit dehydration, extraction, quantification of total phenolics via the Folin-Ciocalteu (F-C) method as gallic acid equivalent (GAE), and quantification of flavonoids via the aluminium chloride method as quercetin equivalent (QE) were performed on tomato fruit as per Wise et al. (2023).

Sensory perception testing

A blinded test was conducted to explore if the biostimulant treatment was associated with changes to the sensory perception of fruits. Untrained volunteers (n = 9) were asked to score the fruits (on a 9-point scale) based on their texture, taste, aroma, mouthfeel, and taste after swallowing (Fig. S1).

Statistical analysis

Statistical analyses of the data were performed in the Minitab 19 statistical software package (Minitab Inc., State College, PA). This involved paired t-tests for sensory analysis data (paired on a per-panellist basis), whilst fruit chemical and colour measures were assessed by ANOVA.

RESULTS AND DISCUSSION

Fruit colour is an important quality metric as it is the consumer's first impression and tomato fruit colour has been identified as a driver of fruit liking (Sinesio et al., 2010). Herein, the impact of FE to tomato fruit colour (Fig. 1, Table S2) was explored, which found that no differences were observed with statistical significance. Of potential note was the intensity of yellow, which was on average 2.16-fold higher in the FE-treatment group than the control group ($p = 0.120$). Therefore, the biostimulant treatment did not cause a significant change in tomato fruit colour.

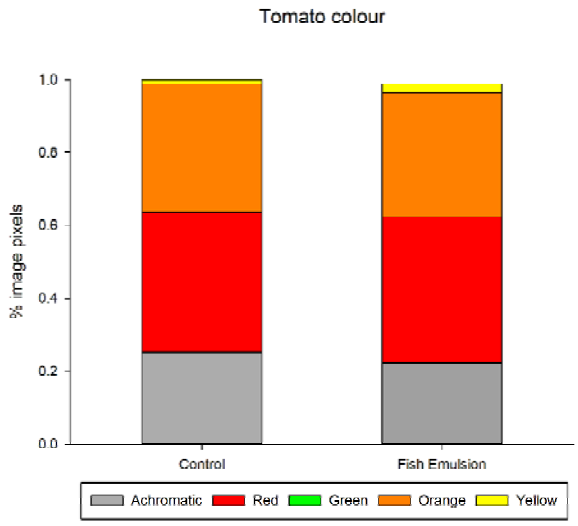

Fig. 1 : Impact of fish emulsion to fruit colour. Profile of fruit colour, represented as mean image colour proportion (n = 3).

The biostimulant treatment produced fruits with a significant 1.25-fold increase to total phenolics ($p = 0.001$), but did not change flavonoids ($p = 0.418$, Fig. 2, Table S3), indicating potential increases to common non-colour-associated tomato phenolics such as the hydroxycinnamic acids; chlorogenic acid, gallic acid, p-coumaric acid, or sinapic acid (Castagna et al., 2014; Perea-Domínguez et al., 2018). It has been recently identified that supplementation of fertiliser with FE during plant growth induced molecular changes associated with pathogen defence responses (Wise et al., 2024) and phenolics are known to be involved in the plant defence response (Kumar et al., 2020). Accordingly, the observed increase to phenolics in tomato grown with FE-supplementation may be indicative of induction of plant defence mechanisms.

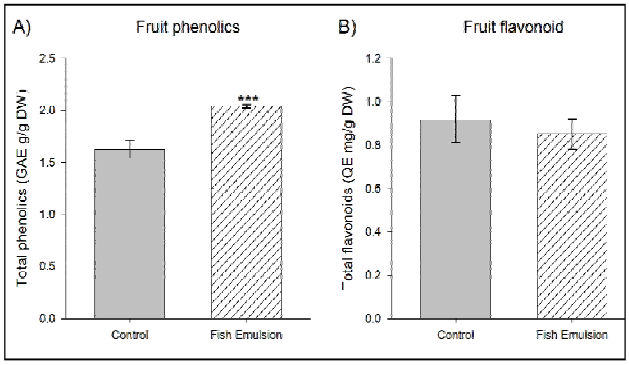

Fig. 2 : Fish emulsion impact to tomato antioxidants. A) total phenolic, and B) flavonoids of tomato fruit. Data presented as mean ± standard deviation (n = 3), with significant differences indicated by *** for $p < 0.001$.

Table 3 : Impact of fish emulsion to tomato chemical measures

Measure	N	Control		Fish emulsion		P-value
		Mean	SD	Mean	SD	
Brix (°)	3	4.367	0.306	4.000	0.100	0.187
pH (20-fold diluted)	3	4.167	0.289	3.833	0.289	0.230

Table 4 : Impact of fish emulsion to tomato sensory measures

Measure	N	Control		Fish emulsion		P-value
		Mean	SD	Mean	SD	
Visual - Skin Colour	9	6.000	1.414	5.778	2.224	0.645
Touch - Firmness	9	3.889	1.453	3.889	1.269	1.000
Smell - Desirability of aroma	9	6.333	1.658	5.111	1.691	0.202
Smell - Strength of aroma	9	5.667	1.323	5.444	1.740	0.777
Mouth feel - Firmness	9	4.333	1.581	4.000	1.500	0.545
Mouth feel - Juiciness	9	5.667	1.118	6.222	1.787	0.499
Mouth feel - Fruit outer skin texture	9	4.667	1.414	4.556	1.590	0.813
Mouth feel - Overall	9	5.444	1.590	5.667	2.179	0.772
Taste - Sweet	9	5.444	1.130	5.000	1.871	0.403
Taste - Sour/acid	9	4.333	1.871	5.000	2.121	0.081
Taste - Salty	9	2.667	1.803	3.333	2.236	0.316
Taste - Overall strength	9	6.000	1.581	6.333	1.500	0.500
Taste - Overall desirability	9	5.667	0.866	5.667	1.803	1.000
Taste after swallowing - strength	9	5.667	0.707	6.444	1.740	0.193
Taste after swallowing - desirability	9	6.778	1.302	5.333	2.179	0.109

Differences in Brix between control and FE-treated fruit extracts were not statistically significant ($p = 0.119$, Table 3), which is consistent with the non-significant differences in sweet taste ($p = 0.403$, Table 4), as Brix (or "total soluble solids") strongly correlate with fruit sweet perception (Harker et al., 2002; Malundo et al., 2001). Similarly, differences in pH between control and FE-treated fruit extracts were not statistically significant ($p = 0.230$, Table 3), which is consistent with non-significant differences in sour taste ($p = 0.081$, Table 4), as pH strongly correlates with fruit sour perception (Malundo et al., 2001).

Within tomatoes, the phytochemicals associated with fruit colour are the carotenoids; lycopene, β-carotene and lutein. Lycopene is the primary contributor to red colour of tomatoes (Kotikova et al., 2009), β-carotene contributes to orange colour, and xanthophylls (such as lutein) contribute to yellow colour (Becerra et al., 2020; Giuliano et al., 1993). Accordingly, the unchanged levels of red and orange colour (Fig. 1) indicate no significant changes to lycopene and beta-carotene, but the on-average 2.16-fold increase to yellow ($p = 0.120$) suggests that FE-treatment may be associated with promotion of lutein production. Although lutein is relatively lipophilic (Li et al., 2020) and the F-C assay has a tendency not to capture contributions of lipophilic antioxidants (Berker et al., 2013), lutein is known to contribute to antioxidant activity (Krinsky et al., 2003), and Everette et al. (2010) showed that compounds related to lutein demonstrate activity for the F-C assay. Accordingly, it is possible that lutein has contributed to the apparent increase in phenolics via antioxidant activity. Nonetheless, the increase in measured phenolics, either as a genuine increase in phenolics, or an increase to antioxidant-active phytochemicals (such as carotenoids), suggests that the FE-treatment has enhanced the functional food value of tomato fruit. Thus, inclusion of FE during tomato cultivation may produce fruit with enhanced antioxidant potential which is associated with improved potential to promote health via mediation of diseases associated with oxidative stress and inflammation (Bungau et al., 2019).

CONCLUSION

Benefits to crop production from application of FE has tended to focus on nutritional supplementation, however, recent publications indicate that this complex functions as a biostimulant to promote yield quality. The results herein indicate that supplementation of hydroponic nutrients with FE during the growth of tomatoes resulted in increased antioxidant potential (attributed to phenolics and potentially also carotenoids), thereby increasing functional food value without significantly impacting customer perceptions of fruit quality or desirability. Accordingly, FE appears to be a valuable opportunity for tomato farmers to increase the functional food value of their crops and thereby provide increased health benefits to their customers.

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Original Research Paper

Studies on production of Anaheim pepper in greenhouse media supplemented with organic and inorganic nutrient sources, and water conservation

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ABSTRACT

An experiment was conducted in the greenhouse to study growth, development, yield and water-use by Anahein pepper grown in a potting mixture supplemented with MiracleGro® (9:4:12) and milled alfalfa (3:1:2) as sources of nutrient. The study was comprised of 5 treatments, control (C), 3 gL^{-1} MiracleGro® (MG), 9 g alfalfa mill supplement (SA-1), 18 g (SA-2), and 27 g (SA-3), and replicated 6 times. Ten physiological and morphological parameters were used to evaluate growth, development and yield of the Anaheim pepper, and two parameters used to evaluate the water holding capacity of the potting mixture. The results indicate that the potting medium supplemented with alfalfa mill required significantly less water to support growth and development of the species. Also, growth, development and yield of Anaheim pepper was significantly higher in the organic supplements at SA-2 and SA-3.

Keywords: *Capsicum* species, alfalfa, MiracleGro, growth and development, greenhouse

INTRODUCTION

Anaheim pepper is an important versatile chilli peppers of the *Capsicum annum* species. It grows mostly in hardiness zones five through twelve (USDA, 2012). It is rich in vitamins A & C, and used as therapy to reduce the risk of heart diseases, diabetes, and cancer (Bray, 2019). It is a perennial grown as annual by farmers and greenhouse growers. It grows up to 46 cm and can yield fruits (15.24-25.4 cm long) over three years (Stephens, 2018), and is one of the two economically important varieties of the *Capsicum* species (Blanco-Rios et al., 2017). Climatic and edaphic conditions affect its pungency and flavor resulting to differences in the variety's pungency (Lillywhite et al., 2013). The Scoville Heat Units of the variety ranges from 500 and 1000 depending on location. Anaheim pepper tolerates a wide range of temperatures and grows best in 18°C - 30°C on sandy loamy well-drained soils (Olatunji & Afolayan, 2018).

Allabi (2006) and Chellemi & Lazarovits (2002) noted that macro and micro-nutrients are crucial for boosting productivity of pepper crops. Nagavardhanam (2017) concurred by adding that chemical fertilizers release nutrients fast for plant uptake and the result was a higher crop yield. However, Rajasekharan et al. (2012) cautioned that excessive use of chemical fertilizers might have a negative impact on soil fertility, while Tiwari et al. (2000) warned that continuous cropping supplemented only with inorganic fertilizers may not meet the expected yield without the addition of organic matter. Pandey et al. (2020) noted that both soil fertility and crop production are adversely affected by misuse of inorganic fertilizers, while, Amen (2020) noted the abundance of plant and animal biomass as organic soil amendments, but Bhatia & Prasad (2005) stressed that maintenance of soil fertility using plant and animal biomass could only occur by the process of microbial decomposition and gradual release of nutrients. This single factor is the main attraction to inorganic fertilizers by farmers and greenhouse growers. Most chemical fertilizers solubilize instantaneously to release their nutrient load thereby making nutrients readily available for plant absorption (Brust, 2019).

MiracleGro® is the inorganic fertilizer used in this study because of its popularity and wide spread use in home gardens and greenhouses across the United States. The NPK composition of the fertilizer is 9:4:12 while alfalfa (*Medicago sativa*) used as the organic nutrient source is 3:1:2. Alfalfa is a member of the *Fabaceae* family and is often used as a soil amendment because of its biological nitrogen fixation (BNF) propensity. Qian et al. (2011) noted that

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powdered alfalfa was highly successful at providing nutrients, particularly N, for plant growth and development.

The main objectives of the study are to compare growth, development and yield of Anaheim pepper grown in a potting mixture supplemented with inorganic fertilizer (MiracleGro®) and organic soil amendment (alfalfa), and to understand freshwater retention of potting mixture supplemented with the two plant nutrient sources.

MATERIALS AND METHODS

The study was conducted in a greenhouse with average day and night time temperatures of 29°C and 21°C throughout the plant growth phase. The study design comprised of five treatments *viz.*, control (C) plants grown without either nutrient supplement, MiracleGro® (MG) plants grown with inorganic nutrient supplement, soil amendment (SA) plants grown with milled alfalfa supplement in three concentrations, SA-1, SA-2, and SA-3.

The recommended application rate of the MiracleGro® was 3 gL^{-1}. Because the inorganic fertilizer NPK is 9:4:12, and 3:1:2 for alfalfa, we used 9, 18, and 27 g of milled alfalfa for SA-1, SA-2, and SA-3, respectively. The 9 g of alfalfa for SA-1 was intended to bring the concentration of nitrogen per unit of potting mixture close to the inorganic nitrogen content. SA-2 and SA-3 were intended to double and triple the nitrogen content of the inorganic fertilizer per unit of potting mixture. The inorganic fertilizer was dissolved in water and given every 14 days at the rate of 3 gL^{-1} for 7.5 pots until the crops were harvested. There was a total of 30 pots per treatment *i.e.* 5 pots per replication. Each pot in the SA treatments received either 9, 18 or 27 g of milled alfalfa based on the treatment group. The milled alfalfa was mixed with the potting medium before transplantation into 0.003 m^3 pots.

Anaheim pepper seedlings were transplanted into 0.003 m^3 pots containing 2:1:1 top soil:peat moss:perlite 35 days after sowing. The transplants were randomly divided into 5 treatments and replicated 6 times in 6 blocks in a randomized complete block design (RCBD). The pots were placed in saucers for leachate collection. Watering was 300 mL/pot and leachate, collected 10 minutes after watering, was measured to determine the amount of water retained by the potting mixture.

Physiological and morphological measurements were collected every two weeks. Potting mixture pH was measured immediately after transplanting and every two weeks thereafter. Average potting mixture pH was 7.03 for the C, 7.33 for MG, 7.21 for SA-1, 7.24 for SA-2, and 7.21 for SA-3. Height was measured from plant base to stem tip, and used to calculate relative growth rate (RGR) computed as the height difference between succeeding measurements divided by the interval between the measurements.

Rate of transpiration was measured by gravimetric water loss. This involves drenching the potting mixture and sealing the pots in plastic after draining so water loss is by transpiration. The pots are weighed immediately after sealing and left for 7 days or longer and weighed again before the plastic sealing is removed. The rate of transpiration per hour was computed as weight difference divided by the interval in hours between the measurements.

Stomatal conductance was measured using the leaf porometer by Decagon Devices, Washington, USA. Similarly, chlorophyll content was measured using SPAD-502 Plus chlorophyll meter (Konica Minolta Inc., Japan) in SPAD Units. Leaf area was measured using CI-202 Portable Laser Leaf Area Meter by CID Bio-Science.

Nitrate nitrogen (NO_3^- N) was determined from the leachate using DR300 pocket colorimeter, method No. 8039 (Hach Company, Colorado, USA). The leachate was filtered with Whatman No.1 filter paper immediately after collection and 10 ml of the filtrate, used as blank, was used to zero the instrument. Another 10 mL of the filtrate was mixed with Nitra Ver 5 powder and shaken vigorous for a few minutes for color development. Color intensity depends on the amount of NO_3^- N in the leachate which was measured for NO_3^- N content.

At maturity, harvested fruits and vegetative parts were weighed fresh and dried at 80°C until constant weight to obtain the dry weight. The roots were washed and weighed fresh, and dried at 80°C to obtain dry root biomass weight. All physiological and morphological measurements were analyzed using SAS 9.40.

RESULTS AND DISCUSSION

Repeated measure ANOVA, unpaired t-test and regression analyses were adopted to decipher the effects of the treatments on the species and water

Table 1 : Parameter indicators of growth and development of Anaheim pepper

Parameter	C	MG	SA-1	SA-2	SA-3	P-value
Chlorophyll content (SPAD units)	30.75$^{(a)}$	39.66 $^{(c)}$	37.90 $^{(b)}$	39.31 $^{(c)}$	41.01 $^{(c)}$	<0.0001
Transpiration rate (mL/h)	1.04 $^{(a)}$	2.88 $^{(c)}$	2.15 $^{(b)}$	2.98 $^{(c)}$	3.69 $^{(d)}$	<0.0001
RGR (cm/day)	0.33 $^{(a)}$	0.6 $^{(c)}$	0.54 $^{(b)}$	0.69 $^{(c)}$	0.71 $^{(c)}$	<0.0001
Stomatal conductance (mmol/m^{2}s)	44.46$^{(a)}$	173.74 $^{(c)}$	163.48$^{(b)}$	188.13$^{(c)}$	219.66 $^{(c)}$	<0.0001
Nitrate nitrogen (mg/L)	6.78 $^{(a)}$	12.39 $^{(b)}$	9.65 $^{(c)}$	10.94 $^{(c, b)}$	9.35 $^{(c)}$	0.0005
Leachate (mL)	136.36$^{(a)}$	88.55 $^{(c)}$	92.11 $^{(c)}$	77.24 $^{(b)}$	74.70 $^{(b)}$	<0.0001
Flower count	0.83 $^{(a)}$	1.73 $^{(b)}$	1.31 $^{(b)}$	1.46 $^{(b)}$	2.02 $^{(b)}$	0.0480
Fruit count	0.53 $^{(a)}$	1.57 $^{(b)}$	1.21 $^{(b)}$	1.34 $^{(b)}$	1.33 $^{(b)}$	0.0388
Fruit Fresh weight (g)	2.50 $^{(a)}$	8.36 $^{(b)}$	3.89 $^{(a)}$	11.74 $^{(b)}$	9.29 $^{(b)}$	0.0110
Fruit dry weight (g)	1.00 $^{(a)}$	2.28 $^{(a)}$	1.25 $^{(a)}$	2.56 $^{(a)}$	3.51 $^{(a)}$	0.1136
Vegetative fresh weight (g)	18.23 $^{(a)}$	28.77 $^{(b)}$	25.77 $^{(c)}$	31.70 $^{(d)}$	36.17 $^{(e)}$	<0.0001
Vegetative dry weight (g)	13.97 $^{(a)}$	18.77 $^{(b)}$	17.73$^{(b,c)}$	20.57 $^{(b, d)}$	22.53 $^{(d)}$	<0.0001
Fresh root weight (g)	2.00 $^{(a)}$	5.17 $^{(b)}$	6.17 $^{(b)}$	7.50 $^{(b)}$	18.33 $^{(c)}$	<0.0001
Dry root weight (g)	0.70 $^{(a)}$	1.92 $^{(b)}$	2.23 $^{(b)}$	3.17 $^{(b)}$	9.17 $^{(c)}$	<0.0001
Leaf area (cm^2)	363.73$^{(a)}$	398.24 $^{(b)}$	380.61$^{(b)}$	390.80 $^{(b)}$	401.28 $^{(b)}$	0.03635

Rows with the same letter superscripts are not significantly different

holding capacity of the medium (Table 1). The values indicate that all parameters are statistically significantly different except fruit dry weight. Fruit dry weight indicates that increased water absorption was induced by the treatments and the rate of transpiration bears out this result. The treatments resulted to increased rates of transpiration which was significantly different from the control. Also, the rate of transpiration was different between the treatments but similar for MG and SA-2 (Table 1). SA-2 and MG results are similar in most of the parameters measured, while, SA-3 outperformed MG, an indication that greenhouse growers can achieve the same or higher yields with media amended with organic nutrient source on short rotation agriculture crops.

We used a simple regression to validate our transpiration method. The leaf porometer used to measure stomatal conductance is a modern cutting-edge instrument, while, transpiration was measured with the gravimetric method. The analysis indicates an R^2 of 92% with Y= -4.895+63.889x predictability of the transpiration rate (Fig. 1). This is a confirmation of the reliability of the information obtained with the experimental design and methodology used in the study.

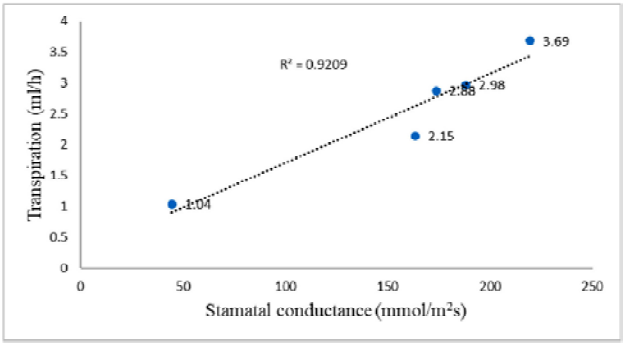

Fig. 1: Relation between stomatal conductance and transpiration rate of Anaheim pepper

The leachate result has real-life significance in freshwater conservation. Freshwater constitutes only 3% of the earth's water resource (Manzoor et al., 2007) and agriculture uses 75% of the freshwater. Agricultural use goes up to 90% in some parts of the world (Sophocleous, 2004). Additionally, there are 463,365 ha of fresh harvest producing greenhouses in the United States (Wright, 2021), and each hectare uses 83 kl of fresh water per day (Bilderback, 2017). This amounts to 38,459,295 kl of fresh water per day. Leachate analysis indicates that the potting mixture amended with milled alfalfa (SA-2 and SA-3) had higher water holding capacity and thus, less watering

for crop use. This saves greenhouse operational fund including labor cost. These cost savings increase the grower's profit margin. The result is also significant for fresh water conservation in bourgeoning human population and its associated increases in fresh water demand.

The results of NO_3^- N in the leachate are not surprising with the highest nitrogen in the MG treatment leachate. The similarity between MG, SA-2 and SA-3 in the other parameter indicators further confirm that greenhouse growers can eliminate inorganic fertilizers without compromising yield, if the organic nutrient source is treated to shorten or circumvent decomposition. Because agricultural crops are short rotation crops, a nutrient amendment must be capable of releasing nutrients readily else yield will be compromised. Nutrient release delay due to decomposition creates the attraction to inorganic fertilizers by growers. We circumvented decomposition in this study by milling alfalfa and thus increased cellular hydration and nutrient leach-out of alfalfa cells into the potting medium.

We considered nitrogen only in factoring the comparable amounts of alfalfa mill per unit of potting mixture. Because the MG used has NPK ratio of 9:4:12, and 3:1:2 for alfalfa and the factory recommended application rate of the MG is 3 gL^{-1}, we used 9 g (SA-1), 18 g (doubled for SA-2), and 27 g (tripled for SA-3). This amount, example 9 g of alfalfa, is theoretically designed to supply a comparable amount of nitrogen in the potting mixture as 3 g of MG dissolved in a liter of water. Because cellulose cell walls of milled alfalfa cannot dissolve in water, it was added directly into the potting mixture, while, the MG was dissolved in water and treated every two weeks until the crops were harvested. The results indicate that the 9 g of alfalfa, intended to produce similar results as the MG, failed to do so. Instead the doubled concentration (18 g alfalfa) gave similar results as the MG, while, the tripled concentration (27 g of alfalfa) outperformed the MG in most parameters. Erisman et al. (2008) reported that about 50% of the global human population is fed with agricultural produce grown with inorganic nitrogen. This study has shown that humans fed with produce grown with inorganic fertilizers can be reduced because organic nutrient sources can be used without compromising crop yield. Additionally, the adverse consequences of inorganic nitrogen in the environment (Rajasekaran et al., 2012; Nagavardhanam, 2017; Pandey et al., 2020) can be reduced.

Randomization experiment in the greenhouse ensures that micro environmental conditions are equally distributed. Greenhouse environmental conditions are fairly uniform but the apparent movement of the sun casts shadow through the day which could result to different effects in parts of the same greenhouse. This could become a problem for north-south and east-west facing parts of a greenhouse. The randomized complete block design took care of this potential experimental error. Also, based on the initial dry run of the experiment we collected leachate 10 minutes after watering to avoid loss of leachate by evaporation. The time is long enough for complete drainage of excess water but short to avoid loss of leachate to evaporation.

CONCLUSION

Human population growth and concerns about food insecurity, sustainable agriculture, and environmental health, the study concludes that organic nutrient sources can be used in short rotation cropping in greenhouse production. This, by extension, can be applied to field agriculture as long as the organic nutrient source is treated to shorten and/or circumvent the natural decomposition delay which limits timely release of plant nutrients. Decomposition time required before organic materials release their nutrient load is one single reason greenhouse growers and farmer rely on inorganic fertilizers. Also, the study concludes that supplementing greenhouse potting mixtures with treated organic nutrient source conserves fresh water use in greenhouse crop production and so reduces labor cost and increases the grower's profit margin.

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Original Research Paper

Effect of fertigation on growth and yield on Chilli hybrid Arka Meghana

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ABSTRACT

A field experiment was conducted to study the effect of fertigation on chilli F_1 Hybrid Arka Meghana during *kharif* of 2017 and 2018, with ten treatments, including different doses, sources of fertilizers and its frequency of application, in randomized block design with three replications. The pooled analysis revealed that application of fertilizer dose (125:100:125 kg N: P_2O_5: K_2O ha^{-1}) through fertigation on bi-weekly basis resulted in higher plant height (76.3 cm) at 80 days after transplanting, which was on par with the same dose and source applied at weekly interval (74.0 cm). These two treatments recorded higher fruit length (12.63 and 12.27 cm), number of fruits per plant (153.33 and 169.67) and dry weight of 10 fruits (9.00 and 8.63 g), respectively. All the fertigation treatments recorded higher yields over the conventional soil application of fertilizers to the tune of 14.84 to 61.55%. Among the fertigation treatments, application of 100% of fertilizer dose using water soluble fertilizers at bi-weekly interval resulted in significantly higher yield (32.44 t ha^{-1}) compared to all treatments except the treatment where the weekly application of same dose of fertilizer through the same sources (31.81 t ha^{-1}) and 75% of 125:100:125 kg N: P_2O_5: K_2O ha^{-1} was applied weekly or bi-weekly intervals (29.23 and 30.01 t ha^{-1}). Biweekly and weekly application of 100% fertilizer dose of 125:100:125 kg N: P_2O_5: K_2O ha^{-1} through fertigation could yield maximum net income (Rs. 400151 and Rs. 387551 ha^{-1}) with B: C (1.61 and 1.56). However, fertilizer applied to soil resulted in minimum net income of Rs.183054 ha^{-1} and B: C (0.84).

Keywords: Chilli, economics, fertigation, growth, yield

INTRODUCTION

Chilli (*Capsicum annuum* L.), a member of Solanaceae family, is an important spice crop in India. The states such as Andhra Pradesh, Karnataka, Bihar, Tamil Nadu, Uttar Pradesh and Maharashtra, account for 3/4 of the total area. The green chilli is grown in 4.05 lakh hectares with the production of 42.72 lakh tonnes. The productivity of green chilli is approximately 10.54 t ha^{-1} in India, which is low (Anonymous, 2022). The fruits of chilli are rich in vitamin A, C and minerals. Fresh green and ripe chilli are used to make pickles, sauces and paste. The essential oil, oleoresin is used in the food and beverage industries.

Although, it is one of the major crops grown, its yield is quite low. Increase in chilli production can be achieved either bringing more area under its cultivation or by adopting improved varieties and better cultural practices. The second approach is more often preferred and among various cultural practices, proper fertilizer application is one of the quickest and easiest ways of increasing the yield per unit area (Natsheh and Mousa, 2014). Balanced nutrition is one of the most important factor affecting the growth and productivity of the crops. The optimum levels at which the nutrients are to be applied and source from which they have derived are equally important. Nutrients applied to the crop contribute to crop production through yield increase and quality of the produce. Fertigation is an effective means of controlling timing and placement of fertilizers and improving fertilizer use efficiency by reducing losses through leaching, volatilization and fixation in the soil to less available forms (Papadopoulous, 1994). Source of nutrient is also a major contributing factor in yield increase and in the economics of production. Hence, the present experiment was conducted to study the influence of fertigation, its frequency and the source of nutrients on yield of green chilli.

MATERIALS AND METHODS

The experiment was conducted at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India during *kharif* of 2017 and 2018. The institute is situated at 13° 7^2 N latitude,

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72° 29² E longitude and an elevation of 890 meters above mean sea level. The experimental soil was well drained sandy loam (pH 6.60 and electrical conductivity 0.25 dSm^{-1}) characterized by medium organic carbon (0.63%), low available N (169 kg ha^{-1}), high available P (68 kg ha^{-1}) and medium available K (260 kg ha^{-1}). The soil has available water holding capacity of 130 mm in one-meter soil depth. The experiment was laid out in randomized block design with ten treatments and three replications. Prior to planting, a uniform amount of farmyard manure @ 25.0 t ha^{-1} was applied as basal application to all the treatments as common practice. The treatment details and quantity of different fertilizers applied have been presented in Table 1 and Table 2. The entire dose of P and half of N and K were applied as basal and remaining half of N and K was side dressed to soil in equal splits at 30 and 60 days after transplanting in T_1. Urea, 19:19:19 and sulphate of potash were used as water soluble fertilizers for treatments T_3 to T_{10}, while, urea, single super phosphate and muriate of potash were used as common fertilizers for treatments T_1 and T_2.

Thirty-five days old seedlings of chilli hybrid Arka Meghana were transplanted at 80-40 x 50 cm, under paired row system during first week of July in both the years. Drip irrigation was provided depending on the rate of evaporation and amount of effective rainfall received. The fertigation treatments started after two weeks of planting and fertilizers were applied through drip system at weekly and bi-weekly interval. The treatments were imposed dissolving desired amounts of fertilizers and applied via venturi system through drip irrigation. A total of 16 and 32 number of fertigation were given for weekly and bi-weekly interval, which was continued up to 15 days before completion of crop growth period. Five plants per replication in each of the treatments were selected randomly for recording yield parameters. Recommended package of practices including agronomic and plant protection measures were adopted to raise the crop (Prabhakar et al., 2010). Fertilizer use efficiency of chilli was calculated by using the following formula.

FUE (kg yield kg-NPK^{-1}) = Economic yield (kg ha^{-1}) / total NPK applied (kg ha^{-1})

The experimental data were statistically analysed (Gomez and Gomez, 1983) and compared using critical difference at 5% probability level.

Table 1 : Fertigation treatment details in chilli

Note	Treatment	Fertilizer	Application dose	Basal dose (kg ha^{-1})	Top dressing (kg ha^{-1})	Fertigaton (kg ha^{-1})	Frequency
T_1	100 % fertilizer dose	Common	100% soil application	62.5:100: 62.5	62.5: 0: 62.5	-	-
T_2	(125: 100: 125 kg N:	Common	50% NK fertigation	62.5: 100: 62.5	-	62.5: 0: 62.5	-
T_3	P_2O_5: K_2O ha^{-1})	WSF	100% NPK fertigation	-	-	125: 100: 125	Weekly
T_4		WSF	50% NK fertigation	62.5: 100: 62.5	-	62.5: 0: 62.5	Weekly
T_5	75 % fertilizer dose	WSF	100 % NPK fertigation	-	-	93.75: 100: 93.75	Weekly
T_6	(93.75: 75: 93.75 kg	WSF	50% NK fertigation	46.8: 75: 46.8	-	46.8: 0: 46.8	Weekly
	N: P_2O_5: K_2O ha^{-1})						
T_7	100 % fertilizer dose	WSF	100% NPK fertigation	-	-	125: 100: 125	Bi-weekly
T_8	(125:100:125 kg N:	WSF	50% NK fertigation	62.5: 100: 62.5	-	62.5: 0: 62.5	Bi-weekly
	P_2O_5: K_2O ha^{-1})						
T_9	75 % fertilizer dose	WSF	100% NPK fertigation	-	-	93.75: 100: 93.75	Bi-weekly
T_{10}	(93.75:75:93.75 kg	WSF	50% NK fertigation	46.8: 75: 46.8	-	46.8: 0: 46.8	Bi-weekly
	N: P_2O_5: K_2O ha^{-1})						

WSF: Water soluble fertilizers

Table 2 : Treatment wise fertilizers applied (kg ha^{-1}) under fertigation in chilli

Treatment	Basal dose			Top dressing		Fertigation			
	Urea	Single super phosphate	Muriate of potash	Urea	Muriate of potash	Urea	Muriate of potash	Sulphate of potash	19:19:19
T_1	135.5	625.0	104.5	135.5	104.5	0.00	0.00	0.00	0.00
T_2	135.5	625.0	104.5	-	-	135.5	104.5	-	-
T_3	0.0	0.0	0.0	-	-	54.0	-	50.0	526.0
T_4	135.5	625.0	104.0	-	-	135.5	-	125.0	-
T_5	0.0	0.0	0.0	-	-	40.50	-	37.5	394.0
T_6	102.0	469.0	78.0	-	-	102.0	-	94.0	-
T_7	0.0	0.0	0.0	-	-	54.00	-	50.0	526.0
T_8	135.5	625.0	104.0	-	-	135.5	-	125.0	-
T_9	0.0	0.0	0.0	-	-	40.50	-	37.5	394.0
T_{10}	102.0	469.0	78.0	-	-	102.0	-	94.0	-

RESULTS AND DISCUSSION

Growth parameters

The data pertaining to plant growth at 35, 80 days of transplanting (DAT) and harvest are presented in Fig. 1. Significant differences among the treatments were observed at 80 days after transplanting and harvest. However, 50% of N & K fertigation of the recommended dose through fertigation using water soluble fertilizers (T_4) has recorded the taller plants (55.4 cm) at initial stage of the growth (35 DAT) than other treatments, however, shortest plants (48.1 cm) were observed with T_2, where the common fertilizers were used for soil and fertigations. The treatments shown significant differences for the plant height at 80 DAT and harvest. Application of 100% fertilizer

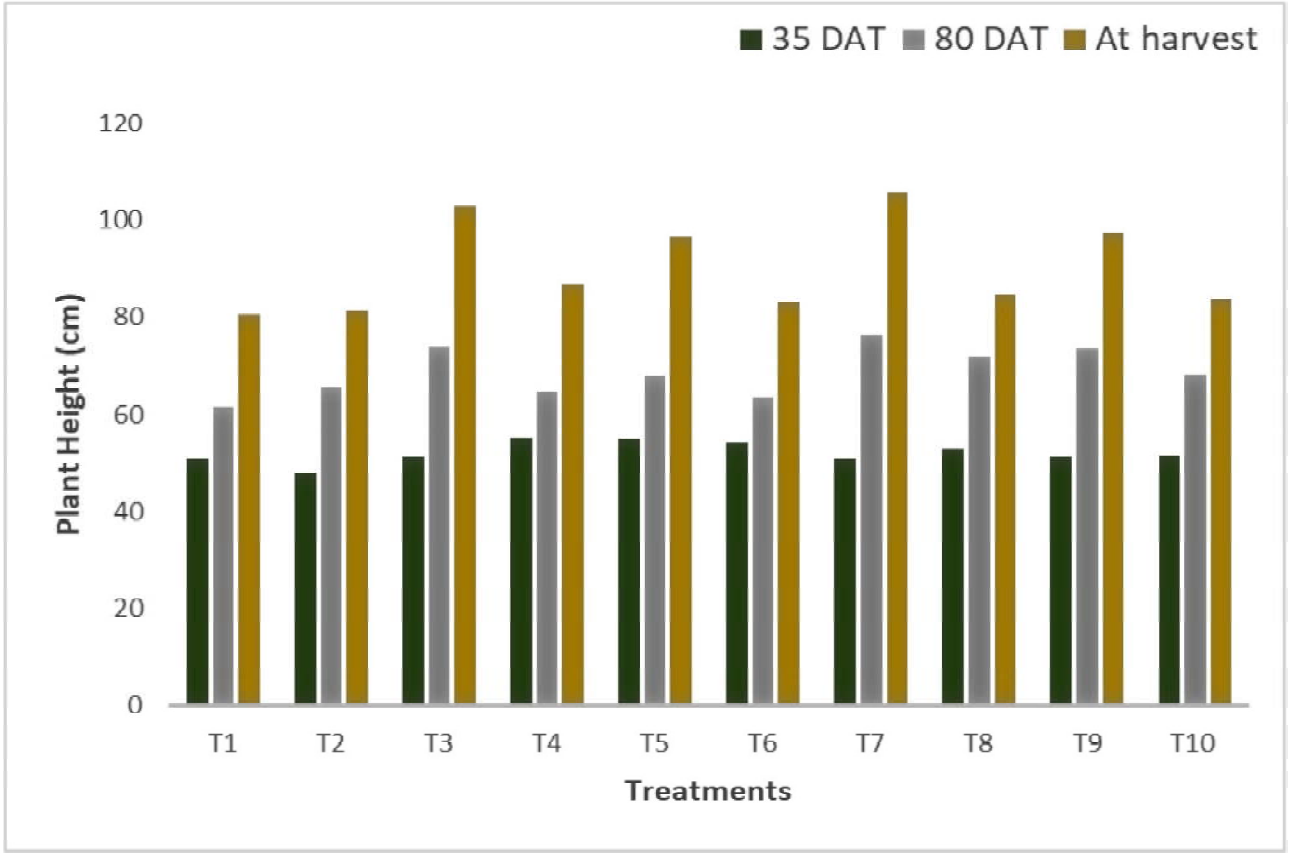

Fig. 1 : Effect of fertigation treatments on plant height (cm)

dose through fertigation using water soluble fertilizers at bi-weekly interval (T_7) recorded significantly taller plants at 80 DAT (76.3 cm) and harvest (105.7 cm), which remained on par with T_8 (72.0 cm), T_9 (73.6 cm) and T_3 (74.0 cm) at 80 DAT and with only T_3 (103.0 cm) at harvest. The minimum values (61.7 and 80.6 cm) for the plant height was recorded with soil application of nutrients through common fertilizers at 80 days after transplanting and at harvest, respectively.

Different treatments produced significant differences for the number of branches per plant at harvest only (Fig. 2). However, application of 100% fertilizer dose through fertigation using water soluble fertilizers at bi-weekly interval (T_7) recorded higher number of branches per plant (7.8 and 9.2) at 35 and 80 DAT, respectively. The same treatment recorded significantly higher number of branches per plant (13.2) than other treatments except T_3, T_9 and T_{10} (11.8). However, minimum number of branches (9.7) was recorded with soil application of nutrients.

Application of higher dosage of water soluble fertilizers through fertigation produced best results in growth parameters like plant height and number of branches per plant, which might be due to better nutritional environment in the root zone for growth and development of plants, as nitrogen and phosphorus are considered as major nutrients required for proper growth and development of plant. Beside this, nitrogen is the main constituent of protoplasm, cell nucleus, amino acids, chlorophyll and many other metabolic processes like transpiration (Godara et al., 2013). The similar results were also reported by Vinayak et al. (2019) and Chandramohan Reddy et al. (2016).

Yield attributes

The data related to yield attributes has been presented in Table 3. Significantly higher number of fruits per plant was observed in T_3 (169.67), which was on par with T_7 (153.33), T_4 (150.0), T_8 (138.67) and T_9 (132.67), whereas, minimum number of fruits per plant was recorded with T_1 (72.67). The higher availability of soil moisture, optimum NPK nutrients and uptake when supplied through fertigation might have increased the number of fruits per plant. Fertigation might have increased the number of primary branches, shoot growth and potential sites

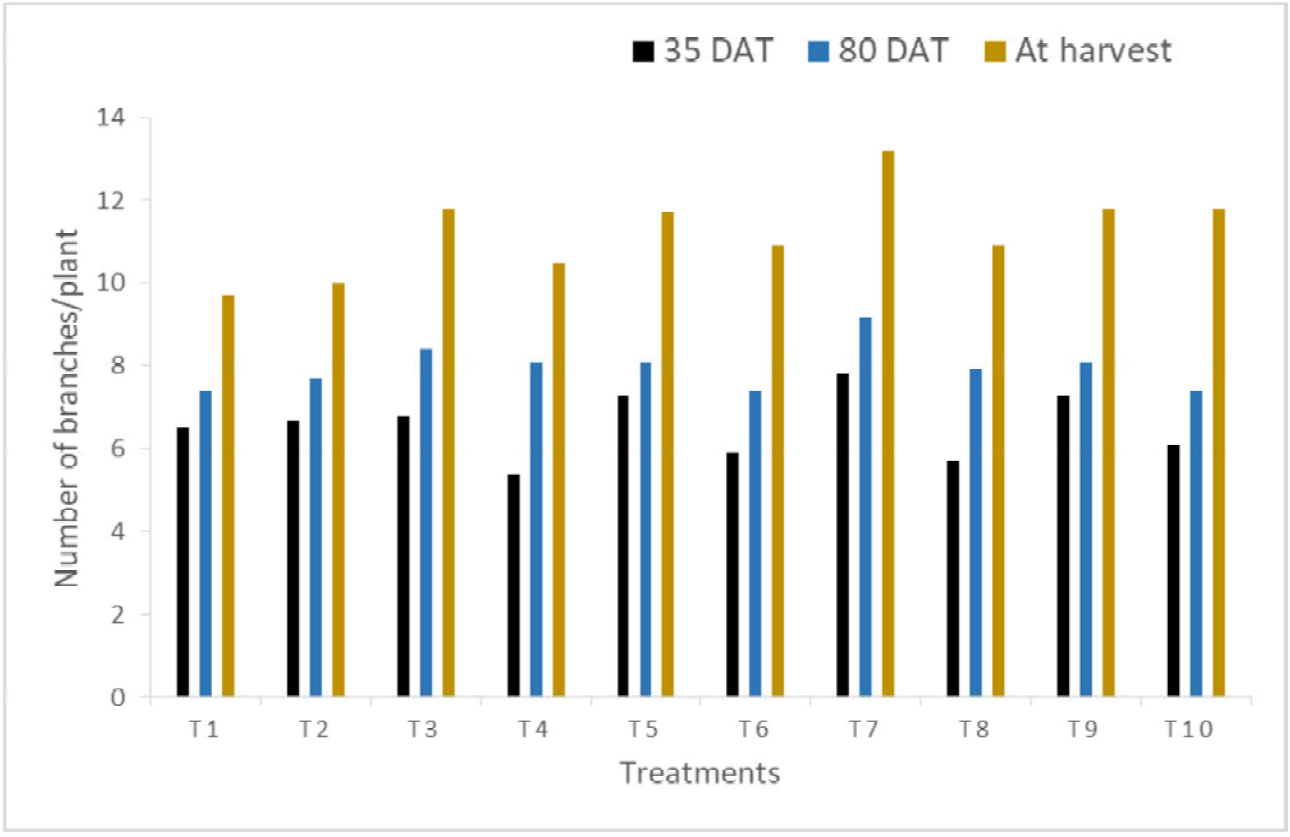

Fig. 2 : Effect of fertigation treatments on number of branches plant^{-1}

Table 3 : Effect of fertigation treatments on yield attributing characters, yield and FUE in chilli

Treatment	Fruits/plant (Nos.)	Fruit length (cm)	Fruit girth (cm)	Ten fruit weight (g)	Dry weight of ten fruits (g)	Yield (t ha^{-1})	Fertiliser use efficiency (kg kg^{-1})
T_1	72.67	10.77	4.05	77.27	6.90	20.08	57.37
T_2	112.33	10.99	4.22	79.33	7.16	23.06	65.89
T_3	169.67	12.27	4.01	86.67	8.63	31.81	90.89
T_4	150.00	11.92	3.89	81.77	8.06	23.67	67.63
T_5	109.67	11.98	4.33	84.53	8.10	29.23	111.35
T_6	112.67	11.03	3.84	79.57	7.43	23.30	88.76
T_7	153.33	12.63	3.81	91.90	9.00	32.44	92.69
T_8	138.67	11.96	4.01	82.27	7.83	27.64	78.97
T_9	132.67	12.19	3.93	85.23	8.40	30.01	114.32
T_{10}	114.67	11.37	3.80	80.60	7.43	24.40	92.95
SEm±	13.69	0.455	0.227	1.63	0.11	1.478	
CD (P=0.05)	40.99	1.36	NS	4.87	0.34	4.424	

where flower could develop. Similar results were also reported by Krishnamoorthy & Noorjehan (2014) and Chandramohan Reddy et al. (2016).

Significantly higher fruit length (12.63 cm) was observed in T_7, which was on par with most of the treatments except T_1 (10.77 cm), T_2 (10.99 cm) and T_6 (11.03 cm). There were no significant differences among the treatments for the fruit girth. However, T_5 recorded the maximum (4.33), while T_{10} recorded the minimum fruit girth (3.80 cm). Similarly, there were significant differences for ten fruit weight and T_7 resulted in significantly higher ten fruit weight (91.90 g), than all other treatments, while minimum ten fruit weight was recorded with T_1 (77.27 g). Bi-weekly application of 100% fertilizer dose through fertigation using water soluble fertilizers (T_7) resulted in significantly higher (9.00 g) dry weight of ten fruits than all other treatments. Weekly application of same amount of fertilizers through fertigation (T_3) recorded the second highest value (8.63 g) followed by T_9 (8.40 g), whereas minimum value (6.90 g) recorded with soil application of nutrients. This may be due to continues nutrient supplied through fertigation in the required and optimum form, which must have helped in healthy growth of plants and increased the fruit length and girth. The present findings are in accordance with the results of Krishnamoorthy and Noorjehan (2014) and Vinayak et al. (2019).

Yield

Irrespective of dosage and source of fertilizer, fertigation treatments were superior to conventional soil application treatment with respect to yield. All the fertigation treatments recorded higher yields over the conventional soil application of fertilizers to the tune of 14.84 to 61.55% (Table 3). Among the fertigation treatments, bi-weekly application of 100 % fertilizer dose through fertigation using water soluble fertilizers (T_7) resulted in significantly higher yield (32.44 t ha^{-1}) than all the other treatments except the treatment T_3, where the weekly application of same amount of fertilizer was given through the same sources (31.81 t ha^{-1}), T_9 (30.01 t ha^{-1}) and T_5 (29.23 t ha^{-1}), where 50% N and K of 75% fertilizer dose was applied through water soluble fertilizers at bi-weekly and weekly interval. Reducing the dosage of NK or NPK fertigation by 25% reduced the yield substantially.

This can be explained on the basis that fertigation saves fertilizer nutrients as it permits applying for fertilizer in small quantity at a time matching with the plants nutrient need. This contributes to an improved availability of moisture, nutrients, and uniform distribution of fertigated nutrients in the crop root zone throughout the growth stages leading to better uptake of nutrients. The enhancing effects

Table 4 : Economics of green chilli crop in relation to fertigation treatments

Treatment	Average yield (t ha^{-1})	Gross investment (Rs. ha^{-1})	Gross income (Rs. ha^{-1})	Net income (Rs. ha^{-1})	B:C ratio
T_1	20.08	218546	401600	183054	0.84
T_2	23.06	218546	461200	242654	1.11
T_3	31.81	248649	636200	387551	1.56
T_4	23.67	224199	473400	249201	1.11
T_5	29.23	238419	584600	346281	1.45
T_6	23.30	220150	466000	245950	1.12
T_7	32.44	248649	648800	400151	1.61
T_8	27.64	224199	552800	328601	1.46
T_9	30.01	238419	600200	361881	1.52
T_{10}	24.40	220150	488000	267850	1.21

Sale price = Rs.20/kg

of NPK on vegetative growth might be attributed to their vital contribution in several metabolic process in plants related to growth (Marschner, 1986). It stimulates the plant vegetative growth to generate leaves, which are able to produce photosynthetic products accumulation required for fruits formation and development and subsequently fruit yield and its attributes. Gireesh et al. (2020) and Vinayak et al. (2019) reported the similar results in chilli crop.

Fertilizer use efficiency

The fertilizer use efficiency was ranged between 65.89 to 114.32 kg kg^{-1} for the fertigation treatments (Table 3). Though, application of 100% fertilizer dose using water soluble fertilizers at bi-weekly interval (T_7) recorded the highest yield (32.44 t ha^{-1}), but the fertilizer use efficiency was higher (114.32 and 111.35 kg kg^{-1}) with the treatments where the 75% of fertilizer dose was applied through fertigation using water soluble fertilizers at bi-weekly and weekly intervals, which was followed by T_{10} (92.95 kg/kg), T_7 (92.69 kg/kg) and T_3 (90.89 kg kg^{-1}), however, minimum fertilizer use efficiency was recorded in soil application of common fertilizers (57.37 kg/kg). Ramachandrappa et al. (2010) also recorded higher fertilizer use efficiency at 75% recommended dose of NPK through fertigation than 100% recommended NPK fertigation in green chilli.

Economics

The averaged data pertaining to economic returns and benefit: cost ratio is presented in Table 4. All the fertigation treatments with water soluble fertilizers resulted in higher gross income than soil application (T_1) and fertigation with common fertilizers (T_2). Among the fertigation treatments, application of 100% fertilizer dose through fertigation on bi-weekly basis (T_7) has resulted in highest gross income (Rs. 648800 ha^{-1}) followed by T_3 *i.e.* same amount of fertilizer given on weekly basis (Rs. 636200 ha^{-1}). As far as net income is concerned, higher values were recorded with T_7 (Rs. 400151 ha^{-1}) and T_3 (Rs. 387551 ha^{-1}). Irrespective of dosage and frequency, fertigation with water soluble fertilizers resulted in higher B: C ratio (1.11 to 1.61) compared to soil application (0.84). Gireesh et al. (2020) and Suman Kumari et al. (2020) also reported maximum net returns and cost : benefit ratio with the application of 100% recommended dose of NPK through fertigation.

CONCLUSION

It can be concluded that application of water soluble fertilizers @ 125:100:125 kg N: P_2O_5: K_2O ha^{-1} during the cropping period through fertigation at bi-weekly intervals resulted in higher yield (32.44 t ha^{-1}), net income of (Rs. 400151 ha^{-1}) and B: C ratio (1.61) in *kharif* grown chilli, which remained on par with application of same amount of water soluble fertilizers through fertigation on weekly basis.

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Original Research Paper

Aeroponics approach for production of gladiolus (*Gladiolus hybridus* Hort.) corms and cormels

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ABSTRACT

Gladiolus is a popular commercial flower crop among top ten cut flowers of international trade. In order to improve the rate of multiplication, eleven gladiolus varieties with three nutrient regimes (N1- 96:18:108, N2-128:24:144 and N3-160:30:180 ppm of NPK) were evaluated under aeroponics system. The results revealed that different varieties and nutrient regimes had significant influence on plant growth, corm and cormel production. Among the genotypes, Arka Naveen recorded maximum plant height (109.44 cm), on par with Arka Amar (104.77 cm). The genotype Arka Amar (1.89) recorded highest number of corms per plant, on par with Arka Ranjini (1.78), Arka Aayush (1.44), Arka Naveen (1.44) and Arka Shobha (1.67). However, number of cormels were recorded highest in Arka Aayush (1.67), on par with Arka Amar (1.67) and Arka Ranjini (1.44). Among the nutrient regimes, N2 (128:24:144 ppm NPK) registered maximum plant height (85.44 cm), on par with N3 (160:30:180 ppm NPK) (77.42 cm). The number of corms (1.39) and cormels (3.56) per plant were recorded highest in N2 (128:24:144 ppm of NPK). The genotype Arka Manorama did not produce cormels under aeroponics system. Gladiolus varieties Arka Amar, Arka Aayush and Arka Naveen were found as best suited under aeroponic system for better corm multiplication under the nutrient regime of N2 (128:24:144 ppm of NPK).

Keywords : Aeroponics, corm, cormel, gladiolus, multiplication, nutrient regime

INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.) is an important commercial cut flower crop cultivated for its elegant flower spikes, vibrant colours, better vase life and wide adaptability. It is one of the top ten cut flowers of international trade. Propagation and rate of multiplication is important for the adoption and popularisation of any crop. Gladiolus is propagated vegetatively through underground storage organs called corms and cormels. The *in vitro* propagation of cormels is expensive which increases the production cost per corm. Generally, the mother corm produces one or two corms and minimum of 25 cormels per season (Sinha & Roy, 2002). The cormels need one season (six to seven months) to reach a standard plantable size of corm. After harvest, the corms and cormels are dormant (Priyakumari & Sheela, 2005) which need proper cold storage to release dormancy to facilitate planting.

The scarcity of quality planting material supply in gladiolus is primarily due to the low multiplication efficiency and greater spoilage of corms during storage (Singh & Dohare 1994). Gladiolus is prone to *Fusarium* wilt disease, which leads to the loss of corm and cormels. Added to that, the multiplication of corms under soil cultivation increases the risk of *Fusarium* wilt infestation in the propagules. The Fusarium infected mother corms produce disease-ridden daughter corms which succumbs to death in the storage or in the field during subsequent cropping season. To overcome the soil borne disease problems and to improve the multiplication efficiency of gladiolus, an innovative method *i.e.*, aeroponics is used. Aeroponics is the method of growing plants in an air or mist of nutrients devoid of soil or any media (Farran & Castel, 2006; Chiipanthenga et al., 2012). Studies on aeroponics mediated potato seed production indicated that this technique has advantage over conventional method in terms of energy conservation, survival rate, growth rate, maturation time, disease free planting material

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production and tuber yield (Stoner, 1983; Otazu, 2010; Tsoka et al., 2008).

The nutrient solution or mixture plays a key role in multiplication of gladiolus under aeroponics which is primarily soilless. The composition of nutrient mixture was another major phenomenon in the production and multiplication of gladiolus corms and cormels under aeroponics. Keeping the above in view, the study on corm and cormels production in gladiolus through aeroponics using different nutrient mixtures were carried out to standardize the nutrient solution and to find the suitable gladiolus variety for aeroponics system.

MATERIALS AND METHODS

The experiment was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru during the year 2019 to 2021. The eleven gladiolus varieties namely Arka Amar, Arka Aayush, Arka Naveen, Arka Poonam, Arka Shobha, Arka Kesar, Arka Tilak, Arka Pratham, Arka Gold, Arka Manorama and Arka Ranjini were used for the study. The aeroponic system was designed and fabricated at ICAR-IIHR, Bengaluru (Hemlata, 2019), was used for this study. The aeroponic system consisted of root chamber (18 x 90 x 90 cm) with sliding door, nutrient tank (80 litre capacity) and automated nutrient misting system with 50-micron sprinklers. The nutrient tank was connected with motor (0.5 hp) to pump the nutrient solution to the root chamber with the pressure of 60 psi. The motor was connected with the automated timer to manage the misting time and duration.

The uniform size of corms (4 to 5 cm) was planted in the net pot and burnt clay balls were placed over the corms for the support. Three different nutrient regimes viz., N1-96:18:108 ppm, N2-128:24:144 ppm and N3-160:30:180 ppm of NPK were applied. The pH of the nutrient solutions N1, N2 and N3 were 6.5, 6.7 and 6.9 and EC were 1.1, 1.2 and 1.3 dSm^{-1} respectively. For all nutrient regimes, the secondary and micronutrients were kept unchanged. The mean temperature observed during the crop growth period in the aeroponics system was 25 to 30 ºC. The experiment was conducted using factorial completely randomized design with three replications. The flower spikes emerged were removed without disturbing the leaves as experiment was carried out for corm and cormels production, The observations on plant height (cm), leaf length (cm), leaf width (cm), root length (cm), root growth (visual scoring), corm weight (g), corm size (cm), number of corms per plant, number of cormels per corm and weight of cormels (g) were recorded. The data collected were analysed statistically.

RESULTS AND DISCUSSION

The effect of eleven different gladiolus varieties, three different nutrient regime and their interaction on multiplication efficiency under aeroponics system was studied. The results revealed the significant differences for the growth parameters (Table 1). Among the varieties, Arka Naveen recorded the highest plant height (109.44 cm), on par with Arka Amar (104.77 cm), Arka Aayush (99.50 cm), Arka Kesar (96.55 cm) Arka Shobha (78.59), and Arka Ranjini (78.61 cm), while, lowest was recorded in Arka Manorama (10.33 cm). Among the three different nutrient regimes, N2 registered maximum plant height (85.44 cm), on par with N3 (77.42 cm). There was no significant influence noticed for the plant height with respect to the interaction of variety and nutrient regime.

Leaf length was recorded highest in Arka Amar (68.66 cm), on par with Arka Naveen (61.23 cm), Arka Aayush (54.68 cm) and Arka Ranjini (51.38 cm). The variety Arka Kesar (2.22 cm) recorded highest leaf width, on par with Arka Aayush (2.11 cm), Arka Naveen (1.82 cm) Arka Amar (1.79 cm) and Arka Shobha (1.78 cm). Among the three different nutrient regime N2 recorded the highest leaf width (1.71 cm), on par with N3 (1.47 cm). Nutrient regime alone and interaction of varieties with nutrient regime have not recorded significant differences for leaf length and width. The variation in plant growth among the varieties are due to the inherent genetic character of the individual genotype. Apart from that the different concentrations of nutrients present in the three different nutrient regime has greater influence on the plant growth parameters. Effat et al. (2018) reported that the plant height of gladiolus was increased under aeroponic culture with the increasing flow rate of nutrients. As per Ritter et al. (2001), the plants grown under aeroponics grow fast and absorb nutrients better than the plants grown in hydroponics. The growth of gladiolus was reduced in nutrient regime (N1) with low concentrations of nutrient solution, which might be probably due to low availability of mineral nutrients.

Table 1 : Effect of different nutrient regime on growth parameters of gladiolus varieties under aeroponics

Treatment	Plant height (cm)				Leaf length (cm)				Leaf width (cm)			
	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Arka Amar	104.33	103.00	106.97	104.77	68.47	66.17	71.33	68.66	1.47	2.27	1.63	1.79
Arka Aayush	90.80	104.67	103.03	99.50	44.17	58.57	61.30	54.68	1.63	2.83	1.87	2.11
Arka Naveen	97.17	117.50	113.67	109.44	61.40	65.17	57.13	61.23	1.40	1.83	2.23	1.82
Arka Poonum	68.67	33.33	67.00	56.33	36.73	18.83	45.33	33.63	1.37	0.70	0.90	0.99
Arka Shobha	72.67	92.93	70.17	78.59	35.60	42.43	47.27	41.77	1.73	1.80	1.80	1.78
Arka Kesar	71.50	108.33	109.83	96.56	37.10	44.37	55.50	45.66	1.93	2.53	2.20	2.22
Arka Tilak	0.00	85.17	106.00	63.72	0.00	51.67	69.87	40.51	0.00	1.13	1.93	1.02
Arka Pratham	51.60	65.47	53.70	56.92	21.33	26.70	39.13	29.06	0.87	1.10	1.20	1.06
Arka Gold	67.00	104.50	37.33	69.61	42.30	58.40	21.53	40.74	1.30	2.07	0.63	1.33
Arka Manorama	0.00	31.00	0.00	10.33	0.00	21.00	0.00	7.00	0.00	0.53	0.00	0.18
Arka Ranjini	58.00	93.93	83.90	78.61	42.67	60.07	51.40	51.38	0.80	1.97	1.77	1.51
Mean	61.98	85.44	77.42		35.43	46.67	47.26		1.14	1.71	1.47	
	C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)	
Variety	32.01	15.99	11.31		20.83	10.41	7.36		0.661	0.330	0.234	
Nutrient	16.72	8.35	5.90		NS	5.43	3.84		0.345	0.173	0.122	
V x N	NS	27.71	19.59		NS	18.03	12.75		NS	0.572	0.405	

Rooting parameters are important to understand the performance of gladiolus varieties under aeroponics system (Table 2). Profuse root growth was observed in majority of the gladiolus varieties under three different nutrient regimes. The gladiolus varieties susceptible to *Fusarium* wilt are not able to withstand the continuous water spray and subsequently rotten and not survieved in all the nutrient regimes under aeroponics system. The root length was recorded maximum in Arka Aayush (80.94 cm), on par with Arka Naveen and Arka Kesar. Among the nutrient regime N2 recorded the highest root length (62.10 cm) followed by N3 (50.13 cm). In contrary to present results, Souret & Weathers (2000) reported that the root growth of *Crocus* under aeroponics culture was less as compared to soil culture. Root growth of gladiolus varieties was recorded by visual scoring, the density and appearance of the roots under aeroponics system. Root growth was profuse and recorded maximum in Arka Amar with the score of 3.44 out of 4.0, on par with Arka Naveen (3.00), Arka Gold (2.89), Arka Naveen and Arka Kesar (2.78), minimum was recorded in Arka Manorama (0.44). The *Fusarium* wilt susceptible varieties have produced few roots initially and as the disease progressed, they succumbed to wilt.

The nutrient regimes alone had no significant influence on the root growth, whereas, the interaction between the varieties and nutrient regime had the significant influence on the root growth (Fig. 1). The results of the interaction effect revealed that the variety Arka Amar recorded the highest root growth with the score of 5.00 in the N2 nutrient regime. The variety Arka Manorama which was susceptible to *Fusarium* wilt could not survive under the nutrient regime N1 and N3. Though survived in the nutrient regime N2, it recorded lowest score for root growth (1.33). As a whole, the root growth in gladiolus was better under aeroponics and this might be due to the fact that the plants are placed in air gets good root aeration and 100% of existing oxygen (Sun et al., 2004).

Among varieties, Arka Amar recorded the highest number of corms (1.89), on par with Arka Ranjini (1.78), Arka Aayush (1.44), Arka Naveen (1.44) and Arka Shobha (1.67) (Table 3 & 4). The nutrient regime alone has not recorded any significant influence on the corm production. The interaction between varieties and nutrient regime revealed that the variety Arka Amar under nutrient regime N2 recorded the highest number of corms per plant (2.67), on par with Arka Naveen (2.00), Arka Pratham (2.00), Arka

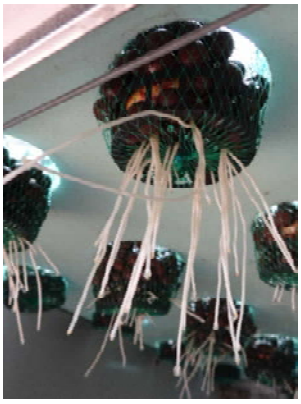
Table 2 : Effect of different nutrient regime on root parameters of gladiolus varieties under aeroponics

Treatment	Root length (cm)				Root growth (visual scoring)			
	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Arka Amar	44.70	59.50	72.17	58.79	3.00	5.00	2.33	3.44
Arka Aayush	65.33	102.00	75.50	80.94	3.00	3.00	3.00	3.00
Arka Naveen	64.90	81.33	60.33	68.86	4.33	1.67	2.33	2.78
Arka Poonum	46.47	21.00	37.33	34.93	3.00	0.33	0.67	1.33
Arka Shobha	48.43	75.00	50.17	57.87	3.00	3.00	2.67	2.89
Arka Kesar	45.00	85.30	74.23	68.18	3.00	2.33	3.00	2.78
Arka Tilak	0.00	52.13	72.90	41.68	0.00	2.00	2.33	1.44
Arka Pratham	40.83	47.67	32.00	40.17	1.33	2.67	1.33	1.78
Arka Gold	39.03	84.13	28.33	50.50	2.67	4.33	1.67	2.89
Arka Manorama	0.00	14.00	0.00	4.67	0.00	1.33	0.00	0.44
Arka Ranjini	26.13	61.03	48.17	45.11	0.67	3.00	2.33	2.00
Mean	38.26	62.10	50.13		2.18	2.61	1.97	
	C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)	
Variety	21.29	10.64	7.52		1.27	0.636	0.449	
Nutrient	11.12	5.55	3.93		NS	0.332	0.235	
V x N	NS	18.43	13.03		2.20	1.10	0.778	

Table 3 : Effect of different nutrient regime on corm parameters of gladiolus varieties under aeroponics

Treatment	Corms per plant (Nos.)				Corm size (cm)				Corm weight (g)			
	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Arka Amar	2.00	2.67	1.00	1.89	3.57	4.47	3.00	3.68	14.00	26.67	10.00	16.89
Arka Aayush	1.33	1.33	1.67	1.44	3.83	4.17	4.53	4.18	22.33	24.00	29.67	25.33
Arka Naveen	1.33	2.00	1.00	1.44	3.60	3.77	4.07	3.81	13.67	22.00	20.00	18.56
Arka Poonum	1.00	0.67	2.00	1.22	2.60	1.77	3.67	2.68	5.67	3.67	15.33	8.22
Arka Shobha	1.33	1.00	1.67	1.33	4.17	3.40	3.43	3.67	25.33	15.67	11.33	17.44
Arka Kesar	1.00	1.00	1.00	1.00	4.70	4.10	4.07	4.29	27.00	20.33	19.67	22.33
Arka Tilak	1.67	0.67	0.00	0.78	3.87	3.90	0.00	2.59	18.33	18.00	0.00	12.11
Arka Pratham	0.67	2.00	0.67	1.11	2.10	3.30	1.43	2.28	8.00	17.33	4.33	9.89
Arka Gold	0.67	1.67	1.00	1.11	2.73	4.97	3.73	3.81	15.33	39.67	17.33	24.11
Arka Manorama	0.00	0.33	0.00	0.11	0.00	0.73	0.00	0.24	0.00	2.00	0.00	0.67
Arka Ranjini	2.00	2.00	1.33	1.78	3.70	4.60	2.87	3.72	16.00	26.33	8.33	16.89
Mean	1.18	1.39	1.03		3.17	3.56	2.80		15.06	19.61	12.36	
	C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)	
Variety	0.622	0.311	0.220		0.818	0.409	0.289		6.09	3.04	2.15	
Nutrient	NS	0.162	0.115		0.427	0.213	0.151		3.18	1.58	1.12	
V x N	1.07	0.538	0.381		1.41	0.708	0.501		10.54	5.27	3.72	

a. Vegetative growth of gladiolus



b. Root initiation

c. Profuse root growth of gladiolus

d. Corm and cormels production under aeroponics

e. Corm and cormels production under aeroponics

Fig. 1 (a-e) : Gladiolus multiplication under aeroponics system

Ranjini (2.00) and Arka Gold (1.67). The varieties Arka Amar and Arka Ranjini recorded 2.0 corms per plant in the N1 nutrient regime. The considerable number of corms of the varieties Arka Manorama was died due to *Fusarium* infestation which could not produce adequate root tissues to absorb nutrients. The tissue cultured plants of potato with well-established root system were transplanted in aeroponics for further multiplication, but in case of gladiolus the corms were planted and allowed to root under aeroponics for further establishment and multiplication which would have limited the survival of *Fusarium* wilt susceptible variety. The corm multiplication of gladiolus varieties under aeroponics was in line with the studies of Hemlata (2019).

The corm size is one of the economic characters which plays major role in deciding the standard size and cost of planting material. Among the different varieties studied, the corm size was recorded maximum in Arka Kesar (4.29 cm), on par with Arka Aayush (4.18 cm), Arka Naveen (3.81 cm), Arka Gold (3.81 cm), Arka Ranjini (3.72 cm) and Arka Amar (3.68 cm), however, minimum corm size was recorded in Arka Manorama (0.24 cm). The nutrient regime N2 registered the highest corm size (3.56 cm), on par with N1 (3.17). The interaction effect revealed that the corm size of Arka Gold (4.97 cm) was recorded maximum under N2, on par with Arka Amar (4.47 cm), Arka Aayush (4.17 cm), Arka Kesar (4.10 cm), Arka Tilak (3.90 cm) and Arka Shobha (3.40 cm).

The corm weight among the varieties, nutrient regime and interaction effect were significant. The variety Arka Aayush recorded the highest corm weight (25.33 g), on par with Arka Gold (24.11 g) and Arka Kesar (22.33 g), while, lowest corm weight (0.667 g) was observed in Arka Manorama. The corm weight was recorded maximum under the

Table 4 : Effect of different nutrient regime on cormel parameters of gladiolus varieties under Aeroponics

Treatment	No. of cormels per corm				Weight of cormels (g)			
	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Arka Amar	1.33	3.33	0.33	1.67	0.33	1.33	0.33	0.67
Arka Aayush	2.33	3.67	2.00	2.67	2.67	3.33	1.00	2.33
Arka Naveen	0.00	0.67	0.00	0.22	0.00	0.67	0.00	0.22
Arka Poonum	0.00	0.00	1.67	0.56	0.00	0.00	0.67	0.22
Arka Shobha	0.33	2.33	0.00	0.89	0.33	0.67	0.00	0.33
Arka Kesar	0.67	0.00	0.00	0.22	1.00	0.00	0.00	0.33
Arka Tilak	1.00	0.33	0.00	0.44	0.67	0.33	0.00	0.33
Arka Pratham	0.67	1.67	0.00	0.78	0.67	0.67	0.00	0.44
Arka Gold	0.00	0.67	0.00	0.22	0.00	0.33	0.00	0.11
Arka Manorama	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arka Ranjini	0.00	4.33	0.00	1.44	0.00	4.00	0.00	1.33
Mean	0.57	1.55	0.36		0.52	1.03	0.18	
	C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)	
Variety	1.61	0.808	0.571		0.994	0.497	0.351	
Nutrient	0.845	0.422	0.298		0.519	0.259	0.183	
V x N	NS	1.40	0.990		NS	0.861	0.609	

nutrient regime N2 (19.61 g) followed by N1 (15.06 g). The interaction effect showed that the corm weight was highest in the variety Arka Gold (39.67 g) under N2 followed by Arka Aayush (29.67 g) under N3. Different varieties of gladiolus had diverse effect under the nutrient regimes, the variety Arka Poonum performed better in N3, while, Arka Tilak performed well under N1 and N2. The aeroponic culture has advantages such as elevated leaf stomatal conductance, intercellular carbon dioxide concentration and higher photosynthesis rate (Sun et al., 2004). The increase in corm growth under aeroponics culture system was reported by Souret & Weathers (2000) in *Crocus* and Hemlata (2019) in gladiolus.

The influence of varieties and nutrient regime had significant effect on number of cormels and weight of cormels per plant (Table 4). Among the varieties, Arka Aayush (2.67) recorded the highest number of cormels per plant, on par with Arka Amar (1.67) and Arka Ranjini (1.44) and no cormels were produced in Arka Manorama. The nutrient regime N2 has produced highest number of cormels (1.55) per plant. The number of cormels produced were very meagre as compared to soil cultivation. Weight of cormels was recorded maximum in Arka Aayush (2.33 g), followed by Arka Ranjini (1.33 g). Among the nutrient regime, N2 recorded the highest weight of cormels per plant (1.03 g), on par with N1 (0.52 g). The cormels are produced from the axillary bud of the mother corms (Teixeira da & Silva, 2003), which are placed around the daughter corms inside the net pot and though roots are vigorous, the cormels are not produced on fibrous roots as like potato. There was no difference on crop duration of the varieties under aeroponics, and cormels production was very low as compared to the soil production. The aeroponics system can also be experimented further in gladiolus to increase the size of the cormels in shorter period instead of soil planting.

CONCLUSION

The study indicated that gladiolus varieties Arka Amar, Arka Aayush and Arka Naveen and nutrient regimes N2 (128:24:144 ppm of NPK) found best for corm and cormels production under aeroponic system. The aeroponics system can be studied further to standardize the interval, frequency of nutrient spray and nozzle size as well as the suitability of other gladiolus varieties for multiplication under aeroponic system.

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Original Research Paper

Response of gerbera under soil and soil-less production systems

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ABSTRACT

Response of gerbera var. Arka Nesara was studied under different soil-less production systems with the aim to improve quality, productivity and water use efficiency, in completely randomized design with eight treatments *viz*., deep flow technique (DFT), nutrient film technique (NFT), aggregate wick system (AWS) with pots on NFT bench, AWS with pots on ground, AWS with grow bags on ground, AWS on soil bed, aeroponics and conventional soil-based drip system, which were replicated five times. Results revealed that AWS with pots on ground recorded maximum plant height (31.66 cm), leaf length (21.31 cm), leaf breadth (9.96 cm) and stalk diameter near neck (4.75 mm), minimum stem deviation (1.27 cm) and stem deflection (2.2°), prolonged vase life (9.2 days), greater water use efficiency (92 mL/plant/day) and water saving (83.46%). Hence, cultivation of gerbera var. Arka Nesara under aggregate wick system with pots on ground could be the superior alternative for traditional soil cultivation.

Keywords: Capillary action, gerbera, water saving, wick system

INTRODUCTION

Gerbera jamesonii, family Asteraceae, is an important cut flower ranked one among the most-traded ornamental plants in the global market. In India, it is cultivated in an area of 5,730 ha with the production of 28,620 MT (Anon., 2023). Gerbera is usually grown on conventional soil beds under protected structures. However, soil as a substrate has several inherent problems like compaction, nutrient immobilization, soil alkalinity, salinity, soil-borne pests and diseases limiting the crop productivity. Therefore, a soil-less cultivation system could be highly helpful for lucrative gerbera cultivation.

Deep flow technique, nutrient film technique, floating technique, root dipping technique, capillary action (wick) technique and aeroponics are the different types of soil-less cultivation. Adopting the appropriate soil-less system is imperative to achieve better yield and quality in gerbera. Moreover, focus should be turned towards amplifying the water use efficiency considering the diminishing water availability. Wick irrigation system has been known to have superior advantages in terms of water and nutrient use efficiency along with reduced cultivation cost. Keeping these points in view, a study was carried out to identify the suitable soil-less system for growing gerbera under protected condition.

MATERIALS AND METHODS

The experiment was conducted in a naturally ventilated polyhouse at Division of Flower and Medicinal Crops, ICAR-IIHR, Bengaluru from November 2021 to February 2023. The experiment was laid out with eight treatments in completely randomized design with five replications. The treatments comprised of T_1 - deep flow technique (DFT), T_2 - Nutrient film technique (NFT), T_3 - aggregate wick system (AWS) with pots on NFT bench, T_4 - AWS with pots on ground, T_5 - AWS with grow bags on ground, T_6 - AWS on soil bed, T_7 - aeroponics and T_8 - conventional soil-based drip system.

The DFT structure used was an A-shaped frame consisting 7 cylindrical PVC pipes of food grade stacked horizontally one over the other with three tiers on each side. NFT structure had a vertical stand 1 meter above the ground level with 6 rectangular channels rested over it. In AWS with pots on NFT bench, 8-inch round pots with one nylon wick per pot were placed on the NFT bench. In AWS with pots on ground, PVC pipes of 2-inch diameter drilled with holes were laid beneath the ground and 10-inch pots

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with two nylon wicks each were inserted onto the holes. In AWS with grow bags on ground, HDPE grow bags of 12-inch diameter with 2 nylon wicks each were kept on drilled PVC pipes. AWS on soil beds was setup by placing the PVC pipes inserted with nylon wicks 5-10 cm below the ground level on conventional soil bed. A low cost aeroponic structure was fabricated in which the semi-transparent rigid PVC panel with planting holes was rested over the vertical stand of 3 m length, 1 m width and 50 cm height. Net pots were used to hold plants in liquid culture systems. The growing medium comprising Arka fermented cocopeat: FYM (1:1) was employed for AWS with pots on NFT bench, pots on ground and grow bags on ground. Arka fermented cocopeat was prepared as per Selvakumar et al. (2016).

Uniform, hardened tissue-cultured plants of gerbera var. Arka Nesara were used as planting material. Water soluble fertilizer, 19:19:19 N: P_2O_5: K_2O was supplied at the TDS 600-650 ppm (vegetative stage) and 900-1000 ppm (flowering stage) via nutrient reservoir. Further, calcium nitrate (0.5%) and magnesium sulphate (0.5%) were sprayed once in a week. Also, a micronutrient mix 'Fertilon Combi-1®' (0.3%) (containing 0.5% boron, 1.5% copper, 4% iron, 4% manganese, 0.1% molybdenum and 1.5% zinc) was sprayed twice in a week. Plant protection measures were carried out as and when required.

Observations on plant growth, flowering and yield parameters were noted down. Days to flower bud initiation and days to first flower harvest were counted from the date of planting till the appearance of first flower bud and first harvest, respectively. Whereas, days to bud opening and harvestable maturity were calculated from the date of bud initiation to the date of flower bud opening and the attainment of harvestable maturity (i.e. when the disc florets were perpendicular to the flower stalk), respectively. Stalk diameter near neck and at base were measured using vernier caliper at the point immediately below the flower head and at the distal end of the flower stalk 1 cm above the cut end, respectively. Stem deviation was recorded by measuring the deviation of flower stalk from the straight axis, whereas stem strength was assessed by observing the degree of deflection from the horizontal plane by holding the base of flower stalk horizontally (Anon., 1994). Comparison of treatment means and analysis of variance were worked out in Microsoft Office Excel 2007 as per the statistical method described by Panse & Sukhatme (1985).

RESULTS AND DISCUSSION

Effect on vegetative growth

Different production systems had significant impact on vegetative growth of gerbera var. Arka Nesara (Table 1). Results revealed that plants grown on AWS with pots on ground exhibited maximum plant height (31.66 cm), leaf length (21.31 cm) and leaf breadth (9.96 cm) at 3 months after planting. Conversely, more number of leaves per plant (12.92) was observed in AWS on soil bed. Hence, it can be inferred that AWS with pots on ground showed the enhanced vegetative growth which might be attributed to the continuous

Table 1 : Vegetative growth of gerbera var. Arka Nesara under different production systems at 3 months after planting

Treatment	Plant height (cm)	Leaves per plant (Nos.)	Leaf length (cm)	Leaf breadth (cm)
Deep flow technique	15.54	5.34	11.16	5.94
Nutrient film technique	17.07	6.84	11.78	6.02
AWS with pots on NFT bench	29.70	11.24	18.69	9.20
AWS with pots on ground	31.66	12.28	21.31	9.96
AWS with grow bags on ground	30.70	10.44	19.51	8.65
AWS on soil bed	30.03	12.92	20.93	9.62
Aeroponics	15.86	6.96	11.00	5.97
Drip system on soil bed	26.39	9.68	18.23	8.28
S.E.m±	0.38	0.17	0.17	0.10
C.D. at 5%	1.10	0.49	0.50	0.28
C.V. (%)	7.78	8.16	5.22	6.13

Table 2 : Flowering and yield parameters of gerbera var. Arka Nesara as influenced by different production systems

Parameter	AWS with pots on NFT bench (T_3)	AWS with pots on ground (T_4)	AWS with grow bags on ground (T_5)	AWS on soil bed (T_6)	Drip system on soil bed (T_8)	S.E.m±	C.D. at 5%	C.V. (%)
Days to bud initiation	135.80	133.56	134.28	132.76	137.80	0.71	NS	2.62
Days to bud opening	13.04	11.32	12.20	10.80	14.96	0.18	0.52	7.09
Days to harvestable maturity	20.00	17.68	17.76	17.04	21.96	0.25	0.73	6.59
Days to first flower harvest	155.80	151.24	152.04	149.80	159.76	0.77	2.28	2.51
Stalk length (cm)	44.98	46.54	47.79	50.36	45.56	0.44	1.29	4.65
Flower diameter (cm)	9.66	9.99	9.68	10.32	9.53	0.07	0.21	3.62
Stalk diameter near neck (mm)	4.64	4.75	4.72	4.74	4.08	0.06	0.18	6.48
Stalk diameter at base (mm)	9.54	9.24	9.04	9.32	8.14	0.15	NS	8.23
Stem deviation (cm)	1.50 (1.22)	1.27 (1.13)	1.29 (1.14)	1.44 (1.20)	1.33 (1.15)	0.01	0.03	4.16
Stem strength (°)	2.60 (1.60)	2.20 (1.48)	2.40 (1.54)	3.56 (1.89)	3.40 (1.84)	0.03	0.08	8.05
Vase life (days)	8.0	9.2	8.4	7.8	7.4	0.16	0.46	9.59
No. of flowers/plant/year	25.66	30.16	28.76	36.58	23.90	0.43	1.26	7.35

*Values in the parentheses are square-root transformed.

** T_1, T_2 and T_7 showed 100% plant mortality after 4 months of planting. Hence, flowering and yield parameters were not recorded.

supply of water and nutrients through the capillary action. Uninterrupted water and nutrient supply might have had an indirect role on faster cell division and differentiation resulting in the superior vegetative growth. Moreover, the capillary movement of water and nutrients might have left the media structure undisturbed thus providing better aeration by curbing the media compaction ultimately making the nutrients readily available to the plants. The similar results were also reported by Hahn et al. (2001) and Arathi (2016) in gerbera.

Effect on floral traits

It is obvious from Table 2 that accelerated flower bud opening (10.80 days), harvestable maturity (17.04 days) and first flower harvest (149.80 days) with the highest number of flowers per plant per year (36.58), stalk length (50.36 cm) and flower diameter (10.32 cm) were recorded with AWS on soil bed. However, AWS with pots on ground showed on par results with respect to days to harvestable maturity (17.68) and days to first flower harvest (151.24). The earlier bud initiation might be due to the greater source-sink relationship, faster translocation of nutrients and growth stimulating substances leading to earlier floral bud differentiation (Ruby et al., 2020). Further, plants grown on AWS with pots on ground exhibited minimum stem deviation (1.27 cm) and stem strength (2.20 cm) with thicker neck (4.75 mm) and prolonged vase life (9.2 days). The extended vase life could be related to the higher accumulation of photosynthates and metabolic substances in the flower (Arunesh et al., 2020).

On the perusal of data presented in Table 3 revealed that days to bud initiation showed the strongest correlation with days to bud opening (0.995), days to harvestable maturity (0.988) and days to first flower harvest (0.997). Further, leaf length (-0.910, -0.914 & -0.892) and number of flowers per plant per year (-0.905, -0.890 & -0.884) were negatively correlated with days to flower bud initiation, days to bud opening and days to first flower harvest. Also, number of leaves per plant exhibited positive correlation with the flower diameter (0.937). Similar results were reported by Hahn et al. (2001), Arathi (2016) in gerbera, and Wahome et al. (2011) in gypsophila. However, plants grown on liquid culture systems failed to produce flowers and started rotting at 4 MAP, which might be due to inadequate mechanical support and poor aeration near root environment.

Table 3 : Correlation data on vegetative, flowering and yield parameters of gerbera var. Arka Nesara

Trait	PH	LN	LL	LB	DBI	DBO	DHM	DFH	SL	FD	ND	BD	SD	SS	VL	FNY
PH	1.00	.662	.764	.753	-.851	-.867	-.873	-.864	.338	.518	.956*	.800	-.102	-.677	.857	.548
LN		1.00	.866	.937*	-.854	-.889	-.763	-.810	.600	.937*	.737	.726	.310	.011	.408	.855
LL			1.00	.863	-.910*	-.914*	-.869	-.892*	.639	.870	.722	.505	-.199	-.191	.666	.834
LB				1.00	-.777	-.828	-.692	-.736	.335	.785	.739	.740	.152	-.271	.642	.661
DBI					1.00	.995**	.988**	.997**	-.764	-.860	-.897*	-.697	-.018	.200	-.571	-.905*
DBO						1.00	.973**	.987**	-.717	-.865	-.914*	-.747	-.067	.224	-.590	-.890*
DHM							1.00	.997**	-.754	-.780	-.907*	-.667	.064	.275	-.601	-.859
DFH								1.00	-.761	-.822	-.905*	-.684	.024	.239	-.588	-.884*
SL									1.00	.817	.477	.244	.070	.395	-.014	.927*
FD										1.00	.614	.511	.205	.246	.240	.965**
ND											1.00	.899*	.151	-.497	.668	.664
BD												1.00	.507	-.412	.479	.496
SD													1.00	.384	-.483	.159
SS														1.00	-.847	.235
VL															1.00	.211
FNY																1.00

PH- plant height, LN- number of leaves/plant, LL- leaf length, LB- leaf breadth, DBI- days to bud initiation, DBO- days to bud opening, DHM- days to harvestable maturity, DFH- days to first harvest, SL- stalk length, FD- flower diameter, ND- stalk diameter near neck, BD- basal stalk diameter, SD- stem deviation, SS- stem strength, VL- vase life, FNY- number of flowers/plant/year

Effects on plant water consumption

Data presented in Table 4 revealed that plants grown on AWS with pots (T_4) and grow bags (T_5) on ground consumed minimum amount of water (92 and 96 mL/ plant/day), thus, saving around 83.46 and 82.70% of water as compared to soil-based drip system, respectively. The better water use efficiency in AWS might be associated with reduced drainage loss and efficient water usage attributed by capillary action. Yeager and Henley (2004) reported similar results in capillary wick irrigation systems.

Table 4 : Water consumption in different production systems

Treatment	Water consumption per plant per day (mL)	Water saving over drip system (%)
AWS with pots on NFT bench	103	81.39
AWS with pots on ground	92	83.46
AWS with grow bags on ground	96	82.70
AWS on soil bed	307	44.75
Drip system on soil bed	555	0.00

Table 5 : Economics of different production systems

Treatment	Annual (amortized) cost of cultivation (Rs.)	*No. of flowers/unit/ year	**Gross returns (Rs.)	Annual net returns (Rs.)	BC ratio
AWS with pots on NFT bench	1,22,908	15,396	76,980	-45,928	0.63
AWS with pots on ground	1,27,028	36,192	1,80,960	53,932	1.43
AWS with grow bags on ground	1,45,052	34,512	1,72,560	27,508	1.19
AWS on soil bed	1,47,212	52,675	2,63,375	1,16,163	1.79
Drip system on soil bed	1,33,148	34,416	1,72,080	38,932	1.29

*1 unit = 180 m^2 ; **price @ Rs. 5/ flower; T_1, T_2 and T_7 showed 100% plant mortality at 4 months after planting.

Economics

It is evident from Table 5 that the annual net returns (Rs. 1,16,163) and benefit cost ratio (BCR) (1.79) were higher in AWS on soil beds (T_6) followed by AWS with pots on ground (T_4) with the annual net returns of Rs. 53,932 and BCR of 1.43. Greater returns in T_6 were exclusively associated with higher flower yield whereas in T_4, it was contributed by both cost saving and yield returns when compared with control (T_8).

CONCLUSION

Gerbera plants of var. Arka Nesara grown under aggregate wick system on pots, grow bags and soil bed showed better results compared to the liquid culture systems. Aggregate wick system with pots on ground exhibited superior vegetative growth, flower stem quality and water use efficiency, while, aggregate wick system on soil bed resulted in earlier harvest and higher production with bigger flowers and longer stalks. Considering the maximum water saving, aggregate wick system with pots on ground could be employed as an alternative for conventional soil-based drip system.

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Original Research Paper

Effect of land configuration and fertilizer dosage on growth and yield of African marigold under vertic ustochrept soil regimes

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ABSTRACT

Adopting proper land management systems and nutrient levels could improve growth, yield and quality by rendering better soil physical structure and mineral nutrition under heavy rainfall areas. An experiment was conducted to identify appropriate land configuration and fertilizer dose for African marigold var. Punjab Gainda-1, in split plot design with three land configuration methods *viz.*, flat bed, raised bed and ridge & furrow system as main plots, and three fertilizer doses (RDF/ha) *viz.*, 100%, 80% and 60% as subplots with recommended dose of fertilizer (RDF) being 10 t FYM + 150:100:100 kg/ha NPK. Results showed that adopting raised bed method of land configuration with the application of 8 t FYM + 120:80:80 kg/ha NPK exhibited enhanced vegetative growth and flower yield (16.26 t/ha) with greater benefit cost ratio (2.88) and, hence, found economically best for commercial production of African marigold.

Keywords : African marigold, fertilizer dose, land configuration, quality, yield

INTRODUCTION

African marigold (*Tagetes erecta* L.) belonging to the family Asteraceae, holds considerable importance in commercial floriculture due to its profuse flowering, short duration, ease of cultivation and wider acceptability across agro-climatic zones of the country. In India, marigold is being cultivated in 81,540 ha with the production of 9,23,430 MT (Anon., 2023). It is regarded as the 'State flower of Gujarat' since most of the festival celebrations in Gujarat rely on marigold loose flowers for decoration and worship. South Gujarat region receives relatively heavy rainfall with an average of 1675 mm. Its soil type is vertic ustochrept characterized by low infiltration, poor internal drainage, low organic matter and high cation exchange capacity. Thus, improving the physical conditions of soil to enhance crop production seems extremely important. This can partly be accomplished through land management system. Land management system plays a significant role in minimizing soil erosion, improving water use efficiency and increasing nutrient availability to crops (Chiroma et al., 2008).

Apart from land configuration, nutrition plays an important role in determining the growth and yielding ability of crop. However, their indiscriminate application alters the soil fertility leading to pollution of soil and water bodies, and also increases the cost of cultivation. Thus, deriving an optimal dose of fertilizers based on the fertility status of the above-mentioned soils could help the farmers of heavy rainfall area save additional expenditure and earn better revenue out of their produce. Keeping these issues in view, an experiment was conducted to assess the different land configuration methods and nutrient levels with respect to growth, quality and yield of African marigold var. Punjab Gainda-1 under south Gujarat conditions.

MATERIALS AND METHODS

The present investigation was conducted at Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari during *kharif* season of 2018. The experiment was laid out with nine treatment combinations in which three land configuration *viz.*, flat bed (L_1), raised bed (L_2) and ridge & furrow system (L_3) as main plots, and three fertilizer doses (RDF/ha) *viz.*, 100% (10 t FYM/ha + 150:100:100 kg, N_1), 80% (8 t FYM/ha + 120:80:80 kg NPK/ha, N_2) and 60% (6 t FYM/ha + 90:60:60 kg NPK/ha, L_3) as sub-plots with recommended dose of fertilizer (RDF) being 10 t FYM + 150:100:100 kg/ha NPK, were replicated five times in split plot design.

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One month old marigold seedlings with 3-4 leaves were transplanted at the spacing of 60 cm x 40 cm at different land configuration treatments. Plants were applied with two equal doses of N in the form of urea during bed preparation and after pinching, whereas, full dose of P and K were applied as basal dose in the form of single super phosphate and muriate of potash, respectively as per the treatment. The observations were recorded on various vegetative, flowering and yield parameters from the net plot of each treatment and analyzed statistically as described by Panse & Sukhatme (1985) in Microsoft Office Excel 2007.

Observations on plant height, plant spread and number of branches per plant were recorded at 90 days after transplanting, whereas, fresh weight and dry weight of the plant were recorded at the end of experiment. Days to flower bud initiation, flower bud opening and 50% flowering were calculated from the date of transplanting to the date of first bud initiation, bud opening and the stage when the plot shows 50% flowering, respectively. Number of flowers per plant was counted during each picking and summed up at the end of the season. Similarly, flower yield was calculated by weighing flowers obtained from the single plant with the help of weighing balance. Fresh weight of 10 flowers and flower diameter were recorded at the time of second picking. The harvested flowers were kept under ambient conditions and the shelf life was calculated from the date of harvesting to flower senescence. Number of days taken from the date of first flower opening to the last flowering was recorded as the flowering duration.

RESULTS AND DISCUSSION

Effect of land configuration

Marigold plants grown on raised beds recorded significantly increased plant height (104.41 cm), plant spread (72.06 cm), number of branches per plant (11.91), fresh weight (1083.60 g) and dry weight (594.80 g) of plant when compared with flat bed system, thus, resulting in improved vegetative growth (Table 1). This might be associated with better aeration, drainage, root environment, nutrient utilization, soil plant water relationship and soil aggregate stability during water-logging conditions (Gudge, 2015). Moreover, plants on raised beds achieved first flower bud initiation, bud opening and 50% flowering by 12.87, 10.52 and 10.80 days earlier than those on flat beds, respectively, which might be due to hastened metabolic activities, cell differentiation and enzymatic reaction associated with structural change of leaf primordia to floral primordia.

Table 1 : Effect of land configuration on growth, flowering and yield of African marigold

Parameter	Flat bed	Raised bed	Ridge & furrow system	S.E.m±	C.D. at 5 %
Plant height (cm)	89.47	104.41	92.87	1.82	5.92
Plant spread (cm)	61.57	72.06	61.98	0.96	3.13
Number of branches per plant	8.26	11.91	8.63	0.24	0.79
Fresh weight of plant (g)	736.93	1083.60	890.53	30.10	98.16
Dry weight of plant (g)	338.33	594.80	428.53	13.65	44.52
Days to bud initiation	59.93	47.06	58.51	1.28	4.17
Days to bud opening	71.47	60.95	69.68	1.14	3.73
Days to 50% flowering	80.73	69.93	79.93	1.56	5.08
Flower diameter (cm)	4.44	5.36	4.60	0.12	0.38
Fresh weight of 10 flowers (g)	55.17	65.05	55.69	1.50	4.90
Number of flowers per plant	103.72	139.53	105.36	2.81	9.16
Flowering duration (days)	87.47	99.67	89.27	1.49	4.87
Shelf life (days)	2.73	4.40	2.87	0.09	0.29
Flower yield per plant (g)	358.76	567.11	436.33	11.32	36.91
Flower yield per ha (t)	8.99	14.62	9.03	0.29	0.95

Raised bed enhanced the flower production (139.53/ plant) by 34.53% than the flat beds along with maximum flower diameter (5.36 cm) and fresh weight of 10 flowers (65.05 g). Maximum food reserves, better water and nutrient uptake and reduced starvation in the plants grown on raised bed system attributed by good soil structure and physical conditions also might have exerted pronounced effects on flower quality of marigold. Further, flowering duration and shelf life were extended to 12.20 and 1.67 days, respectively, which might be associated with the consistent soil moisture availability under raised bed system. Enhanced flower yield (567.11 g/plant and 14.62 t/ha) in raised bed method which was 58-62% higher than the flat bed system could be associated with maximum number of branches and earlier bud development leading to maximum number of flowers per plant and more number of pickings, respectively. These findings were in agreement with Kumar et al. (2016) in marigold, Chawla et al. (2018) in tuberose and Kumar & Sharma (2018) in saffron.

Effect of nutrient management

Plants supplied with 10 t/ha FYM + 150:100:100 kg NPK/ha recorded maximum plant height (98.46 cm), plant spread (67.14 cm), number of branches per plant (10.13), plant fresh weight (966.53 g) and plant dry weight (473.60 g) followed by 80% RDF (Table 2). This might be because of higher availability of N and P which are directly involved in improving the vegetative growth and root development. The 100% RDF also resulted in maximum number of flowers per plant (128.16), flower diameter (4.91 cm), fresh weight of 10 flowers (61.55 g), flowering duration (96.93 days), flower yield (500.68 g/plant and 11.30 t/ha) and extended shelf life up to 3.73 days with minimum days to bud initiation (53.97), days to bud opening (66.27) and 50% flowering (72.47). The enhanced vegetative growth might have accelerated the photosynthesis, carbohydrate production and food accumulation in vegetative parts resulting in rapid transition to reproductive structures and higher number of pickings with amplified flower production. These findings are in accordance with the earlier reports of Kishore (2016) and Ahmed et al. (2017) in marigold, and Kitty et al. (2019) in chrysanthemum.

Interaction effect

Plant spread (78.33 cm) and the number of branches per plant (13.14) was significantly increased by 21 & 46%, respectively when the plants were grown on raised beds with the supply of 80% RDF compared to flat bed + 100% RDF. Similarly, number of flowers (165.68/ plant) and flower yield (16.26 t/ha) were enhanced by 29.11 and 59.10% with the raised bed system + 80% RDF (Table 3). These remarkable

Table 2 : Effect of different fertilizer doses (RDF/ha NPK) on growth, flowering and yield of African marigold

Parameter	100%	80%	60%	S.E.m ±	C.D. at 5 %
Plant height (cm)	98.46	97.24	91.05	1.51	4.42
Plant spread (cm)	67.14	66.97	61.51	0.87	2.53
Number of branches per plant	10.13	9.90	8.77	0.23	0.66
Fresh weight of plant (g)	966.53	925.60	818.93	23.55	68.73
Dry weight of plant (g)	473.60	470.87	417.20	15.45	45.08
Days to bud initiation	53.97	54.98	56.54	0.67	1.95
Days to bud opening	66.27	66.56	69.27	0.83	2.42
Days to 50% flowering	72.47	75.93	82.20	1.37	4.01
Flower diameter (cm)	4.91	4.89	4.62	0.09	0.25
Fresh weight of 10 flowers (g)	61.55	61.48	52.89	1.40	4.08
Number of flowers per plant	128.16	127.97	92.48	1.58	4.60
Flowering duration (days)	96.93	94.53	84.93	1.82	5.31
Shelf life (days)	3.73	3.67	2.60	0.10	0.30
Flower yield per plant (g)	500.68	477.16	384.35	10.65	31.09
Flower yield per ha (t)	11.30	11.25	10.09	0.17	0.50

Table 3 : Interaction effect of land configuration and fertilizer dosage on African marigold

Parameter	L_1N_1	L_2N_1	L_3N_1	L_1N_2	L_2N_2	L_3N_2	L_1N_3	L_2N_3	L_3N_3	S.Em.±	C.D. at 5 %
Plant height (cm)	95.50	104.74	95.14	88.10	109.86	93.75	84.81	98.63	89.71	2.62	NS
Plant spread (cm)	64.39	70.52	66.51	62.19	78.33	60.39	58.14	67.34	59.05	1.50	4.38
No. of branches per plant	9.00	11.88	9.50	8.18	13.14	8.38	7.60	10.70	8.02	0.39	1.14
Fresh weight of plant (g)	832.40	1125.60	941.60	699.20	1148.00	929.60	679.20	977.20	800.40	40.79	NS
Dry weight of plant (g)	377.20	571.60	472.00	323.40	659.20	430.00	314.40	553.60	383.60	26.75	NS
Days to bud initiation	59.08	45.80	57.04	61.42	46.56	56.96	59.28	48.83	61.52	1.16	NS
Days to bud opening	67.90	60.84	70.08	72.96	58.84	67.88	73.56	63.16	71.08	1.43	NS
Days to 50% flowering	74.80	66.00	76.60	84.20	65.20	78.40	83.20	78.60	84.80	2.38	NS
Flower diameter (cm)	4.32	5.47	4.92	4.56	5.62	4.47	4.44	5.00	4.42	0.15	NS
Fresh weight of 10 flowers (g)	61.08	66.98	56.58	56.78	69.80	57.86	47.66	58.38	52.64	2.42	NS
No. of flowers per plant	128.32	138.48	117.68	105.00	165.68	113.24	77.84	114.44	85.16	2.73	7.97
Flowering duration (days)	95.80	104.40	90.60	85.20	106.20	92.20	81.40	88.40	85.00	3.15	NS
Shelf life (days)	3.20	4.80	3.20	3.00	4.80	3.20	2.00	3.60	2.20	0.18	NS
Flower yield per plant (g)	422.66	545.97	533.41	361.39	657.37	412.72	292.22	497.98	362.86	18.45	53.86
Flower yield per ha (t)	10.22	13.14	10.55	8.36	16.26	9.13	8.38	14.47	7.42	0.30	0.87

Table 4 : Effect of land configuration and nutrient management on economics

Treatment	Yield (t/ ha)	Fixed cost (Rs./ha)	Variable cost (Rs./ha)	Total cost of cultivation (Rs./ha)	Gross returns* (Rs./ha)	Net returns (Rs./ha)	Benefit cost ratio
L_1N_1	10.22	61559	36415	97974	255475	157501	1.61
L_1N_2	8.36	61559	29666	91225	209075	117850	1.29
L_1N_3	8.38	61559	25845	87404	209600	122196	1.40
L_2N_1	13.14	61559	42175	103734	328425	224691	2.17
L_2N_2	16.26	61559	43200	104759	406425	301666	2.88
L_2N_3	14.47	61559	36553	98112	361725	263613	2.69
L_3N_1	10.55	61559	38725	100284	263625	163341	1.63
L_3N_2	9.13	61559	32658	94217	228150	133933	1.42
L_3N_3	7.42	61559	26131	87690	185375	97685	1.11

*Price of marigold flowers = Rs. 25/ kg

results might be due to cumulative effect of better soil conditions and deep root system attributed by raised bed configuration and continuous availability of soil nutrients throughout the growing season, better fertilizer use efficiency and nutrient uptake achieved with the nutrient application. However, the interaction effect remained non-significant for rest of the other parameters. Similar findings were also reported by Augustina et al. (2017) in guar gum and Sodavadiya et al. (2017) in Indian bean.

Economics

Data presented in Table 4 revealed that raised bed system along with application of 80% RDF gained higher net returns of Rs. 3,01,665 per ha with the benefit cost ratio of 2.88 in African marigold var. Punjab Gainda-1. The reduced cost of cultivation could be attributed to optimal fertilizer application and higher returns to the greater flower yield obtained by better nutrient availability and soil physical conditions.

CONCLUSION

It can be concluded that growing of marigold on raised bed system along with the application of 8 t/ha FYM + 120:80:80 kg/ha NPK (80% RDF) could enhance the vegetative, flowering and yield attributes with maximum net returns in African marigold var. Punjab Gainda-1 under high rainfall areas having vertic

ustochrept type of soil. Thus, the farmers could be benefitted by better plant growth with the adoption of proper land configuration and less production costs with the application of optimized fertilizer dose. This would also be environmentally sustainable as it curbs the indiscriminate application of fertilizers and maintains the soil health.

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Original Research Paper

Effect of osmotic stress on *in vitro* plant growth hormone production by osmotolerant bacteria isolated from chilli phyto microbiome

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ABSTRACT

The present study was conducted to determine the effect of osmotic stress on the plant growth hormone production by six osmotolerant plant growth promoting bacterial strains. These strains originated from the phytomicrobiome of chilli cultivated in the drought prone areas of Andhra Pradesh. They possessed multiple plant growth promotion traits including the ability to produce a variety of plant growth hormones. The effect of osmotic stress on the plant growth hormone production was determined by High Performance Liquid Chromatography (HPLC) under normal and *in vitro* osmotic stress conditions using 25% Poly Ethylene Glycol (PEG) 8000. In general, it was observed that osmotic stress impacted the plant growth hormone production of the isolates, but nevertheless plant hormones were detected in all the bacterial strains. An exception to this was the cytokinin molecule zeatin riboside, which was produced at higher levels by five of the six bacterial isolates under osmotic stressed conditions.

Keywords: Chilli, cytokinin, gibberellic acid, indole acetic acid, osmotolerant bacteria, PEG 8000

INTRODUCTION

Chilli (*Capsicum annum*) an important vegetable crop of India, is used as an ingredient in various culinary preparations is valued for its pungency, conferred by the alkaloid capsaicin. Chilli cultivated in the Palnadu area of Andhra Pradesh, is impacted by low moisture during its growth and yields are impacted, thereby requiring interventions. The utilization of stress tolerant plant growth promoting rhizobacteria (PGPR) for overcoming deficit irrigation stress (Bouremani, 2022) has gained traction worldwide. Such bacterial strains improve plant growth through improved shoot and root biomass, root length and root surface area as a result of multiple plant growth promotion traits (Masood et al., 2020). The improved plant growth performance can be attributed IAA production (Zhang et al., 2020), phosphate solubilization (Audipudi et al., 2021), siderophore production (Ashry et al., 2022), ACC deaminase activity (Danish et al., 2019), production of gibberellins (Selvakumar et al., 2015) and cytokinins (Di et al., 2023). To select potential bacterial strains for deficit irrigation stress alleviation, initial screening under *in vitro* conditions using an osmoticum like Poly Ethylene Glycol (8000) is a prerequisite (Zhang et al., 2020; Ashry et al., 2022). Therefore, this study was undertaken to study the effect of PEG 8000 induced osmotic stress on the plant growth hormone production by six osmotolerant bacterial isolates.

MATERIALS AND METHODS

Osmotolerant bacterial strains

Six osmotolerant bacterial strains (previously isolated using 25% PEG 8000 as a selection agent), originating from chilli phytomicrobiome samples collected from the Palanadu region of Andhra Pradesh, India, were used. They were identified by Sanger sequencing of the 16S rRNA gene as *Atlantibacter hermannii* R11, *Enterobacter* sp. R19, *Achromobacter* sp. T26, *Lysinibacillus composti* T55, *Atlantibacter hermannii* S12 and *Pseudomonas mosselii* S13L.

Estimation of indole acetic acid production and gibberellic acid production

To estimate the indole acetic acid (IAA) production, the media combinations *viz.*, nutrient broth; nutrient broth + tryptophan (100 µg mL^{-1}); nutrient broth + 25% PEG 8000; nutrient broth + 25% PEG 8000 + tryptophan (100 µg mL^{-1}) were used. For the

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Table 1 : Instrumentation parameters for the detection of IAA and GA_3 by HPLC

Parameter	IAA	GA_3
Stationary phase	C18 column	C18 column
Flow rate	1 mL/min	0.8 mL/min
Mobile phase	Methanol: water (80:20)	Methanol: water (70:30)
Wavelength	270 nm	208 nm
Column Temperature	30ºC	30ºC

estimation of gibberellic acid (GA_3), the media combinations *viz.*, nutrient broth and nutrient broth + 25% PEG 8000 were used as suggested by Selvakumar et al. (2015). The respective media were inoculated with 24-hour old cultures of individual isolates and incubated at 30°C for 7 days under dark conditions. After seven days, cultures were centrifuged at 6000 rpm for 10 minutes and 1N HCl was added to the supernatant, and pH adjusted to 2.8. To the acidified supernatant an equal volume of diethyl ether was added and incubated in dark for 4 hrs and stored overnight at 4°C in a separating funnel. Subsequently, the organic phase (lower phase) was discarded and the solvent phase (upper phase) was collected. To the solvent phase, a pinch of sodium sulphate was added and kept overnight and evaporated in a rotary flash evaporator. After evaporation, 2-3 mL of HPLC grade methanol was added and the resultant extract was filtered through a PVDF (polyvinylidene difluoride) filter (0.22 µm pore size, 47 mm diameter) (Selvakumar et al., 2015). The IAA and GA_3 concentrations were quantified by HPLC (Prominence, Shimadzu, Japan) as described by Kelen et al. (2004) with slight modifications. A photodiode array detector (Shimadzu, model: SPD M 20 A Japan) and 4 µm-Fusion RP-C18 column (Phenomenex, USA, 250 × 4.6 mm) were used for the assay. The IAA and GA_3 contents were quantified using external standards (Sigma-Aldrich, MO, USA). The conditions for the HPLC analysis are mentioned in Table 1.

Estimation of cytokinin production

For estimation of cytokinins, individual isolates were cultivated in M9 medium supplemented with 20% glucose, 0.2% casamino acid and 2 pg/mL biotin at 28ºC for 72-96 hours. The M9 medium supplemented with 25% PEG 8000 was used to determine the effect of osmotic stress. After incubation, the cultures were centrifuged at 16000 rpm for 10 min at 4ºC and the supernatant was filtered through a cellulose acetate filter (0.22 µm pore size, 47 mm diameter). The pH of the cell free supernatant was adjusted to 8.0 by the addition of 1N NaOH, in a separating funnel to which 30 mL of butanol was added and shaken thoroughly and allowed to settle till the clear organic phase (lower phase) and solvent phase (upper phase) were separated. The butanol fraction in the solvent phase was evaporated to dryness in a rotary flash evaporator at 40ºC and the remnants were dissolved in 2.5 mL HPLC grade methanol and filtered through a cellulose acetate filter (0.22 µm pore size, 47 mm diameter) (Selvakumar et al., 2018). The cytokinins in the filtrate were analysed by HPLC (Prominence, Shimadzu, Japan) using a PDA detector as described by Chen et al. (2010). The analysis run time was 60 min, with a flow rate of 0.2 mL/min, using a detection wavelength of 270 nm. All estimations were replicated thrice. The data was analysed with the SAS 9.3 statistical package (SAS Institute Inc, 2011).

RESULTS AND DISCUSSION

Effect of osmotic stress on indole acetic acid (IAA) production by elite osmotolerant bacterial isolates

Indole-3-acetic acid (IAA) is a primary auxin in plants which along with indole butyric acid, are collectively known as auxins. They control a variety of critical physiological processes such as seed germination, cell division, cell elongation, cell differentiation, root formation, photosynthesis and drought reaction of plants (Ullah et al., 2018). Several plant associated rhizobacterial genera such as *Microbacterium, Rhizobium, Mycobacterium* and *Sphingomonas* produce IAA (Etminani and Harighi, 2018). In the present study, IAA production was affected by the imposition of osmotic stress, but nevertheless, all the isolates produced IAA under osmotic stressed conditions. In general, it was observed that the addition of tryptophan to the growth medium enhanced IAA concentrations under both normal and osmotic stress

Table 2 : Effect of osmotic stress on indole acetic acid (IAA) production by osmotolerant bacterial isolates

Stress/ Isolate	IAA (ng mL^{-1})					
	Normal (without 25% PEG 8000)	Osmotic stress (with 25% PEG 8000)	Mean	Normal (without 25% PEG 8000 + Tryptophan)	Osmotic stress (with 25% PEG 8000 + Tryptophan)	Mean
Atlantibacter hermannii R11	912	860	886	1249	866	1058
Enterobacter sp. R19	973	851	912	998	859	929
Achromobacter sp. T26	982	855	919	1082	861	972
Lysinibacillus composti T55	1215	864	1040	1234	879	1057
Atlantibacter hermannii S12	1191	883	1037	1215	895	1055
Pseudomonas mosselii S13 L	1152	895	1024	1229	899	1064
Mean	1071	868	-	1168	877	-
Factor	Stress	Isolate	Stress x Isolate	Stress	Isolate	Stress x Isolate
SEm	1.453	2.517	3.55	1.70	2.95	4.18
C.D.	4.266	7.389	10.45	5.01	8.68	12.27

conditions. Under normal conditions in the absence of tryptophan, *Lysinibacillus composti* T55 recorded the highest concentrations of IAA (1215 ng mL^{-1}), whereas, under osmotic stress conditions *Pseudomonas mosselii* strain S13L recorded the highest concentration (895 ng mL^{-1}). With the addition of tryptophan, *Atlantibacter hermannii* strain R11 recorded the highest IAA concentration (1249 ng mL^{-1}) under normal conditions, while *Pseudomonas mosselii* strain S13L recorded the highest IAA concentration (899 ng mL^{-1}) under stress conditions. (Table 2).

The effect of osmotic stress on *in vitro* IAA production has not been reported much in the past. Selvakumar et al. (2015) reported that the plant growth promoting *Citricoccus zhacaiensis* strain B-4, produced 419.4 ng mL^{-1} of IAA under normal conditions and 301.4 ng mL^{-1} under osmotic conditions, which is in concurrence with the present study. Similar results were reported by Arun et al. (2020) who observed that osmotic stress reduced IAA production in *Bacillus megaterium* PB50 under osmotic stress conditions (15.4 µg mL^{-1}) compared to normal conditions (23.2 µg mL^{-1}).

Effect of osmotic stress on gibberellic acid (GA_3) production by elite osmotolerant bacterial isolates

Gibberellins (GAs) are an important group of plant growth regulators in higher plants. They are usually derived from gibberellic acid and stimulate many metabolic events such as germination, flowering, stem elongation and fruit formation (Shahzad et al., 2016). Several plant growth promoting rhizobacterial strains produce gibberellic acids (GAs) and the most widely recognized amongst them is GA_3. In the present study, it was observed that, in general osmotic stress caused a reduction in the bacterial production of GA_3. Among the isolates *Atlantibacter hermannii* strain R11 recorded highest GA_3 concentration both under normal (4983 ng mL^{-1}) and osmotic stress (4883 ng mL^{-1}) conditions (Table 3).

Table 3 : Effect of osmotic stress on gibberellic acid (GA_3) production by osmotolerant bacterial isolates

Stress / Isolate	Gibberellic acid (ng mL^{-1})		Mean
	Normal (Without 25% PEG 8000)	Osmotic stress (With 25% PEG 8000)	
Atlantibacter hermannii R11	4983	4883	4933
Enterobacter sp. R19	4972	4679	4826
Achromobacter sp. T26	4967	4796	4882
Lysinibacillus composti T55	4970	4765	4868
Atlantibacter hermannii S12	4979	4732	4856
Pseudomonas mosselii S13 L	4963	4843	4903
Mean	4972	4783	-
Factor	Stress	Isolate	Stress x Isolate
SEm	1.82	3.16	4.47
C.D.	5.36	9.29	13.14

The reduction in the gibberellic acid production due to the imposition of osmotic stress was reported by Selvakumar et al. (2015), who observed that GA_3 production by *Citricoccus zhacaiensis* B-4 declined from 589.7 ng mL^{-1} under normal conditions to 176.2 ng mL^{-1} under osmotic conditions. Kumar et al. (2019) assessed eight osmotolerant bacterial isolates for the production of gibberellic acid and observed that isolate PB50 recorded the highest GA production of 69 µg mL^{-1} under non-stress conditions and 10.3 µg mL^{-1} under osmotic stress conditions (-0.73 MPa). Arun et al. (2020) reported that gibberellic acid production by *Bacillus megaterium* PB50 reduced to 10.2 µg mL^{-1} under osmotic stress conditions when compared to normal conditions (16.4 µg mL^{-1}). Ghosh et al. (2018) quantified the growth and phytohormone secretion abilities of *Pseudomonas aeruginosa* PM389, *Pseudomonas aeruginosa* ZNP1, *Bacillus endophyticus* J13 and *Bacillus tequilensis* J12 under osmotic stress induced by 25% polyethylene glycol (PEG). In general, they observed that osmotic stress retarded growth of all the bacterial strains, which in turn negatively impacted the auxin and cytokinin production in both the *Pseudomonas* strains. A possible reason for the reduced production of plant growth promoting hormones such as auxins and gibberellins under osmotic stress by the bacteria could be the reduced cell count per unit volume of the medium as a result of the imposition of osmotic stress.

Effect of osmotic stress on cytokinin production by elite osmotolerant bacterial isolates

Cytokinins are a class of plant hormones that regulate cell division and stimulate a variety of plant developmental processes (Waldie and Leyser, 2018). Regulation of growth (root and shoot) and branching, control of shoot apical dominance, development of chloroplasts, regulating the relocation of nutrients from leaves to reproducing seeds are some of the crucial activities regulated by cytokinins (Vaten et al. 2018). Cytokinins also alter the size and activity of meristems through cell division activity of embryonic and mature plants (Martins et al. 2019). In the present study, it emerged that all six isolates produced the cytokinin molecules *viz*., zeatin and zeatin riboside under both normal and osmotic stress conditions (Table 4). While osmotic stress negatively impacted the zeatin production by five of the isolates, *Lysinibacillus composti* strain T55 produced higher levels of zeatin under osmotic stress conditions (94 ng mL^{-1}) compared to normal conditions (49 ng mL^{-1}). When zeatin riboside production was assayed under normal and osmotic stressed conditions, five of the six isolates with the exception of *Enterobacter* sp. strain R19 produced enhanced levels of zeatin riboside under osmotic stressed conditions compared to normal conditions.

The highest levels of Zeatin Riboside (ZR) under both normal condition (27 ng mL^{-1}) and osmotic stress condition (22 ng mL^{-1}) was recorded by *Enterobacter* sp. strain R19. The suppressive effect of osmotic stress on cytokinin production has been documented by Selvakumar et al. (2018) who reported that osmotolerant *Citrococcus zhacaiensis* B-4 produced zeatin (Z) (7.15 ng mL^{-1} and 5.65 ng mL^{-1}), dihydrozeatin riboside (DHZR) (9.65 ng mL^{-1} and

Table 4 : Effect of osmotic stress on cytokinin production by osmotolerant bacterial isolates under normal and osmotic stress conditions

Stress / Isolate	Zeatin (Z) (ng mL^{-1})		Mean	Zeatin Riboside (ZR) (ng mL^{-1})		Mean
	Normal (Without 25% PEG 8000)	Stress (With 25% PEG 8000)		Normal (Without 25% PEG 8000)	Stress (With 25% PEG 8000)	
Atlantibacter hermannii R11	55	37	46	12	18	15
Enterobacter sp. R19	79	59	69	27	22	25
Achromobacter sp. T26	77	58	68	13	20	17
Lysinibacillus composti T55	49	94	72	14	16	15
Atlantibacter hermannii S12	42	41	42	17	19	18
Pseudomonas mosselii S13 L	45	33	39	11	14	13
Mean	58	54	-	15.7	18.2	-
Factor	Stress	Isolate	Stress x Isolate	Stress	Isolate	Stress x Isolate
SEm	1.435	2.48	3.51	0.71	1.23	1.74
C.D.	NS	7.30	10.32	2.09	3.62	5.13

7.32 ng mL^{-1}), zeatin riboside (ZR) (16.95 ng mL^{-1} and 12.11 ng mL^{-1}) under normal and osmotic conditions, respectively. They also reported that the osmotolerant strain *Bacillus amyloliquefaciens* P-72 produced the cytokinin molecules *viz.*, zeatin (Z) (5.01 ng mL^{-1} and 3.21 ng mL^{-1}), dihydrozeatin riboside (DHZR) (15.22 ng mL^{-1} and 12.01 ng mL^{-1}), zeatin riboside (ZR) (21.66 ng mL^{-1} and 16.82 ng mL^{-1}) under normal and osmotic conditions, respectively. Conversely osmotic stress has also been shown to positively impact plant growth hormone production by rhizobacteria. Bhatt et al. (2015) reported that *Enterobacter* strains P-41 and P-46 reported increased IAA and GA_3 production under osmotic stress. Similarly, Ghosh et al. (2018) reported that *Bacillus* strains showed a stress-induced increase in the levels auxins, gibberellins and cytokinins. The reason for the enhanced production of zeatin riboside under osmotic stressed conditions by five of the six bacterial isolates in this study could not be deciphered clearly.

The earliest report on the enhancement of plant drought tolerance by PGPR was made by Timmusk and Wagner (1999) in *Arabidopsis thaliana* inoculated with *Paenibacillus polymyxa* B2. The inoculation of IAA producing rhizobacteria promotes root growth and enhances uptake of nutrients and water in a number of crops (Mantelin and Touraine, 2004). Marulanda et al. (2009), reported the survival of plants under drought stress inoculated with IAA producing *Pseudomonas putida*. Maize (Cohen et al., 2009) and wheat (Creus et al., 2004) plants had better survival under drought stress when inoculated with the GA producing, *Azospirillum lipoferum*. Inoculation of cytokinin producing *Bacillus subtilis* improved shoot cytokinins in lettuce under water stress conditions (Arkhipova et al., 2007). Cytokinin producing *Micrococcus luteus* chp37 inoculation in maize plants under water stress resulted in enhanced shoot/root biomass and increased photosynthetic pigments (Raza & Faisal, 2013). Selvakumar et al. (2018) reported that inoculation of cytokinin-producing *Citricoccus zhacaiensis* and *Bacillus amyloliquefaciens* improved physiological parameters and yield of tomato plants under deficit irrigation conditions.

CONCLUSION

This study clearly indicates the ability of osmotolerant bacterial isolates to produce plant growth promoting hormones under *in vitro* osmotic stressed conditions. This needs to be reconciled with their plant growth promotion abilities under deficit irrigation stress conditions under field conditions.

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Original Research Paper

Morpho-physiological changes in oil palm (*Elaeis guineensis* Jacq.) tenera hybrid seedlings raised under different shade levels

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ABSTRACT

Climatic conditions prevailing in oil palm growing states of India indicate the need for shade during primary stage of oil palm nursery for optimum growth and vigour of seedlings. Experiments were conducted to standardize the shade requirement based on growth/quality of oil palm seedlings in summer, rainy and winter seasons by providing 25%, 50% and 75% ultra violet stabilized high density poly ethylene (HDPE) shade nets. Results were found significant among the treatments for most of the growth parameters studied over the seasons. Higher growth for key characters like seedling height, leaf area, collar girth and dry matter production were recorded at 75% shade level. Similarly, higher chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and inter cellular CO_2 concentration were observed at 75% shade. Among the season, seedling growth was vigorous in rainy season followed by summer and winter seasons. Hence, provision of 75% shade found to be ideal for raising seedlings during primary stage of nursery in oil palm.

Keywords: Growth, oil palm, seedling and shade

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the highest edible oil yielding crop and perennial in nature with 30 years productive life. It is a fast-growing plantation cum oil seed crop with an area of 4.00 lakh ha under cultivation in India (Anonymous, 2022) and there is a lot of demand for planting material in the country. Production of high-quality planting material is of prime importance for establishing productive oil palm plantations in the country. Oil palm is mainly propagated by seedlings which are raised in poly bags round the year in double stage nursery system *i.e.* primary nursery for 4 months under shade net and secondary nursery for 8 months in open conditions. Frequent modification in shade net house as per the seasons is not possible as the shade net houses/pandals are semi-permanent structures. Oil palm is grown in diversified agro-climatic zones of India and these are different from traditional oil palm countries like Indonesia and Malaysia. Temperature and sun light decide the quality of seedlings during nursery stage in oil palm. Harsh climatic conditions particularly high temperature (38-45ºC) and light intensity during summer are detrimental to the growth of oil palm seedlings. So, shading is must for getting good growth in oil palm seedlings in primary nursery stage. Shade net reduces both light and heat intensity and this modified environment/microclimate can protect seedlings from heat stress and promote plant growth. Shading promotes the vigour and growth of seedlings in oil palm (Samuel et al., 2017). But the information on level of shade requirement for primary nursery and its impact over seedling growth is not available and this information is very much required for oil palm nursery growers in India. Hence, series of experiments were conducted to standardize the optimum shade level and quantify its effect on growth and development of oil palm seedlings during primary nursery stage in summer, rainy and winter seasons.

MATERIALS AND METHODS

The studies were conducted at ICAR-Indian Institute of Oil Palm Research, Pedavegi, West Godavari district, Andhra Pradesh during 2020-21. Experimental site is located at 16º 43 N′ and 81º 09' E′ with 13.41 meter above mean sea level. The location Pedavegi experiences hot and humid weather owing to proximity to the sea Bay of Bengal. Average annual temperature ranges from 21.8 ºC to 34.8 ºC, relative humidity 69.3 per cent and average rainfall 1215 mm/annum. Weather parameters recorded during the study period are furnished in Fig. 1, 2 & 3.

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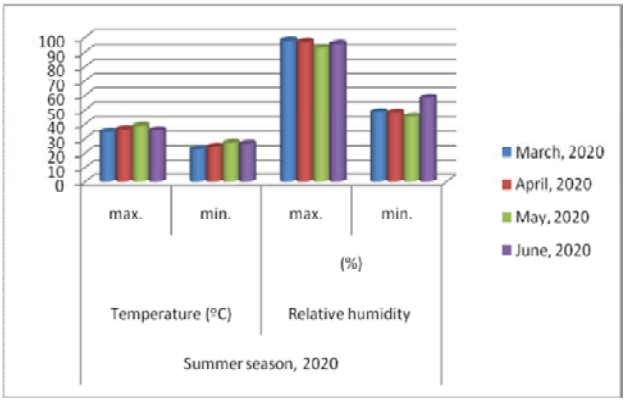

Fig. 1 : Weather conditions prevailed during the summer season at ICAR-IIOPR, Pedavegi, Andhra Pradesh

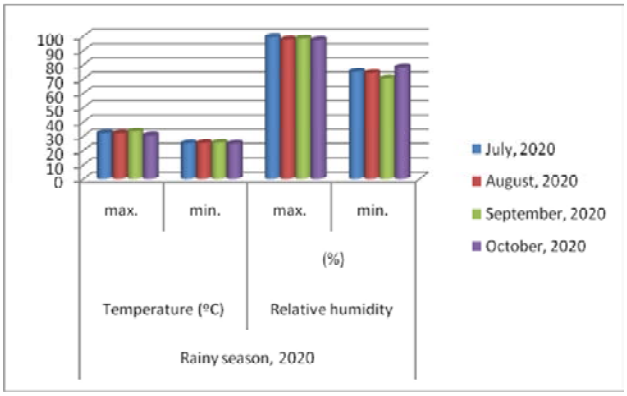

Fig. 2 : Weather conditions prevailed during the rainy season at ICAR-IIOPR, Pedavegi, Andhra Pradesh

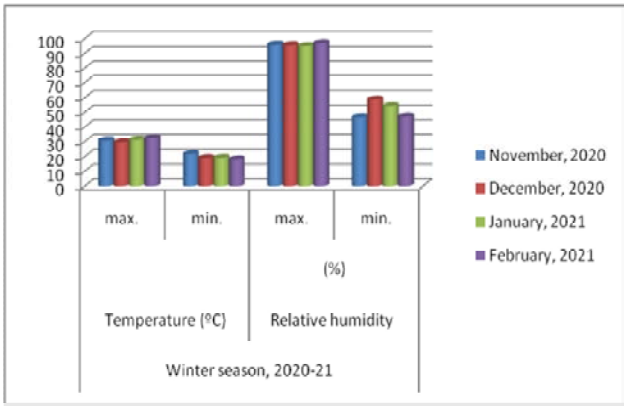

Fig. 3 : Weather conditions prevailed during the winter season at ICAR-IIOPR, Pedavegi, Andhra Pradesh

Experiment was laid out in completely randomized design (CRD) factorial with 4 treatments and 5 replications with 20 seedlings in each replication. Village base silt and farmyard manure mixed in 2:1 (v/v) ratio were used as a growing medium. Seedlings were raised at four shade levels *i.e.* 25%, 50%, 75% and no shade (open condition) and in three seasons *i.e.* summer (March-June), rainy (July-October) and winter (November-February). Uniform and healthy seed sprouts of oil palm hybrid *Tenera* (670 *Dura* x 77 *Pisifera*) produced from Oil Palm Seed Garden, Pedavegi, Andhra Pradesh were used for the study. Shade net house/pandal with 2 m x 2 m dimensions were erected with cement concrete poles covered with UV stabilized high density poly ethylene (HDPE) green colour agro shade net (25%, 50% and 75%) on all four sides. Seedlings were grown in poly bags (size 23 cm x 15 cm) by following the recommended nursery practices (Rethinam and Murugesan, 2000) in all the treatments uniformly during the study period in all the seasons.

Vegetative growth of oil palm seedlings was determined by measuring plant height, leaf and root production, leaf area, collar girth, fresh and dry biomass on four-month-old seedlings. Chlorophyll and carotenoids contents were estimated by UV-VIS spectrophotometer (*Shimadzu UV-1800*) by following dimethyl sulfoxide (DMSO) method. Gas exchange parameters *i.e.* photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (gs), intercellular CO_2 (ci) and leaf temperature (T_L) were recorded on 4-month-old seedlings by using portable photosynthesis apparatus (*LCA-4, ADC*, Hertfordshire, UK) connected to a *PLC 4* chamber between 9.00-11.00 hrs. Fully opened leaf *i.e.*, 3rd leaf from the top of seedlings was used for measurements. For comparing shade effect over different seasons, combined analysis was carried out using proc generalized linear model (GLM) procedure SAS version 9.3. Post-hoc analysis was conducted using least significant difference (LSD) and effects were considered as significant at $p<0.05$.

RESULTS AND DISCUSSION

Most of the morphological and physiological responses of oil palm seedlings differed significantly among the shade levels, seasons and between shade levels and seasons. Mean height of oil palm seedlings was significantly high during rainy season (41.86 cm) compared to summer (34.22 cm) and winter seasons (22.88 cm) (Table 1). Significantly taller seedlings were observed at 75% shade (37.40 cm), whereas, the shorter ones were recorded in no shade (28.13 cm). Similarly, seedlings grown under 75% shade during rainy season (45.50 cm) were taller compared to seedlings in other treatments except at 75% shade

Table 1 : Influence of shade on growth parameters of oil palm seedlings raised in different seasons

Treatment	Seedling height (cm)				Leaves/seedling			Leaf area (cm^2)			
	summer	rainy	winter	mean	summer	rainy	winter	mean	summer	rainy	winter	mean
25% shade	32.26	41.26	25.06	32.86	5.20	6.60	4.20	5.33	413.84	1004.20	237.37	551.80
50% shade	35.38	42.00	23.28	33.55	5.40	6.60	4.00	5.33	445.07	1132.80	176.69	584.86
75% shade	43.30	45.50	23.40	37.40	5.60	6.40	4.20	5.40	660.16	1101.97	213.07	658.40
No shade	25.94	38.70	19.76	28.13	5.00	6.60	4.20	5.27	291.10	969.812	187.66	482.86
Mean	34.22	41.86	22.88		5.30	6.55	4.15		452.55	1052.20	203.70	
LSD (P=0.05)												
Season				1.84				0.24				78.72
Shade				2.13				NS				90.90
Season x Shade				3.69				0.48				157.43

during summer season. Similarly, taller seedlings were observed under shade in oil palm nursery (Samuel et al., 2017). Present results clearly indicate the increasing trend in seedling height as the shade density increased in summer and rainy seasons. Seedling growth was vigorous in rainy season, medium in summer and slow during winter season. Seedling height was poor in all the seasons at no shade (open condition). Oil palm is basically a tropical plant which needs warm and humid conditions normally present during rainy season and because of this, better seedling height was observed in rainy season.

The results revealed that shade densities over the seasons could not influence the leaf production (Table 1) as also reported by Samuel et al. (2017) in oil palm. Seedlings grown in rainy season (6.55) possessed highest number of leaves as compared to summer (5.30) and winter seasons (4.15). Mean leaf production was significantly higher at all the shade levels during rainy season when compared to summer and winter seasons. This may be attributed to optimum weather conditions like warm and humid conditions prevailed during rainy season.

There were significant variations among the seasons and shade levels for leaf area of seedlings (Table 1). Mean leaf area in rainy season was recorded significantly high (1052.20 cm^2) compared to summer (452.55 cm^2) and winter (203.70 cm^2) seasons. Among the shade levels, maximum leaf area was estimated with 75% shade (658.40 cm^2) which was markedly superior to rest of the treatments but it was at a par with 50% shade level. Similarly, enhanced leaf area in tomato seedlings was reported under shading (Formisano et al., 2022). Mean leaf area was recorded maximum at 50% shade level (1132.80 cm^2) during rainy season and it was on par with other shade levels during rainy season.

Among the seasons, collar girth (6.91 cm) was significantly better in seedlings raised in rainy months (Table 2). There was no impact of shade levels over the collar girth. However, relatively more collar girth (5.24 cm) was recorded under 25% shade level. Seedlings with maximum collar girth were recorded in no shade (7.20 cm) and closely followed by 25% shade level (7.06 cm) in rainy season. It reflected the ability of seedlings to store more quantity of photosynthates in stem which in turn might have happened due to higher photosynthetic rate and better utilization of nutrients (Hastuti 2021).

Table 2 : Influence of shade on growth parameters of oil palm seedlings raised in different seasons

Treatment	Collar girth (cm)				No. of primary roots/seedling				Primary root length (cm)			
	summer	rainy	Winter	mean	summer	rainy	winter	mean	summer	rainy	winter	mean
25% shade	5.14	7.06	3.64	5.24	4.20	5.80	2.80	4.27	32.42	39.44	36.28	36.05
50% shade	4.78	6.60	3.56	4.98	4.60	5.20	2.80	4.20	35.72	36.32	25.52	32.52
75% shade	5.00	6.76	3.44	5.07	4.60	5.60	3.20	4.47	36.86	35.82	27.18	33.29
No shade	4.24	7.20	3.60	5.01	4.00	5.40	2.60	4.00	27.48	34.63	38.76	33.62
Mean	4.79	6.91	3.56		4.35	5.50	2.85		33.12	36.55	31.93	
LSD (P=0.05)												
Season				0.24				0.41				3.85
Shade				NS				NS				NS
Season x Shade				0.47				0.81				7.70

There were no marked differences among the treatments in production of primary roots in all the seasons (Table 2). This indicates that root production was not influenced by shade levels. Significantly higher mean root count was recorded in rainy season (5.50) as compared to summer (4.35) and winter (2.85) seasons. The highest mean root production was observed at 25% (5.80) in rainy season and the differences among shade levels were on par with one another. Poor root production was observed in seedlings grown in winter season at all the shade levels. Samuel et al. (2017) obtained similar results in oil palm. Results were found non-significant among the treatments (Table 2) showing no influence of shade levels over the root length. Primary roots were lengthier in rainy season (36.55 cm) which is markedly higher than summer (33.12 cm) and winter (31.93 cm) seasons. Results for mean root length between summer and winter seasons were at par with each other. Seedlings with longer roots were observed in rainy season at 25% shade level (39.44 cm).

Mean shoot dry weight recorded at 75% shade level (4.76 g) was significantly higher as compared to seedlings raised in open (3.75 g) but not significant with other shade treatments (Table 3). Maximum mean shoot dry weight was recorded in rainy season (8.07 g) which was markedly higher than other seasons. Better shoot dry weight was observed at 75% (8.53 g) and 50% (8.53 g) shade levels during the rainy season and both were significantly superior to rest of the treatments. Though there were significant differences among the seasons (Table 4) for mean root dry weight but the results were not significant among the treatments for mean root dry weight. Rainy season recorded the maximum mean root dry weight (1.85 g) and it was significantly higher than summer (1.10 g) and winter (0.44 g) seasons. Shade level 75% recorded the maximum root dry weight (2.04 g) in rainy season while it was the minimum (0.32 g) at 75% shade level during winter season. Mean root: shoot ratio (Table 3) was significantly higher in summer season (0.34), while, it was lower in rainy season (0.23). Of all the treatments, higher root: shoot ratio was observed in open (0.30) and 25% shade level (0.30), whereas, the lower root: shoot ratio was recorded under 75% shade level (0.25). Khan et al. (2000) also reported similar results in conifer

Table 3 : Influence of shade on biomass of oil palm seedlings raised in different seasons

Treatment	Shoot dry weight (g)				Root dry weight (g)				Root : shoot			
	summer	rainy	winter	mean	summer	rainy	winter	mean	summer	rainy	winter	mean
25% shade	3.30	7.85	1.71	4.29	1.25	1.79	0.51	1.19	0.38	0.23	0.29	0.30
50% shade	3.24	8.53	1.38	4.39	1.17	1.60	0.43	1.07	0.36	0.19	0.31	0.29
75% shade	4.31	8.53	1.43	4.76	1.19	2.04	0.32	1.18	0.28	0.24	0.23	0.25
No shade	2.29	7.37	1.60	3.75	0.77	1.96	0.50	1.08	0.33	0.27	0.28	0.30
Mean	3.29	8.07	1.53		1.10	1.85	0.44		0.34	0.23	0.28	
LSD (P=0.05)												
Season				0.47				0.12				0.03
Shade				0.55				NS				0.03
Season x Shade				0.95				0.25				0.05

species seedlings. Maximum root: shoot ratio at 25% shade level (0.38) was recorded during summer season, whereas, the minimum was noticed at 50% shade level in rainy season (0.19). Harsh weather conditions (Fig. 1) might have promoted the root growth rather than shoot growth during summer. Weather conditions prevailed during rainy season promoted the shoot growth at the expense of root growth. Higher root: shoot ratio during summer season may be because of proper distribution of photosynthates to root portion (Seman et al., 2018).

The treatment 75% shade level recorded the maximum mean fresh (21.54 g) and dry (6.06 g) biomass, whereas, the minimum levels (16.02 g and 4.63 g) were quantified in seedlings grown in no shade condition (Fig. 4 & Fig. 5). Among the seasons, seedlings produced significantly higher mean fresh (33.16 g) and dry biomass (9.87 g) during rainy season and this must be due to vigorous growth of seedlings owing to congenial/ favourable weather conditions prevailed during rainy season. Lower mean fresh (6.81 g) and dry biomass (1.66 g) production was observed in winter season at 75% shade level. Better fresh and dry biomass was estimated in seedlings grown in rainy season (37.88 g and 10.86 g) at 75% shade level. Enhanced biomass production at 75% shade level must be attributed to better seedling vigour in terms of seedling height, leaf area, collar girth and net photosynthetic rate of seedlings. Similarly, maximum total biomass was recorded under 75% shade in *Pongamia pinnata* seedlings (Sankeshwar, 2009). Biomass production was increased with decreasing light intensity probably due to enhanced biomass distribution in *Greenwayodendron suaveolens* (Olajuyigbe & Akhande, 2015). Relatively, high biomass production was observed in all the treatments in rainy season, medium in summer season and least in winter season. Better fresh and dry matter production of seedlings grown under shade reflects the compulsory need for shade particularly in summer season and to some extent in rainy season. However, seedling growth was better in no shade (open condition) as compared with shading due to moderate/mild weather conditions prevailed during winter season (Fig. 3).

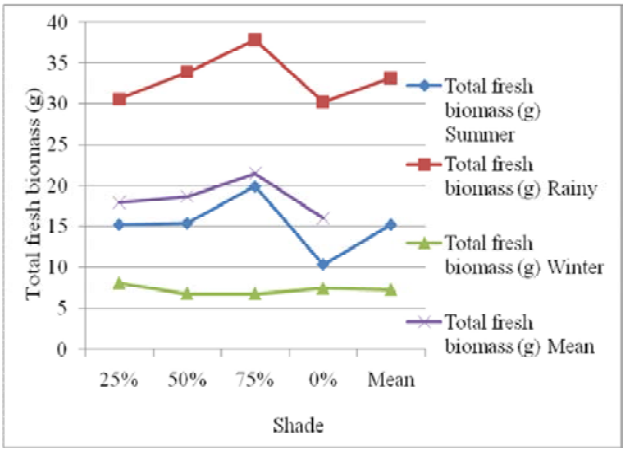

Fig. 4 : Influence of shade on total fresh biomass of oil palm seedlings raised in different seasons

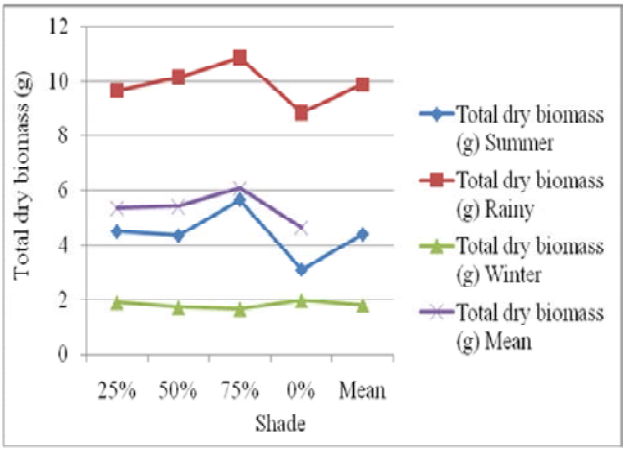

Fig. 5 : Influence of shade on total dry biomass of oil palm seedlings raised in different seasons

Overall, growth of the seedlings was quite vigorous during the rainy season and it was significantly better than summer and winter seasons. Prevalence of favourable weather conditions (Fig. 2) in rainy season might have promoted the growth and development. Being a tropical crop, oil palm needs minimum temperature of 22-24°C for its optimum growth and development (Corley & Tinker, 2016). Poor growth of seedlings during winter might be due to prevalence of cool weather with below 20°C temperature during night times (Fig. 3). Growth of the seedlings in summer was significantly lesser than rainy season but higher than winter season. Harsh weather conditions (Fig. 1) in summer certainly suppressed the growth of the seedlings though sufficient moisture was maintained in growing medium. Among different shade levels, vigorous growth and development of seedlings over the seasons was observed at 75% shade which reflects the dire need for shade during the primary stage of nursery in oil palm. Similarly, 75% shade was found to be ideal for raising *Terminalia arjuna* seedlings in nursery (Prasad, 2002).

Chlorophyll and carotenoids play a vital role in light reactions of photosynthesis and photosynthetic electron transport system in plants (Gardener et al., 2010). Chlorophyll and carotenoid contents were higher in seedlings raised under shade as compared with open condition (Table 4). High light intensity in open condition decreased chlorophyll and carotenoids contents. The treatment 75% shade (3.86 mg g^{-1}) followed by 50% shade (3.86 mg g^{-1}) recorded significantly higher chlorophyll content in leaves when compared with no shade (3.18 mg g^{-1}). Similar trend was observed among the treatments for carotenoid content in leaves. Results were at par between no shade (3.18 mg g^{-1} & 1.04 mg g^{-1}) and 25% shade level (3.50 mg g^{-1} & 1.15 mg g^{-1}) for chlorophyll and carotenoids contents, respectively. Higher concentration of chlorophyll at 75% and 50% shade levels reflects the necessity of shade during summer and present results indicate existence of direct relationship between chlorophyll content and photosynthetic rate (Suresh and Nagamani 2006). Higher chlorophyll level was recorded in naval orange (Incesu et al., 2016) and tomato seedlings (Formisano et al., 2022) under shading.

Gas exchange characters like photosynthetic rate, transpiration rate, stomatal conductance, inter cellular CO_2 level and leaf temperature were found significant among the treatments (Table 4). Maximum values for photosynthetic rate and transpiration rate, stomatal conductance and intercellular CO_2 level were recorded under 75% shade which was significantly superior to other treatments. Whereas, minimum values for the above parameters were observed in no shade (open condition). Increasing trend was observed for above gas exchange parameters as the shade level increased from 25% to 75%. Leaf temperature was significantly lower under shading as compared with open condition. Leaf temperature reduced progressively as the degree of shading was increased from 25 to 75% which reduced the photosynthetic rate and associated parameters (Suresh and Nagamani 2006).

Table 4 : Influence of shade on gas exchange parameters of oil palm seedlings raised in summer season

Treatment	Total chlorophyll content (mg g^{-1})	Carotenoids (mg g^{-1})	Photosynthetic rate (µmol m^{-2} s^{-1})	Transpiration rate (mmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol m^{-2} s^{-1})	Inter cellular CO_2 concentration (ppm)	Leaf temperature (°C)
25% shade	3.50	1.15	3.34	2.48	0.030	211.08	27.25
50% shade	3.86	1.24	3.86	2.75	0.040	226.15	26.55
75% shade	3.86	1.27	4.48	3.05	0.058	262.09	25.16
No shade	3.18	1.01	2.80	2.55	0.036	242.01	33.71
LSD (P=0.05)	0.48	0.11	0.37	0.21	0.010	23.74	0.72

Higher gas exchange measurements at 75% shade reflect the light saturation of seedlings at 75% shade. Higher photosynthetic rate at 75% shade might be attributed to higher CO_2 level and relative humidity inside shade net house when compared with open condition. Lower levels of gas exchange parameters in no shade (open condition) might be due to higher temperature in open condition which in turn might have reduced photosynthetic rate and transpiration rate by closing the stomata in leaves of oil palm seedlings. Higher photosynthetic rate is directly correlated with main growth indicators *viz*., seedling height, collar girth, leaf area and dry matter content. Better growth of seedlings under deep shade indicates morpho-physiological adaptation of oil palm seedlings to the shade. The growth of oil palm seedlings under shade might be due to better stomatal control under partial shade conditions which increased the growth parameters due to higher photosynthetic rate (Suresh and Nagamani 2006). Similar observations were made at 75% shade intensity in oil palm (Osama, 2003) and 50% shade in drumstick (Lamia et al., 2014).

CONCLUSION

The present study revealed that shade exerted significant influence on growth and vigour of oil palm seedlings at primary nursery stage. Findings demonstrated that shading is must for better growth and development of oil palm seedlings particularly during summer and to some extent in rainy season. Better results for main parameters such as seedling height, leaf area, collar girth and dry matter production were observed at 75% shade level. Therefore, 75% shade net can be an optimum shade level for raising seedlings during primary stage of oil palm nursery.

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Original Research Paper

Biochemical quality comparison of forced air dried osmo-dehydrated cashew apple products infused with spice mixture and sugar

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ABSTRACT

Cashew apple is a pseudo-fruit available abundantly during harvest seasons (March to July) and majority of them goes as waste because of their perishability and poor shelf life. However, the absence of distinct exocarp and seeds are some of the potential advantages for processing utility. Hence, in the present study, osmo-dehydrated products were prepared from two maturity stages *i.e.* breaker and ripe stages using sugar, spice mixture and were referred to as cashew fig and chew, respectively. The drying efficiency and product recovery were conquered by cashew chew and fig, respectively. Based on the biochemical and organoleptic qualities, ripe fruits were found suitable for preparation of chew and fig. The tannin content responsible for acridity got reduced (chew of ripe stage 1.18 to 0.53 mg/g and chew of breaker stage 1.85 to 0.68 mg/g) during the process of osmo-dehydration. Excluding total antioxidant activity, all other biochemical properties were found to be improved compared to their respective controls.

Keywords: Bio-chemistry, cashew apple, chew, fig, value addition

INTRODUCTION

Cashew crop was introduced from Brazil in the 16th century and was initially appreciated for its magnificent, juicy false fruit known as the cashew apple. The juice of this fleshy receptacle is rich in minerals, vitamins, and polysaccharides, offering instantaneous energy to the consumers (Runjala & Kella, 2017). However, the distinctive physiological characteristics of cashew apple, *viz*., rapid rates of respiration and ethylene production along with delicate skin hinders their storage, transport, and marketability. Additionally, these fruits pose a sense of acridity/astringency in the throat, while, consuming rendering them moderately acceptable to consumers. There are some other reasons contributing for leaving overripe cashew apples as plantation waste including bulk production in the season, inappropriate harvesting and handling practices, more economic importance for the nut, and decayed cashew apples improving the soil's health (Preethi et al., 2019). The cashew apple fruits are abundant in fibre, minerals, ascorbic acid, antioxidants, phenols and sugars. Though few fermented and non-fermented beverages such as vinegar, cider, fenny, wine, ready-to-serve drinks, syrup, and juice, are readily available in the market, while, technologies for preparation of pickles, candies, probiotic juices, enzyme preparations, emulsions, surfactants, and cattle feed from cashew apple pomace has been standardized (Sobhana et al., 2011) and these processes require manual labour, cumbersome equipment, and substantial investment.

With this outlook, simple and easily adaptable osmo-dehydration techniques for whole and sliced cashew apples using different osmolytes was tried. In order to standardise suitable maturity stage of cashew apple for development of these products, two stages *i.e.* breaker and ripe stages (Fig. 1) were selected (Adiga et al., 2019). Both the stages ensured complete maturity of raw cashew nut (RCN) since it is the major economic part focused for income generation. The biochemical quality and sensory acceptability of these products was studied in comparison to their respective controls (dehydrated fruits without osmolytes).

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Fig. 1 : Breaker stage (a) and ripe stage of cashew apple (b)

MATERIALS AND METHODS

The cashew apples of two different stages *i.e.* breaker stage (BS) and ripe stage (RS) were harvested manually from low lying branches of plantations of ICAR-Directorate of Cashew Research, Puttur, Karnataka, India located at 12º 45¹ N and 75º 15¹ E with 90 meters above mean sea level. Cashew apples were precooled to room temperature and sorted for uniform size, bruise and debris free fruits. Fruits were washed under running water and wiped using a clean, dry cotton cloth to remove the adhering moisture, air dried for 5 to 10 min and used for further processing.

Product preparation

Cashew apple fig

The proximal and distal ends of cashew apples were chopped off using a sharp stainless-steel knife and soaked in sugar solution of 70 °Brix containing 0.06% potassium metabisulphate (KMS) as a preservative (Kaushalya & Weerasooriya, 2011). Since, whole cashew apple is used; gentle horizontal slits are made on four sides of cashew apple to encourage osmosis. The strength of sugar syrup was sustained at 60 °Brix for three days. The cashew apple slices were periodically agitated in sugar syrup to ensure proper soaking and to avoid microbial contamination. After three to four days, the sugar syrup was filtered and the cashew apples were dried in a cabinet dryer at 40 to 45 °C for about 12 to 13 hours. This product was referred as 'Cashew fig' (Fig. 2). The sugar syrup concentration and drying temperature optimized by Azoubel & Murr (2003) were adopted. Similar fruits dried without osmosis act as control and referred to as Cashew apple whole (CAW).

Fig. 2 : Osmo-dehydrated cashew apple products, a. chew and b. fig

Cashew apple chew

The proximal and distal ends of firm cashew apples were removed and cut into cubes of 1 cm^3 size. The cashew apple slices (500 g) were mixed thoroughly with 50 g spice mixture (4 parts of spice powder + 1 part of sugar) of 45 °Brix and left marinated overnight. After 48 h, the coated cashew apple slices were spread as a thin layer over a clean dry stainless steel tray for dehydration in a cabinet dryer at 40-45 °C temperature for 9-10 h (Azoubel & Murr, 2003). To prevent microbial contamination, the slices were often agitated or tossed. The sweet spice mixture functions as an osmolyte, and the aqueous solute liberated from the slices was re-impregnated for the preparation of cashew chews. Similar slices of cashew apples dehydrated without any infusion were considered as control and are referred to as cashew apple slices (CAS). The TSS of the osmolytes was measured using portable hand refractometer (0-90%) at room temperature (30±2°C).

The treatments of the dehydration experiment were Breaker stage - cashew apple whole (BS-CAW), BS-Fig, Ripe stage - cashew apple whole (RS-CAW), RS-Fig, Breaker stage - cashew apple slices (BS-CAS), BS-Chew, Ripe stage - cashew apple slices (RS-CAS) and RS-Chew. The dry product recovery percentage was calculated on weight by weight basis (w/w) using the formula, product recovery (%) = (Initial cashew apple weight - Dry product weight) × 100 ÷ Initial cashew apple weight. Drying rate was calculated using the formula, Drying rate = (initial cashew apple weight - dry product weight) ÷ time taken for drying and expressed in g/min (Reddy et al., 2023).

Biochemical parameters such as ascorbic acid (mg/100 g), Tannin (mg/g), total sugars (%), reducing sugars (%), phenols [mg gallic acid equivalents

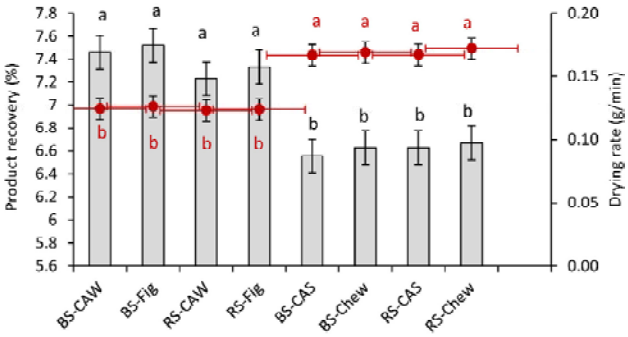
(GAE)/100 g], flavonoids [mg catechin equivalents (CE)/100 g] and total antioxidant activity in cupric ion reducing antioxidant capacity method [mg ascorbic acid equivalents (AAE)/100 g] were estimated referring to Ranganna (1986). The prepared samples were coded and subjected to sensory analysis with a semi-trained panel of 50 members using a nine-point hedonic scale.

In the current investigation, the study of different parameters (n=10) were evaluated with analysis of variance and Fisher's least significant difference at 1% level (p=0.01) using the online SAS (version 9.2) tool, and the results were reported as the mean ± standard deviation of 10 replicates.

RESULTS AND DISCUSSION

The major portion of cashew apple is water accounting for 60-85% depending on the variety, growing conditions, physiological aspects like photo-assimilates synthesis, dry matter accumulation and other biotic and abiotic factors. Since, this is one of the extremely hydrated commodity, the drying rate was comparatively high in cashew apple than in any other fruits and vegetables (Kumar & Sagar, 2014). The product recovery and variation in drying rate using osmolytes *i.e.* sugar and spice mixture is depicted in Fig. 3. Slicing and spice mixture infusion are the processing steps that hastened the drying efficiency of 'Cashew chew' by increasing its surface area exposed to drying environment. Prominent significant difference in drying rate and product recovery was noticed between the osmotic products. In 'Cashew fig', among the control and sugar treated samples, diffusivity, water loss and solid gain were acute in sugar treated osmo-dehydrated product and relative results were obtained by Mini & Archana (2016) in cashew apple and Sujayasree et al. (2022) in Amla. According to Pravitha et al. (2021) while preparing coconut chips, sugar and jaggery were identified as an effective osmolytes and the statement is in line with present experimental result. Likewise, spice mixture inherited with sugars predominantly reducing sugars increased the drying ability and product recovery percentage in 'Cashew chew'.

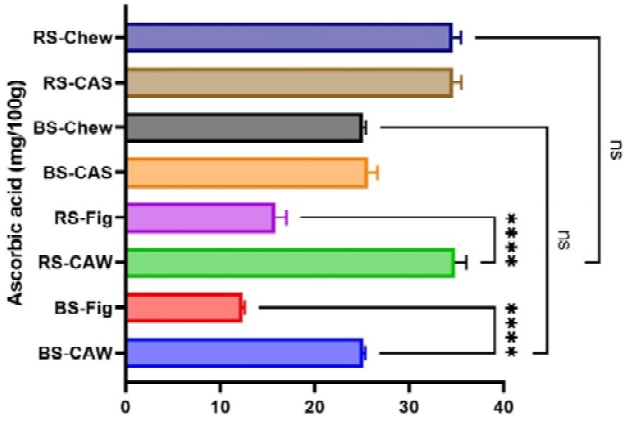
The biochemical properties and their suitability for osmo-dehydrated products development from two maturity stages of cashew apple were studied (Table 1). Cashew apple is one of the predominant bio-source of ascorbic acid similar to fruits like guava,



BS: breaker stage; RS: ripe stage; CAW: cashew apple whole; CAS: cashew apple slices; bars represent the mean values with standard error bars; bars with same different alphabet(s) are significant at 99% confidence level; bars with different alphabets are non-significant (NS) at 99% confidence level

Fig. 3 : Product recovery percentage and drying rate of cashew apple osmo-dehydrated products

citrus and pineapple (Preethi et al., 2019). Between the two maturity stages, ascorbic acid in dehydrated cashew apples of ripe stage (34.85 mg/g) was higher than that of dehydrated cashew apples of breaker stage (25.16 mg/g). Though the ascorbic acid content was lesser in cashew apple slices compared to wholly dehydrated cashew apples, the trend between the maturity stages was similar. In osmo-dehydrated products, the ascorbic acid is tremendously reduced in Cashew fig irrespective of their maturity stages (12.34 and 15.83 mg/g) compared to Cashew chew (25.13 and 34.63 mg/g). In cashew apple, the water soluble vitamins (C and B) were embedded in liquid phase. During sugar infusion, the sugar syrup was



BS: breaker stage; RS: ripe stage; CAW: cashew apple whole; CAS: cashew apple slices

The bars represent the mean values with standard error bar; ****: highly significant at p=0.01

Fig. 4 : Comparison of ascorbic acid content in chew and fig of different maturity stages with their respective whole and slices

Table 1 : Biochemical properties of osmo-dehydrated cashew apple products

Parameter	BS-CAW	BS-Fig	RS-CAW	RS-Fig	BS-CAS	BS-Chew	RS-CAS	RS-Chew
Ascorbic acid (mg/100 g)	25.16^{b}±1.45	12.34^{d}±0.56	34.85^{a}±1.05	15.83^{c}±0.21	25.68^{b}±1.12	25.13^{b}±1.02	34.63^{a}±1.25	34.62^{a}±1.13
Total sugars (%)	15.41^{b}±0.45	16.17^{a}±1.05	15.96^{b}±1.65	16.38^{a} ±0.15	15.54^{b}±0.56	16.08^{a}±0.24	15.56^{b}±1.08	16.25^{a} ±1.00
Reducing sugars (%)	5.45^{e}±0.12	7.42^{d}±0.24	10.13^{b}±0.25	10.56^{a}±0.18	5.63^{e}±0.23	8.90^{c}±0.26	10.28^{b}±0.23	10.40^{b}±0.25
Tannins (mg/g)	1.85^{e}±0.03	0.89^{c}±0.00	1.21^{d}±0.01	0.53^{a}±0.00	1.85^{e}±0.00	0.68^{b}±0.00	1.18^{d}±0.01	0.53^{a}±0.00
Total phenols (mg GAE/g)	1.16^{a}±0.00	0.80^{b}±0.01	0.44^{d}±0.00	1.2^{a}±0.01	1.12^{a}±0.00	0.76^{b}±0.00	0.42^{d}±0.00	0.69^{c}±0.12
Total flavonoids (mg CAE/g)	0.12^{b}±0.00	0.09 ^{c} ±0.00	0.11^{b}±0.00	0.10^{b}±0.00	0.12^{b}±0.00	0.16^{a}±0.00	0.11^{b}±0.00	0.18^{a}±0.00
TAA (mg AAE/g) (NS)	0.02±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.00

TAA: total antioxidant activity; BS: breaker stage; RS: ripe stage; CAW: cashew apple whole; CAS: cashew apple slices

Mean values with the same alphabets and no alphabets are non-significant (NS) at 99% confidence level; mean values with the different alphabet(s) are significant at 99% confidence level

diluted with liquid phase of cashew apple in fig preparation. Whereas, the liquid phase was again and again impregnated in chew making process and thus this product got enriched with ascorbic acid compared to Cashew fig. The ascorbic acid in chew is non-significant compared to the fig of respective maturity stages (Fig. 4).

Though, the variation in total sugar content of BS and RS remains non-significant, there was an increase observed in the content of reducing sugars with due time of cashew apple maturity and thus, the reducing sugar content in dehydrated cashew apples was in the range of 5.63 - 10.28%. Conversion of non-reducing sugars to reducing sugars without altering the total sugar content is a general phenomenon during fruit ripening. Though, cashew apple is a pseudocarp, similar occurrence was noticed here. The sugars were high in both the osmo-dehydrated products prepared from RS cashew apple though they are statistically non-significant with the products of BS cashew apple. Altogether, the soft tissue of RS fruit relatively encouraged osmosis in fruit based osmotic products (Yadav & Singh, 2014) and, similarly in this study, high sugar values in RS based osmo-dehydrated products were recorded. The total and reducing sugars in chews were higher than Cashew fig irrespective of their maturity stages which could be due to the procedural practice of using commercial sugar-sucrose as osmolyte during cashew fig preparation. The results obtained are comparable with the sugar incorporated osmo-dehydrated *Malus* fruit, wherein the polysaccharides like sucrose (non-reducing sugar) and glucose (reducing sugar) were boosted, whereas, the fructose (reducing sugar) remained the same with slight decrease in organic acids (Dixon & Jen, 1977).

The tannin content (3-5 mg/ml) in cashew apple results in its astringency and acridity rendering it as an undesirable character (Preethi et al., 2019). Between the maturity stages, BS has more tannin (1.85 mg/g) than RS (1.18-1.21 mg/g) in dehydrated whole as well as sliced cashew apple. Both the osmolytes *i.e.* spice mixture and sugar, effectively reduced the tannin content to 0.53-0.89 mg/g in cashew apple during product development. Similar reduction of tannins through osmosis was reported (Singh et al., 2019).

Maturity stages of cashew apple played a vital role in the total phenolic content of cashew apple and their products. The dried cashew apple slices of BS had more total phenols (1.12 mg GAE/g) than RS (0.42 mg GAE/g). Whereas in the products, Cashew fig prepared using RS were appraised for the highest total phenols (1.2 mg GAE/g), while, Cashew chew from RS were recorded for greater total flavonoids (0.18 mg CE/g). When the osmo-dehydrated products

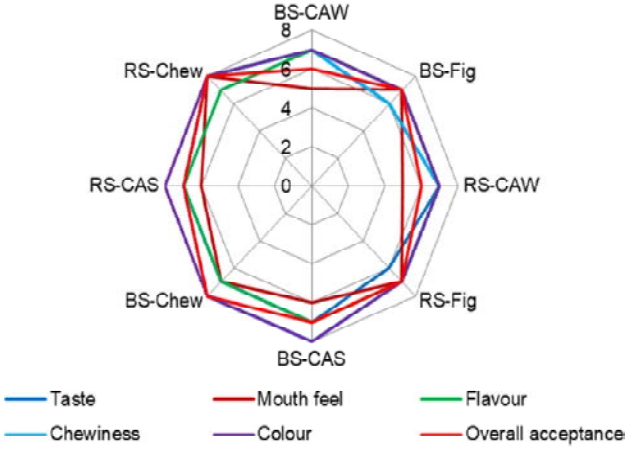


Fig 5 : Organoleptic evaluation of osmo-dehydrated cashew apple products

N=50; BS: breaker stage; RS: ripe stage; CAW: cashew apple whole; CAS: cashew apple slices

were compared, chew had greater flavonoids than fig because of addition of flavonoid rich spices (Ashokkumar et al., 2020) as osmolytes. The total flavonoids in cashew chew of RS cashew apple (0.18 mg CE/g) were on par with that of BS cashew apple (0.16 mg CE/g). The treatments had no significant effect on the total antioxidant activity of osmo-dehydrated cashew apple products.

According to the organoleptic characters (Fig 5), Chew prepared from RS cashew apple scored the highest. The average score of overall acceptance ranged between 6 to 8 indicating chew from both stages secured the highest score and the dehydrated whole cashew apple were the least preferred.

CONCLUSION

The cashew apple processing techniques available currently requires huge investment and are labour intensive. These products *i.e.* cashew chew and fig were developed using simple technology called osmo-dehydration using spices and sugar. Among these two products, chew was found to be highly acceptable with respect to biochemical and organoleptic properties. It also had the potential to be used as a refreshing mouth freshener. Since, very less quantity of sugar was used as osmolyte; this product could be relished by weight watchers and diabetic patients, whereas, cashew apple fig mimics the taste of dates and thus, can be consumed as a regular snack.

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Original Research Paper

Optimization of osmotic dehydration in dragon fruit (*Hylocereus polyrhizus*) slices using response surface methodology

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ABSTRACT

Dragon fruit (*Hylocereus polyrhizus*) is emerging as a super crop because of its several health and therapeutic benefits and ease of cultivation even in degraded land. Using response surface approach, the process parameters for osmotic dehydration of dragon fruit slices included process temperature, syrup concentration and process time. Slices of size 20 x 20 x 5 mm were dipped into sugar syrup with a syrup to dragon fruit slice ratio of 4:1 (w/w). After osmotic dehydration, the initial moisture content of dragon fruit samples was reduced to 27.5-68.49% (wb), demonstrating water loss, solid gain, and mass reduction in the range of 18.01-65.9%, 6.3-17.9% and 9.31-50.6%, respectively. After statistical analysis of the data on water loss, solid gain, and weight reduction, it was shown that regression equations of second order provided the greatest match for all the experimental data. With a syrup to fruit ratio of 4:1 and a syrup concentration of 65.3° Brix at a syrup temperature of 56.5°C, a maximum water loss of 58.2% and a minimum solid gain of 7.7% were expected to occur in 240 minutes of osmotic dehydration.

Keywords : Dragon fruit, optimization, osmotic dehydration, solid gain, water loss

INTRODUCTION

Dragon fruit have numerous nutritional advantages, as well as its ease of growing even on degraded land. It contains essential minerals and nutrients like potassium, iron, sodium, calcium and fiber, and good source of antioxidants. It is able to lower cholesterol concentration to balance blood sugar and to prevent colon cancer to strengthen kidney functioning and bone strength. It has the ability to promote the growth of probiotics in the intestinal tract (Le et al., 2021). The hot air-dried fruits in traditional trays, cabinets or vacuum dryers are not widely accepted due to the low product quality. The issues with products made from air-dried fruits include woody texture, slow or insufficient rehydration of the product, loss of juiciness, and significant shrinkage brought on by the collapsing of cells as a result of significant water loss (Dalla Rosa & Giroux, 2001). It also results in unfavourable colour, flavour, and nutritional quality changes (Jain & Verma, 2003).

One potential preservation method for creating high-quality products is osmotic dehydration (Kar & Gupta, 2001; Rastogi et al., 2002; Sodhi et al., 2006). Numerous variables affect water removal in case of osmotic dehydration (Dalla Rosa & Giroux, 2001) and is used to enhance the fruit's nutritive, sensory and functional qualities (Sujayasree et al., 2022).

MATERIALS AND METHODS

The dragon fruits of var. Taiwan pink were blanched in hot water to ensure that all impurities on the fruit's surface were eliminated. Peeled out fruit was cut into slices of size 20 x 20 x 5 mm and utilised for this study.

Osmotic dehydration of dragon fruit slices

The moisture content of dragon fruit slices was determined as per the method 934.06 (Cunniff & Washington, 1977). Sugar syrup to fruit ratio of 4:1 was used to osmotically dehydrate the dragon fruit slices. The beakers containing the sugar syrup and dragon fruit slices were positioned inside the water bath at a constant temperature. To keep a consistent temperature, the syrup in the beakers was manually stirred at regular intervals. At the predetermined time, one beaker was taken out of the water bath and the samples were quickly washed with running water

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before being placed on tissue paper to absorb any remaining surface moisture. The moisture content of the samples at that condition were determined. For each processing parameter, such as syrup concentration (40, 50, 60, 70° Brix), temperature (40, 50, 60, 70°C) and osmotic duration (30, 60, 90, 120, 150, 180, 210, 240 min), levels were chosen based on recommendations made by prior researchers (Dalla Rosa & Giroux, 2001; Torreggiani & Bertolo, 2001; Ozen et al., 2002; Jain et al., 2011).

Determination of water loss, solid gain and weight reduction

The water loss (WL), solid gain (SG) and weight reduction (WR) of fruit slices were estimated as per the procedure given by Kaleemullah et al. (2002).

$$WL = \frac{M_o X_o - M_\theta X_\theta}{M_o} \times 100 \quad \text{..... (1)}$$

where,

WL = water loss, %

M_o = mass of dragon fruit slices at time zero, g

M_θ = mass of dragon fruit slices at time, $_\theta$, g

X_o = water content as a fraction of mass of dragon fruit slices at time zero

X_θ = water content as a fraction of mass of dragon fruit slices at time θ

The dry matter gain is related to SG and hence, the SG was the net gain in total solids by dragon fruit slices on initial mass basis.

$$SG = \frac{M_\theta(1-X_\theta) - M_o(1-X_o)}{M_o} \times 100 \quad \text{..... (2)}$$

where,

SG = solid gain, %

Weight reduction is defined as the weight lost from the dragon fruit slices during osmotic dehydration process and was calculated on the basis of initial

Table 1 : Central composite design (CCD) experimental treatment combinations obtained by Design Expert for three independent process parameters

Run	Solution concentration (°Brix)	Solution temperature (°C)	Immersion time (min)	Remarks
04	40	40	030	Non centric points
02	70	40	030	
05	40	40	240	
19	70	40	240	
09	40	70	30	
17	70	70	30	
14	40	70	240	
01	70	70	240	
10	40	55	135	
11	70	55	135	
06	55	55	30	
15	55	55	240	
16	55	40	135	
13	55	70	135	
08	55	55	135	Centric points
12	55	55	135	
13	55	55	135	
18	55	55	135	
07	55	55	135	
03	55	55	135	

weight. Weight reduction is the difference between the water loss and solid gain.

$$WR = WL - SG \quad \text{..... (3)}$$

where,

WR = weight reduction, %

WL = water loss, %

SG = solid gain, %

The osmotic dehydration experiments for dragon fruit slices with different osmotic solution concentrations, osmotic solution temperatures and dehydration durations were planned by using the statistical analysis tool Design Expert (Stat Ease, MN 55413, USA, Version 13.0). Osmotic solution concentration, temperature and dehydration duration were chosen as the three independent process factors for this. With the help of fitting models offered by Design Expert software, ANOVA tables were generated, and the coefficient of determination (R^2) and coefficient of variance (CV) were also calculated. In cases where objectives like minimise, maximise, within range, target set to an exact value (factors only), and none (responses only) were available, numerical optimization was employed to improve the responses. Maximizing water loss, reducing solid gain, and maximising weight reduction were the criteria used for optimization. Additionally, the central composite design (CCD) response surface model was chosen with the intention of improving the process parameters. Osmotic dehydration process variables included osmotic solution concentration, temperature and immersion time, which ranged from 40 to 70° Brix, 40 to 70°C and 30 to 240 min, respectively. The response metrics chosen were water loss, solid gain and weight reduction. Twenty randomised treatment combinations were offered by CCD of Design Expert with 14 non-centre points and 6 centre points (Table 1).

Table 2 : Average experimental value of water loss, solid gain and weight reduction at 20 different experimental conditions

Run	Solution concentration (°Brix)	Solution temperature (°C)	Immersion time (min)	Water loss (%)	Solid gains (%)	Weight reduction (%)
01	40	40	30	18.01	8.7	9.31
02	70	40	30	19.7	9.3	10.4
03	40	40	240	25.2	11.5	13.7
04	70	40	240	42.2	7.4	34.8
05	40	70	030	30.4	11.6	18.8
06	70	70	030	48.0	12.3	35.7
07	40	70	240	40.3	16.3	24.0
08	70	70	240	65.9	17.9	48.0
09	55	40	135	27.2	9.0	18.2
10	70	55	135	43.9	6.3	37.6
11	55	55	030	27.7	7.00	20.7
12	55	55	240	59.6	9.0	50.6
13	55	40	135	29.3	7.6	21.7
14	55	70	135	58.9	12.8	46.1
15	55	55	135	44.1	6.4	37.7
16	55	55	135	46.6	6.7	39.9
17	55	55	135	44.1	6.5	37.6
18	55	55	135	44.2	6.4	37.8
19	55	55	135	44.2	6.4	37.8
20	55	55	135	44.3	6.3	38.0

Optimization of independent parameters for osmotic dehydration process

The concentration of the osmotic solution, temperature, and immersion duration were three distinct process parameters that were optimised using the numerical optimization approach of RSM in design expert software. For optimising and choosing the optimal treatment combination, the experimental data of water loss, solid gain, and weight reduction of dragon fruit slices during osmotic dehydration at the various treatment combinations were used. The experimental data were fitted using a second order polynomial equation, which describes the impact of the test variables (A, B, C) as well as their combined and interaction effects on the predicted response (Y).

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC + E \quad ..(4)$$

where,

Y_i (i = 1-3) = predicted responses for water loss, solid gain and weight reduction

β_0 = Estimated coefficient at center of the design

$\beta_1, \beta_2, \beta_3$ = linear coefficients

$\beta_{11}, \beta_{22}, \beta_{33}$ = quadratic coefficients

$\beta_{12}, \beta_{23}, \beta_{13}$ = interaction coefficients

A = Solution temperature, °C

B = solution concentration, °Brix

C = immersion time, min

E = random error

RESULTS AND DISCUSSION

Mass transfer parameters of osmotically dehydrated dragon fruit slices

The mass transfer parameters such as water loss, solid gain and weight reduction for osmotically dehydrated dragon fruit slices at different experimental conditions are presented in Table 2. The analysis of variance (ANOVA) was carried out to evaluate the impact of process factors on the experimental values of response variables and the quadratic model was found significant ($p<0.05$).

The three response variables also produced second order polynomial equations. The terms that had a distinct impact on the response variables are also supplied. By using the programme to display the combined influence of two independent process factors on a specific dependent response variable, the 3-D response surface plots for dependent parameters were created. The numerical optimization method was used to carry out the optimization. All the parameters were given equal weight, and the optimum value was discovered. The regression coefficients for the three response variables were obtained by using Design Expert (Table 3).

Water loss response during osmotic dehydration of dragon fruit slices

When dragon fruit slices were exposed to osmotic solutions with concentrations of 40-70° Brix, solution temperatures of 40-70°C and immersion periods of

Table 3 : Regression coefficient values of response variables

Variables	Water loss	Solid gain	Weight reduction
Intercept	44.28	6.37	37.91
A	11.12****	2.50****	8.62****
B	7.65****	-0.25****	7.90****
C	8.94***	0.9900***	7.95***
AB	3.06**	0.7250**	2.34**
AC	-0.2375**	1.18**	-1.41**
BC	2.91**	-0.4750**	3.39**
A^2	0.7773**	4.66**	-5.44**
B^2	-7.23***	0.709***	-7.94***
C^2	-0.1733**	0.1091**	-0.2864**
R^2	0.9410	0.9485	0.9108

A: osmotic solution concentration, B: solution temperature, C: immersion time.

****p < 0.05, 0.05 ≤ ***p < 0.1, **p ≥ 0.1

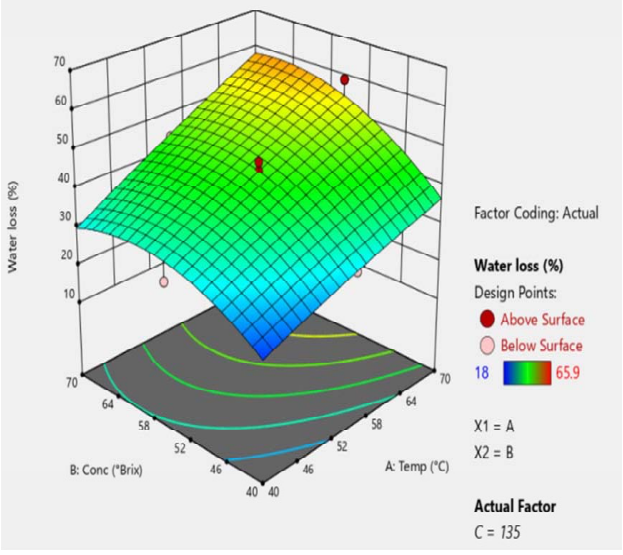
30–240 min, water loss (WL) values ranged from 18.01% to 65.9%. At a solute concentration of 70 °Brix, temperature of 70°C and an immersion time of 240 min, a higher water loss value of 65.9% was attained. At a sugar solution concentration of 40° Brix, temperature of 40°C, and an immersion time of 30 min, a lower water loss value of 18.01% was obtained. The coefficient of determination (R^2) value for the water loss equation was 0.9410. The quadratic model's F-value for the water loss parameter was 17.72, and this result shows that the model's significance for the water loss response was high. The model terms are considered significant ($p<0.05$) if the independent parameter p-value was less than 0.05. For the water loss parameter of slices of dragon fruit, the following model terms are significant in the current study ($p<0.05$): A, B, C, AB, A^2, and B^2. P-values higher than 0.10 denote the absence of significance for the model terms. F-value is 187.64 and P-value is 0.0001 for the water loss parameter's lack of fit value. It suggests that the lack of fit is important for the water loss parameter of slices of dragon fruit. In terms of the actual values of the variables, a regression equation was created to account for water loss.

$$\text{Water loss (\%)} = -90.16209 + (0.393079 \times A) + (0.393079 \times B) + (-0.003928 \times C) + (0.013611 \times AB) + (-0.000151 \times BC) + (0.001849 \times AC) (-0.003455 \times A^2) + (-0.032121 \times B^2) + (-0.000016 \times C^2) \quad \ldots. (5)$$

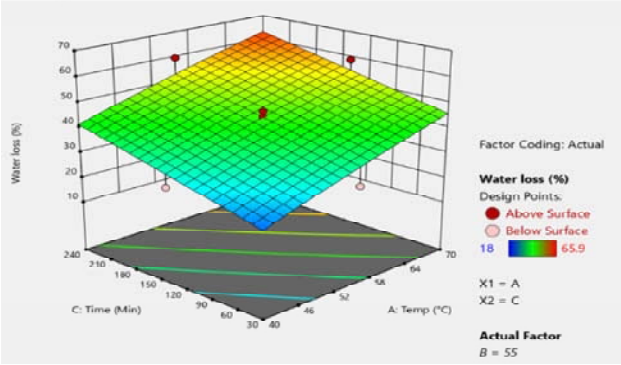
The response surface plots for water loss during osmotic dehydration are depicted in Fig. 1. As solute concentration, immersion time, and temperature increased, water loss also increased. During osmotic dehydration, the water loss value of dragon fruit slices increased as the solute concentration and solution temperature increased. This may be due to the increase of osmotic pressure differential between the fruit and the interface of the osmotic solution and decrease in the sugar solution's viscosity. Increase in water loss with an increase in solute concentration and solution temperature has been reported in osmotic dehydration of papaya (Kaleemullah et al., 2002), pineapple slices (Rastogi & Raghavarao, 2004) and beetroot slices (Kaur & Singh, 2013). All the three process variables (process temperature, syrup concentration and process time) had a substantial favourable impact ($p<0.05$) on the water loss of dragon fruit slices during osmotic dehydration at the linear level. Dragon fruit slices water loss was significantly positively influenced by solution concentration and solution temperature at the interaction level ($p < 0.05$), but not by solution temperature and immersion time. All the three independent process parameters had a negative impact on water loss at the quadratic level, with temperature and concentration having the most notable effects ($p < 0.05$).

Solid gain response during osmotic dehydration of dragon fruit slices

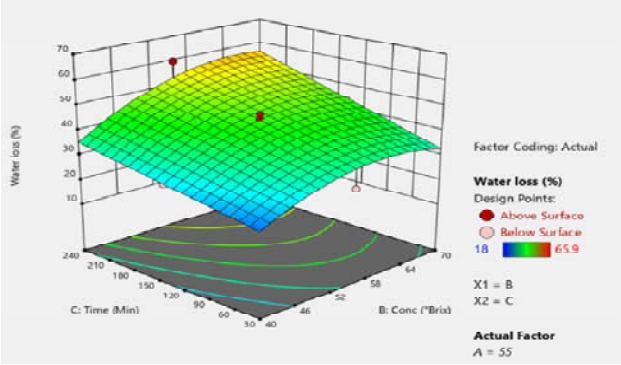
The solid gain (SG) values of dragon fruit slices for osmotic solutions with concentrations of 40-70° Brix, solution temperatures of 40-70°C, and immersion periods of 30-240 min ranged from 6.3% to 17.9%. A sugar solution with a concentration of 70° Brix, a temperature of 70°C, and an immersion duration of 240 minutes produced a higher solid gain value of 17.9%. At a concentration of 55 °Brix, a temperature of 55°C, and an immersion time of 135 minutes, a lower solid gain value of 6.3% was attained. The coefficient of determination (R^2) value for the solid gain equation was 0.9485. The solid gain parameter's F-value from the quadratic model was 20.47, which shows that the solid gain response was significant for the quadratic model ($p< 0.05$). The model terms are significant if the p-value of the independent terms was less than 0.05. In this instance, the solid gain parameter of dragon fruit slices has significant ($p<0.05$) model terms A, B, C, A^2, and B^2. P-values higher than 0.10 denote the absence of significance for the model terms. F-value is 12.09 and p-value is 0.0001 for the solid gain parameter's lack of fit value.



(a) Water loss (%) of dragon fruit slices as a function of solution concentration and temperature



(b) Water loss (%) of dragon fruit slices as a function of solution temperature and immersion time



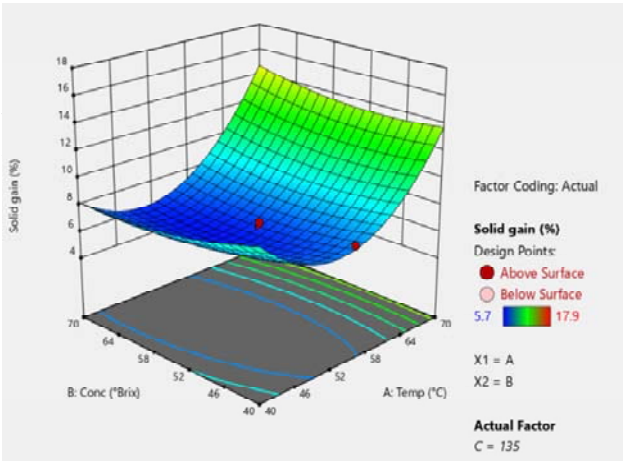
(c) Water loss (%) of dragon fruit slices as a function of solution concentration and immersion time

Fig. 1(a-c) : Response surface plots showing effect of process parameters on water loss of dragon fruit slices during osmotic dehydration

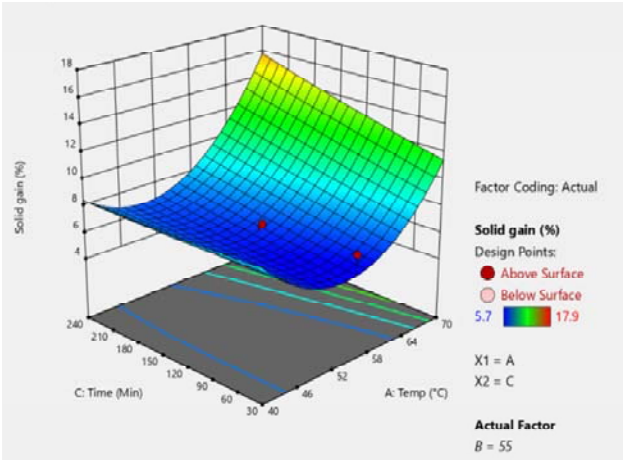
It suggests that the solid gain parameter of dragon fruit slices is significant for the lack of fit. One can predict the outcome of the equation using actual values for the variables as follows:

Solid gain (%) = 82.24328+ (-2.38905× A) + (-0.499841× B) + (-0.499841× C) + (0.003222 × AB) + (0.000746 × BC) + (-0.000302 × AC) +(-0.000302 × A^2) + (0.003152 × B^2) + (9.89487E-0.6×C^2) ….. (6)

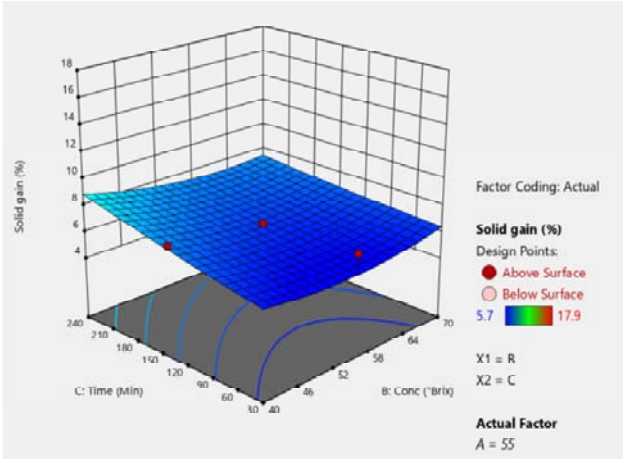
The solid gain increased with an increase in solution concentration, solution temperature and immersion time (Fig. 2). In comparison to solute concentration and immersion period, solution temperature had the greatest impact on the solid gain of dragon fruit slices as documented in carrot slices (Uddin et al., 2004), strawberry slices (Rizzolo et al., 2007) and papaya slices (Jain et al., 2011). All the three independent process parameters (process temperature, syrup concentration and process time) significantly improved the solid gain of dragon fruit slices during osmotic



(a) Solid gain (%) of dragon fruit slices as a function of solution concentration and temperature



(b) Solid gain (%) of dragon fruit slices as a function of solution temperature and immersion time



(c) Solid gain (%) of dragon fruit slices as a function of solution concentration and immersion time

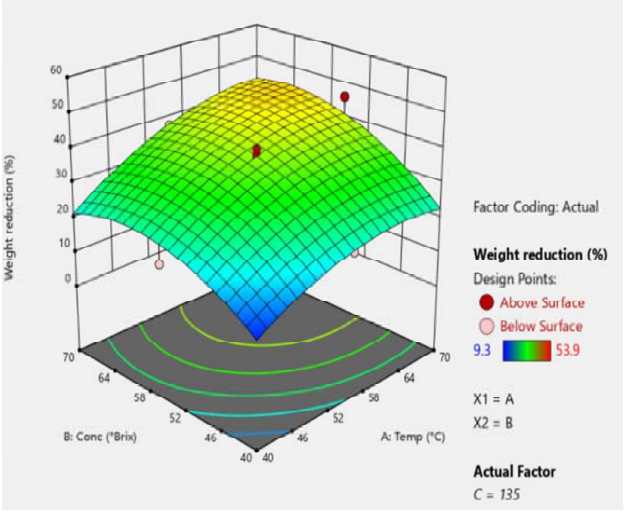
Fig. 2(a-c) : Response surface plots showing effect of process parameters on solid gain of dragon fruit slices during osmotic dehydration

Weight reduction response during osmotic dehydration of dragon fruit slices

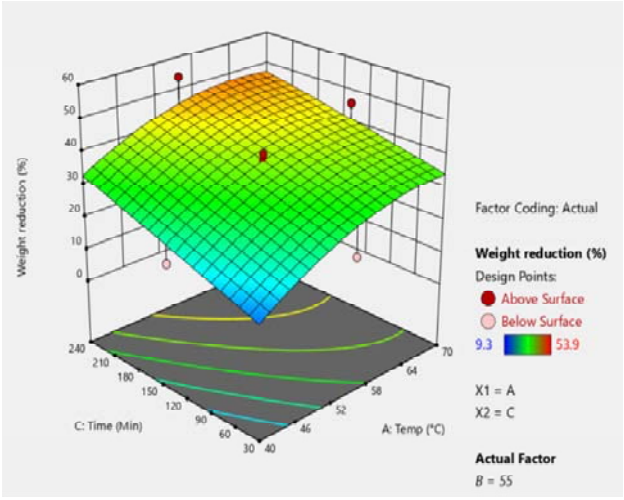
The weight reduction (WR) values of dragon fruit slices for osmotic solutions with concentrations of 40-70° Brix, solution temperatures of 40-70°C, and immersion periods of 30-240 min ranged from 9.31% to 50.60%. At a solution concentration of 70° Brix, solution temperature of 70°C, and immersion period of 240 min, a greater weight loss of 53.9% was obtained. At a solution concentration of 40 °Brix, solution temperature of 40°C and immersion period of 30 min, a lower weight loss of 9.3% was recorded. The coefficient of determination (R^2) value for the weight reduction equation was 0.91. The quadratic model (F-value 11.34) is significant if the $p < 0.05$. P-values higher than 0.10 denote the absence of significance for the model terms. F-value is 27.9 and p-value is 0.0001 for the lack of fit. Making predictions about the response for certain levels of each variable may be done using the equation in terms of real values of the variables as follows:

Weight reduction (%) = -172.40538+ (2.78213×A) +(3.54492×B)+(0.013759×C)+ (0.013759×AB) + (-0.000897×BC)+(0.002151×AC)+(-0.024162 ×A2)+(-0.03527+(0.000026×C2) …….(7)

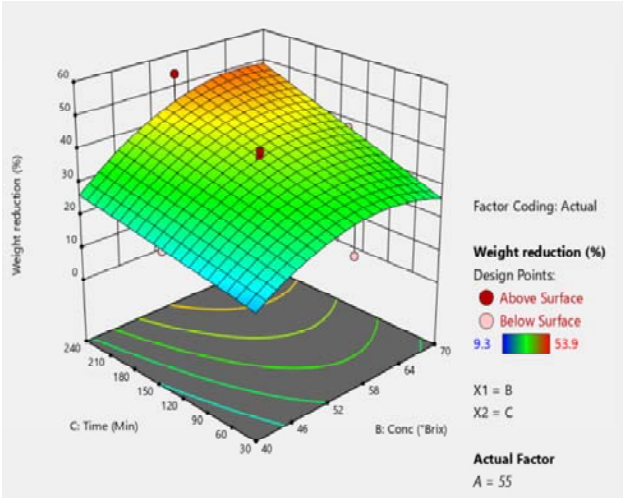
As the solute concentration, solution temperature, and immersion time rose, so did the weight loss of the dragon fruit slices during osmotic dehydration.



(a) Weight reduction (%) of dragon fruit slices as a function of solution concentration and temperature



(b) Weight reduction (%) of dragon fruit slices as a function of solution temperature and immersion time



(c) Weight reduction (%) of dragon fruit slices as a function of solution concentration and immersion time

Fig. 3 (a-c) : Response surface plots showing effect of process parameters on weight of dragon fruit slices during osmotic dehydration

The effect of solution concentration on dragon fruit slice pronounced more weight reduction than the solution temperature and immersion duration ($p< 0.05$) (Fig. 3). All the three process variables had a negative influence on weight reduction at the quadratic level, while, temperature had a statistically significant positive effect ($p< 0.05$) on weight reduction of the dragon fruit slices. Similar observation was quoted by Namrata et al. (2022) in muskmelon cubes. Shrivastava & Gowda (2016) also observed osmotic dehydration of the papaya fruits product with 60 °Brix syrup concentration regarding quality and stability.

CONCLUSION

The effects of sugar syrup concentration, syrup temperature and osmotic time on the osmotic dehydration of dragon fruit slices were investigated. The moisture content of dragon fruit samples was decreased to 27.5-68.49% (wb) after osmotic dehydration, experiments revealing water loss, solid gain, and weight reduction in the ranges of 18.01-65.9%, 6.3-17.9%, and 9.31-50.6%, respectively. It was revealed that the regression equations of second order offered the best fit for all of the experimental data on water loss, solid gain, and weight reduction. A maximum water loss of 58.2% and a minimum solid gain of 7.7% were projected with a syrup to fruit ratio of 4:1 and a syrup concentration of 65.3° Brix at a syrup temperature of 56.5°C in 240 minutes of osmotic dehydration.

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Original Research Paper

Effect of modified atmosphere packaging on quality of minimally processed fenugreek (*Trigonella foenum-graecum* L.) microgreens

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) microgreens is an underutilized vegetable with limited shelf life having good source of antioxidants, carotenoid as well as vitamins. The study deals with nutritional quality and optimization of a suitable passive modified atmosphere packaging (MAP) for improving the shelf life of fenugreek microgreens in its minimally processed form (MPFM) at 8°C Semipermeable plastic films *viz.*, low density polyethylene, polypropylene, Cryovac PD 961® and stretchable PVC cling film with varying thickness were evaluated as packaging materials to obtain different MAP composition inside MPFM packages. Packaging of MPFM in 40 µm thick polypropylene film resulted in development of in-pack equilibrium MA with 10-14% oxygen and 5-8% carbon dioxide during storage. This in-pack MA maintained 'fresh-like' sensory properties, biochemical and nutritional quality in MPFM till 15 days of storage. Significant loss of B vitamins was recorded in all packages during low temperature storage. Packaging in 40 µm thick polypropylene film retained B vitamins significantly better than other semipermeable films. Low temperature storage in modified atmosphere conditions enhanced vitamin E content in MPFM. The outcome of the study will benefit the entrepreneurs and retailers for distant transport and storage of fenugreek microgreens in commercial open chillers maintained in supermarkets in their ready-to-cook form.

Keywords: fenugreek**,** passive modified atmosphere packaging, vitamin, semi-permeable films

INTRODUCTION

Fenugreek microgreens are tiny green vegetable herbs harvested with two fully developed cotyledon leaves and first pair of true leaves. This stage often coincides with growth stage of 10-15 days of seedling emergence (Kyriacou et al., 2016). Microgreens catch the attention of nutritionists, chefs, and consumers due to nutritional quality and bioactive compounds besides their unique flavours; and are considered nutritional enhancers of diet. Microgreens possess a higher content of minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) and lower nitrate content than mature lettuces (Pinto et al., 2015), and are rich sources of antioxidants and vitamins due to abundance of ascorbic acid, phylloquinone, carotenoids, tocopherols, phenolic compounds etc.

Several microgreens are yet to be evaluated for their food use and nutritional quality (Mir et al., 2017). Fenugreek microgreens (FM) is a commodity with market niche restricted to Western parts of India, where it is locally known as *Choti methi* and *Samudra methi*, due to their characteristic tiny size and cultivation in sandy beaches of Arabian sea. The crop is usually sold in loose bundles in local markets, resulting in limited shelf life of less than a day at ambient temperature.

Passive modified atmosphere packaging (PMAP) is a low-cost packaging strategy for enhancement of shelf life in respiring foods such as fruits, vegetables and leafy greens. The basic technique of PMAP relies on modification of the atmosphere inside package to obtain an increased carbon dioxide and low oxygen levels through respiration of the produce. After a certain period, gas composition in the package of fresh product reaches an equilibrium state of gas composition, which is determined by respiration rate, product weight, temperature and permeability of packaging film (Kader et al., 2001; Soltani et al.,

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2015). Semipermeable plastics such as polypropylene, low density poly ethylene, PVC stretchable films, and many coextruded films are found useful for creating modified atmosphere in fresh produce packages (Ranjitha et al., 2018).

There is a potential for obtaining an enhanced shelf life of FM through minimal processing and packaging in passive MA packages. This study is a first ever report on the nutritional quality changes and shelf-life enhancement of minimally processed fenugreek microgreens (MPFM) during storage at open chiller temperature recommended in supermarkets.

MATERIALS AND METHODS

Plant material and minimal processing

Seeds of fenugreek were soaked overnight and broadcasted in aluminum trays filled with Arka fermented coco-peat as medium for seed germination (Kotur, 2014). The crop was harvested by uprooting on 14th-15th day of sowing at microgreen stage. The minimal processing operations included trimming out the roots, washing with tap water, dipping in sodium hypochlorite (100 ppm) for 5-minutes followed by rinsing with potable water and surface drying. Then the processed microgreens were used for further packaging studies.

Standardization of modified atmosphere packaging of MPFM

MPFM prepared with the above procedure were packed in different semi-permeable films having different gas and water vapour permeability characteristics. The films used were stretchable PVC Cling film (12 µm thickness), polypropylene films (25 µm and 40 µm), LDPE (40 µm) and Cryovac film (PD-961®), respectively to create different in-pack modified atmospheres. The MPFM packs were sealed with an impulse sealing machine and stored at 8°C. Twelve replicates were maintained for each type of packaging. Permeability characteristics of the packaging films used in the study are presented in Table 1.

In-pack gas composition analysis

Oxygen and carbon dioxide levels within the packs were recorded at periodic interval using auto gas analyzer (model : Checkmate 3-Dansensor, UK). Sufficient care was taken by affixing a septum on the packages to seal the needle puncture during gas measurement. Ethylene concentration within the packs was recorded at periodic interval using ethylene analyzer (model : ETHAN Bioconservacion, Spain).

Visual quality monitoring and sensory analysis

The unopened packages were observed daily for any changes in visual quality such as wilting, rotting and yellowing. This was carried out since the appearance is one key indicator of freshness in greens. Detailed sensory evaluation was carried out after drawing samples at five days interval. The experiment was terminated on 15th day of storage, as sensory deterioration was observed in all packages, including those which were judged as equal to freshly packed MPFM in previous days of evaluation. Sensory quality of MPFM was carried out in a 5-point Hedonic scale for characteristic *viz*., greenness, fresh-like odor, texture and overall acceptability; where the scores were classified as 5- excellent (exactly similar to freshly harvested), 4- very good, 3- good, 2- average and 1- poor. Score was given to individual quality attributes, and the overall marketability was taken as the most important quality attribute (Ranjitha et al., 2018). The samples were analysed for detailed nutritional quality parameters after 5, 10 and 15 days of storage. The experiment was terminated on 15th day of storage.

Instrumental measures of quality

Table 1 : Permeability characteristics of the packaging films

Film	Monomer	Permeability		
		O_2 (ml mil^{-1} m^{-2} day^{-1} atm^{-1})	CO_2 (ml mil^{-1} m^{-2} day^{-1} atm^{-1})	WVTR (g m^{-2} day^{-1} at 38°C; 90% R.H.)
Polypropylene	Propylene	1300-6400	7700-21,000	16-24
LDPE	Ethylene	3900-13,000	7700-77,000	10-12
PD-961	Polyolefin	7400–8500	21,000–24,000	9-14
PVC Cling	Vinyl chloride	63,555	127,733	140-171

Physiological loss of weight of MPFM was measured gravimetrically. Protein content was measured using modified Lowry's method (Lowry et al., 1951). Parameters *viz.*, total chlorophyll (mg g^{-1}), ferric reducing anti-oxidant potential (mg ascorbic acid equivalent antioxidant capacity, AEAC mg g^{-1}), total phenols (mg gallic acid equivalent g^{-1}, GE mg g^{-1}) and total flavonoids (catechin equivalent mg g^{-1}, CE mg g^{-1}) were measured spectrophotometrically using the standard methods (Benzie & Strain, 1996; Singleton & Rossi, 1965) after 15 days storage. Chlorophyll was measured spectrophotometrically (Haskin, 1942). All biochemical analyses were carried out in triplicates. Water soluble and fat-soluble vitamins were estimated sequentially per Santos et al. (2012).

Statistical analysis

The data obtained on biochemical analysis were statistically analyzed using complete randomized design (CRD) in MS Excel 2007 software. Single factor analysis of Variance (ANOVA) with three replications was carried out to determine the statistical significance and least significant difference (LSD) between treatments on different parameters was found out at 99% confidence level.

RESULTS AND DISCUSSION

Details on the effect of different packaging film on in-pack gas composition of MPFM is presented in Fig. 1. Cling film maintained a nearly normal atmospheric composition throughout storage, with O_2 ranging from 19-20% and CO_2 less than 1% levels. Among the different films, PP 40 µm film packages accumulated carbon dioxide rapidly during initial days of storage, and CO_2 levels in the packages were 12% within 4 days. Thereafter, the EMAP phase began, and CO_2 level balanced in a range of 5-8%. A rapid oxygen depletion was observed concomitant with CO_2 accumulation, reaching O_2 level to 2.5% within 4 days, followed by a further rise to 7.3% on 7th day, marking the EMAP level. The remaining three films, though developed a modified atmosphere, retained a higher oxygen and lower CO_2 than PP 40 µm throughout the storage. Briefly, the equilibrium modified atmosphere formed in the films *viz.*, PP 25 µm, PP 40 µm, LDPE 35 µm, Cryovac PD-961® packs were 11.2-15.1% and 3.7-4.3%, 10.4-14.0% and 5-8%, 13.2-15.3% and 1.7-2.1%, 12.7-14.30% and 2.5-2.7%, respectively. This difference in equilibrium modified atmosphere (EMAP) in these packages is the primary reason for a better shelf life of the MPFM in PP 40 µm packages.

The EMAP development is mainly dependent on respiration of the MPFM and gas permeability characteristics of the packaging film. Microgreens are rapidly respiring fresh produce, and the strategies to reduce respiration gives immense benefit in improving the shelf life (Sharma et al., 2022). It may be inferred that that permeability features of PP 40 µm is suitable for reducing the respiration rate of MPFM to develop a suitable EMAP. Ethylene accumulation inside packages also varied, and was found minimum in PP 40 µm packs (<1-5 mg/L), while, cling film package head space had 20-25 mg/L ethylene during most of the storage period (Fig. 1S). Ethylene is the main senescence hormone in plant tissues; and the accumulation of ethylene is a cause as well as

Table 2 : Effect of MAP on vitamin content of fresh and minimally processed fenugreek micro greens (MFM) stored for fifteen days at 8°C

Packaging film	Vit. B1 (µg g^{-1})	Vit. B2 (µg g^{-1})	Vit. B5 (µg g^{-1})	Vit. B6 (µg g^{-1})	Vit. B9 (µg g^{-1})	Vit. B7 (µg g-1)	Vit. E (µg g^{-1})	Vit. K (µg g^{-1})
Fresh	3.70±0.08	1.42±0.03	2.80±0.01	3.88±0.02	0.0020±0.00	0.07±0.002	700.40±24.50	867.70±28.50
Cling film	3.30±0.10	1.22±0.08	0.10±0.00	3.48±0.03	0.0014±0.000	0.04±0.004	745.10±63.20	604.20±1.60
PP 25 µm	3.30±0.10	1.27±0.05	0.17±0.00	3.52±0.03	0.0013±0.00	0.01±0.003	1005.60±19.1	744.30±17.50
PP 40 µm	3.40±0.10	1.47±0.07	0.19±0.00	3.54±0.03	0.0033±0.000	0.04±0.004	1237.80±6.70	939.50±8.90
LDPE 35 µm	3.00±0.10	1.25±0.01	0.16±0.00	3.44±0.10	0.0046±0.00	0.03±0.006	1170.60±4.90	736.10±8.80
PD-961®	3.40±0.10	1.38±0.08	0.16±0.00	3.39±0.02	0.0030±0.00	0.03±0.009	1147.60±0.30	738.60±17.60
F-test	*	*	*	*	NS	*	*	*
SE (m)	0.04	0.01	0.02	0.02	0.001	0.01	18.50	9.20
CD at 1%	0.16	0.04	0.09	0.11	0.003	0.03	77.80	38.80

Vit. indicates vitamin

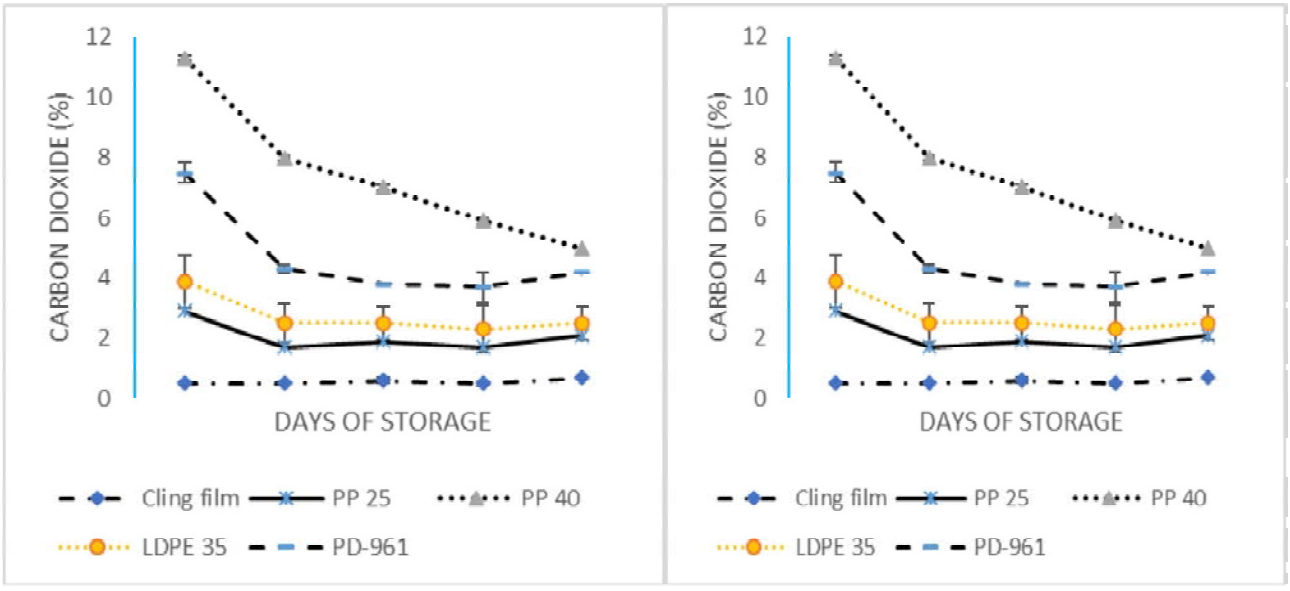

Fig. 1 : In pack O_2 and CO_2 gas composition of the minimally processed fenugreek micro greens packs during storage at 8°C. (Error bars represent standard deviation, n=12

consequence of tissue decay in microgreens (Turner et al., 2020).

Sensory quality of MPFM during storage was influenced by the EMAP conditions in the packaging films. The MPFM stored in PP 40 µm and Cryovac PD-961® possessed acceptable sensory quality till 15 days of storage (Table 3). The change in sensory quality at 5 and 10 days storage is presented in supplementary tables (Table 1S and 3S). In general, cling film wrapped samples scored low due to the poor appearance caused by wilting and yellowing of the produce; while, other packages resulted in slight yellowing of the produce due to extended storage period. This could be correlated well with the difference in chlorophyll levels of different MPFM samples after storage.

Total chlorophyll content was recorded highest in fresh MPFM (4.46 mg g^{-1}) followed by PP 40 µm (3.83 mg g^{-1}) and Cryovac PD-961® (3.27 mg g^{-1}), whereas, lowest was observed in cling film packed MPFM (1.01 mg g^{-1}). The degradative changes began within 10 days of storage in samples drawn from all packages, except PP 40 µm (Table 2S and 4S). Physiological loss of weight (PLW), was recorded highest in MPFM packed in cling film. Colour and turgidity are the main features contributing to the fresh

Table 3 : Effect of MAP on sensory and nutritional quality of fresh and minimally processed fenugreek microgreens (MPFM) stored for fifteen days at 8°C

Packaging film	Sensory acceptability	PLW (%)	Protein (mg g^{-1})	Total Carotenoids (mg g^{-1})	Total chlorophyll (mg g^{-1})	FRAP (mg g^{-1} AEAC)	Total Phenols (mg g^{-1} GAE)	Total flavonoids (mg g^{-1} CE)
Fresh	5.00±0.00	NA.	28.00±0.50	0.41±0.012	4.47±0.23	0.40±0.001	0.54±0.02	0.0011±0.00
Cling film	2.00±0.33	4.53±0.24	25.40±0.50	0.43±0.62	1.01±0.05	0.36±0.010	0.73±0.05	0.0012±0.00
PP 25µm	3.00±0.28	0.24±0.03	26.30±1.40	0.46±0.005	1.18±0.18	0.37±0.001	0.56±0.01	0.0011±0.00
PP 40 µm	4.50±0.19	0.15±0.01	27.20±0.60	0.47±0.006	3.83±0.40	0.42±0.004	0.55±0.07	0.0011±0.00
LDPE 40 µm	3.50±0.22	0.21±0.01	26.30±0.20	0.42±0.01	2.02±0.11	0.39±0.002	0.62±0.00	0.0011±0.00
PD-961	3.70±0.41	0.21±0.01	24.00±1.10	0.44±0.03	3.27±0.51	0.40±0.010	0.62±0.05	0.0011±0.00
F-test		*	NS	NS	*	*	NS	NS
CD at 1%		0.14			1.12	0.037		

All values are expressed as mean of triplicates ± standard deviation; *significant at 1% confidence level; AEAC: ascorbic acid equivalent antioxidant capacity; FRAP: ferric reducing antioxidant potential; GAE: gallic acid equivalent; CE: catechin equivalents; NS: non-significant

Table 4 : Effect of MAP on vitamin content of fresh and minimally processed fenugreek micro greens (MFM) stored for fifteen days at 8°C

Packaging film	Vit. B1 (µg g^{-1})	Vit. B2 (µg g^{-1})	Vit. B5 (µg g^{-1})	Vit. B6 (µg g^{-1})	Vit. B9 (µg g^{-1})	Vit. B7 (µg g-1)	Vit. E (µg g^{-1})	Vit. K (µg g^{-1})
Fresh	3.70±0.08	1.42±0.03	2.80±0.01	3.88±0.02	0.0020±0.00	0.07±0.002	700.40±24.50	867.70±28.50
Cling film	3.30±0.10	1.22±0.08	0.10±0.00	3.48±0.03	0.0014±0.000	0.04±0.004	745.10±63.20	604.20±1.60
PP 25 µm	3.30±0.10	1.27±0.05	0.17±0.00	3.52±0.03	0.0013±0.00	0.01±0.003	1005.60±19.1	744.30±17.50
PP 40 µm	3.40±0.10	1.47±0.07	0.19±0.00	3.54±0.03	0.0033±0.000	0.04±0.004	1237.80±6.70	939.50±8.90
LDPE 40 µm	3.00±0.10	1.25±0.01	0.16±0.00	3.44±0.10	0.0046±0.00	0.03±0.006	1170.60±4.90	736.10±8.80
PD-961®	3.40±0.10	1.38±0.08	0.16±0.00	3.39±0.02	0.0030±0.00	0.03±0.009	1147.60±0.30	738.60±17.60
F-test	*	*	*	*	NS	*	*	*
SE (m)	0.04	0.01	0.02	0.02	0.001	0.01	18.50	9.20
CD at 1%	0.16	0.04	0.09	0.11	0.003	0.03	77.80	38.80

Vit. - indicates vitamin

appearance of greens. Greenness and turgid appearance of the plant tissues represents the integrity of vacuoles and other membrane bound organelles in the cell (Martínez-Sánchez et al., 2011). High water permeability of cling film resulted in loss of turgidity and resulted in tissue disintegration at a rapid rate in cling wrapped MPFM samples. Better retention of chlorophyll content and low PLW were the major contributing features of fresh-like appearance and sensory acceptability of MPFM samples packaged in PP 40 µm film.

Nutritional quality of MPFM was also influenced by EMAP conditions in the packages. Ferric reducing antioxidant capacity of the samples packaged in LDPE, PP 40 and PD-961 remained statistically on par with the fresh FM even after 15 days storage, and significantly superior than cling film wrapped samples. This showed that the antioxidants of the microgreens could be well preserved during postharvest storage using a wide range of modified atmosphere packaging. Antioxidant capacity of microgreens is affected by the storage duration, temperature, relative humidity and the in-pack atmosphere (Xiao et al., 2014). Though, the phenolics in cling wrapped MPFM were on par with other packages, the lower total antioxidant capacity level could be attributed to the loss of other antioxidants such as vitamins.

Vitamin profiling of FM showed that fresh FM is a good source of B vitamins such as B1, B2, B5 and B6 and the fat-soluble vitamins E and K (Table 3). Low temperature storage invariably caused the loss of B vitamins, and the highest loss was visible in vitamin B5. Water soluble vitamins are lost rapidly during postharvest storage of leafy greens such as cabbage (Hounsome et al., 2009). At the same time, low temperature storage enhanced vitamin E content in many MPFM samples. Tocopherol is the main form of vitamin E present in leaves. Vitamin E level enhances during senescence of leaves, cold stress and dark stress (Keles & Oncel, 2002; Kobayashi & Della Penna, 2008). In plant tissues, tocopherols act as antioxidants to protect photosystem II from photoinactivation and cell membranes from lipid peroxidation (Havaux et al., 2005). In the present study, storage in walk-in cold room provided the stresses of low temperature, senescence and dark conditions which have probably helped in accumulation of tocopherols in MPFM in most of the EMAP packages. In cling film wrapped MPFM, where the physiological loss of weight and tissue deterioration was substantially high, vitamin E was less, which may be reasonably attributed to tissue dehydration related metabolic responses.

The present study proved that FM is a rich source of vitamin K. Phylloquinone (vitamin K1) is the major form of vitamin K, which ranges from 90 to 410 µg g^{-1} in different microgreens (Xiao et al., 2012). In MPFM, there was a substantial increase in phylloquinone (vitamin K1) in PP 40 µm packed leaves, which was best MAP for maintaining quality. It can be presumed that the suitability of MAP conditions in PP 40 µm helped to retain freshness in the leaves including accumulation of phylloquinone, while, a range of degradative changes in other suboptimal MA packages have contributed to degradation of phylloquinone too.

CONCLUSION

Present investigation has proven the potential dietary use of FM as source of antioxidants, B vitamins, vitamin E and K. The study has also proven the potential of a PMAP method using a commonly available semipermeable film to obtain an economically viable shelf life. Superior results on shelf-life enhancement and nutritional quality retention of MPFM in fenugreek microgreens was obtained by packaging of MPFM in 40 µm thick polypropylene film, which created an equilibrium modified atmosphere of 10.4-14.0% O_2 and 5-8% CO_2. Besides retaining the consumer acceptability features, this packaging has substantially reduced ethylene accumulation, maintained antioxidants and vitamins, and 15 days shelf life during storage at 8°C. Thus, insights from this study reiterates the importance of underutilized microgreens in local food systems, as well as provides an opportunity for its value addition and packaging suitable for distant transport and marketing.

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Original Research Paper

Effect of edible dyes on value addition and post-harvest life of tuberose spikes

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ABSTRACT

Tuberose is one of the most imperative bulbous crops with white fragrant florets, used for loose as well as cut flowers. All the commercial varieties have white colour flower only which is limiting factor in its popularity and marketing. By using edible dyes this issue may be solved for getting higher price in market as well as consumers satisfaction. An investigation was carried out to study the effect of food dyes on value addition and post harvest life of cut spikes of tuberose cv. Local Double in completely randomized design with factorial concept consisting thirty-six treatment combinations and one absolute control. Different treatments of food dyes and immersion time considerably induced colour in tuberose spikes without affecting postharvest life. The results revealed that 4% lemon yellow food dye with one hour immersion of tuberose cut spikes was found best in improving vase life (6.02 days), floret diameter (3.29 cm), opening of florets (49.66%) and visual appearance with minimum physiological loss in weight (16.91 % and 34.04 % at second and fourth day, respectively). Tinted tuberose cut spikes aids higher net return than white flowers, which will benefit farmers in fetching good prices.

Keywords: Cut spike, food dyes, immersion duration, postharvest life, tinting, tuberose

INTRODUCTION

Tuberose (*Polianthes tuberosa* Linn.) belonging to the family Asparagaceae, is one of the important bulbous flower crops of tropical and sub-tropical regions and popularly known as '*Rajanigandha*' or '*Nishigandha*'. Tuberose, both single and double types, are highly demanded for their waxy white spikes, which have a longer keeping quality; fill the air with their pleasant aroma, and used for loose flowers, cut flowers and oil extraction. The double-flowered tuberose form is highly prized for their usage in floral arrangements and as table decorations. Value addition refers to the process of increasing the value of a raw product by making modifications to the processes or diversifying the product line. Therefore, to make tuberose colourful, tinting is simple and farmer friendly approaches fetch remunerative prices. Dyes can be used for tinting to get a desired colour as they travel through the xylem or veins of a flower, because of its capillary action. This is a vital value-adding technique in flower crops to add colours especially in white cut flowers (Safeena et al., 2016). Inflorescences coloured with edible dyes are more attractive, have a wider colour palette for embellishment, and fetch a higher price. Farmers can increase their earnings by selling tinted tuberose spikes at a premium price over white spikes. In view of this, the present study involved use of several food dyes to transfer varying shades of colour on the petals of aromatic spikes of tuberose cv. Local Double.

MATERIAL AND METHODS

The experiment was conducted at Laboratory, Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, NAU, Navsari during 2017-18, which is located at 20º 572 N latitude, 72º 542 E longitude, and an elevation of about 11.83 meters above mean sea level. The goal of the study was to determine the impact of food dyes on the postharvest life of tuberose spikes cv. Local Double. The experiment was laid out in completely randomised design with factorial concept (FCRD) consisting twelve levels of food dyes with different concentrations *viz*., lemon yellow 4% (C_1), lemon yellow 8% (C_2), kesar yellow 4% (C_3), kesar yellow 8% (C_4), kalakatta 4% (C_5), kalakatta 8% (C_6), orange red 4% (C_7), orange red 8 % (C_8), rose pink 4% (C_9), rose pink 8% (C_{10}), raspberry red 4% (C_{11}) and raspberry red 8% (C_{12}) and three levels of

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Table 1 : Effect of food dyes and immersion period on vase life and quantity of dye uptake of tuberose

Treatment	Vase life (days)				Dye solution uptake (mL/spike)			
	1 hour (I_1)	2 hours x(I_2)	3 hours (I_3)	Mean	1 hour (I_1)	2 hours (I_2)	3 hours (I_3)	Mean
Lemon yellow 4% (C_1)	6.02	5.69	5.60	5.77	3.16	3.58	5.69	4.14
Lemon yellow 8% (C_2)	5.78	5.73	5.51	5.67	2.67	3.58	5.13	3.79
Kesar yellow 4% (C_3)	5.80	5.73	5.24	5.59	3.18	3.62	4.71	3.84
Kesar yellow 8% (C_4)	5.53	5.42	5.42	5.46	3.18	3.33	4.09	3.53
Kalakatta 4% (C_5)	5.38	5.00	5.04	5.14	3.18	3.64	5.07	3.96
Kalakatta 8% (C_6)	5.13	4.96	4.89	4.99	3.22	3.53	4.44	3.73
Orange red 4% (C_7)	5.29	5.09	4.80	5.06	3.24	3.60	5.38	4.07
Orange red 8 % (C_8)	5.02	4.87	4.89	4.93	3.04	3.31	4.38	3.58
Rose pink 4% (C_9)	5.80	5.56	5.24	5.53	3.51	4.13	4.62	4.09
Rose pink 8% (C_{10})	5.47	5.33	5.29	5.36	2.93	2.91	4.62	3.49
Raspberry red 4% (C_{11})	5.40	5.22	5.27	5.30	3.42	3.42	4.93	3.93
Raspberry red 8% (C_{12})	5.51	5.22	4.91	5.21	2.76	3.11	4.13	3.33
Mean	5.51	5.32	5.18	5.34	3.12	3.48	4.77	3.79
Control	6.09	-	-	-	0.00	-	-	-
	C	I	C x I	-	C	I	C x I	-
S. Em±	0.07	0.04	0.12	-	0.10	0.05	0.18	-
CD at 5%	0.20	0.10	NS	-	0.28	0.14	0.49	-
Cont. v/s rest								
S. Em±	0.09	-	-	-	0.12	-	-	-
CD at 5%	0.25	-	-	-	0.35	-	-	-

immersion periods *viz*., 1 hour (I_1), 2 hours (I_2) and 3 hours (I_3) along with one absolute control (distilled water). The experiment was repeated thrice to confirm the results. Dye solutions 4% and 8% were prepared by dissolving 40 g/L and 80 g/L of edible dyes in one litre of distilled water, respectively. Five freshly cut spikes of tuberose were dipped in the different dye solution and placed in a container.

To test the impact of food dye and immersion period on the post-harvest longevity of tuberose, cut spikes were dipped to the dye solutions at ambient conditions as per immersion period and then placed in vases with distilled water. During the time of experimentation, average maximum temperature (28 ºC, 27 ºC and 29 ºC), minimum temperature (20 ºC, 21 ºC and 12 ºC) and relative humidity (55.40 %, 84.35 % and 56.80 %) were recorded in laboratory during March 2017, September 2017 and February 2018, respectively. Throughout the experiment, data were recorded on vase life, physiological weight loss, dye uptake quantity, bud opening, floret diameter and colour intensity. Weight loss due to normal physiological processes was measured on second and fourth day. A digital Vernier calliper was used to measure the diameter of the third set of florets from the base, and the percentage of opened florets on each cut spike was estimated on third day of the experiment. The colour acquired by cut spikes was recorded by using RHS colour chart. Analysis of variance and critical difference (5 %) was used as reported by Gomez and Gomez (1984) to conduct statistical analysis of the data.

RESULTS AND DISCUSSION

Data presented in Table 1 showed impact of various food dyes and immersion period on the vase life and solution uptake of tuberose spikes cv. Local Double. The vase life of control spikes was recorded maximum (6.09 days), while, in dyed tuberose spikes, it was recorded 5.77 days in lemon yellow 4% (C_1), whereas, negatively influenced by a higher concentration (8%) of each food colour. The vase life of spikes immersed in dye solution for 1 hour is increased up to 5.51 days compared to 2 and 3 hours of immersion (5.32 and 5.18 days, respectively). However, non-significant relationship was observed between immersion time and types of food colour used. The poor cell turgidity and water loss linked with the shift in osmotic pressure may account for the shorter vase life as observed with coloured spikes compared to the control. Upon examination of a cross section of the cut spike of coloured tuberose, it was found that the dye

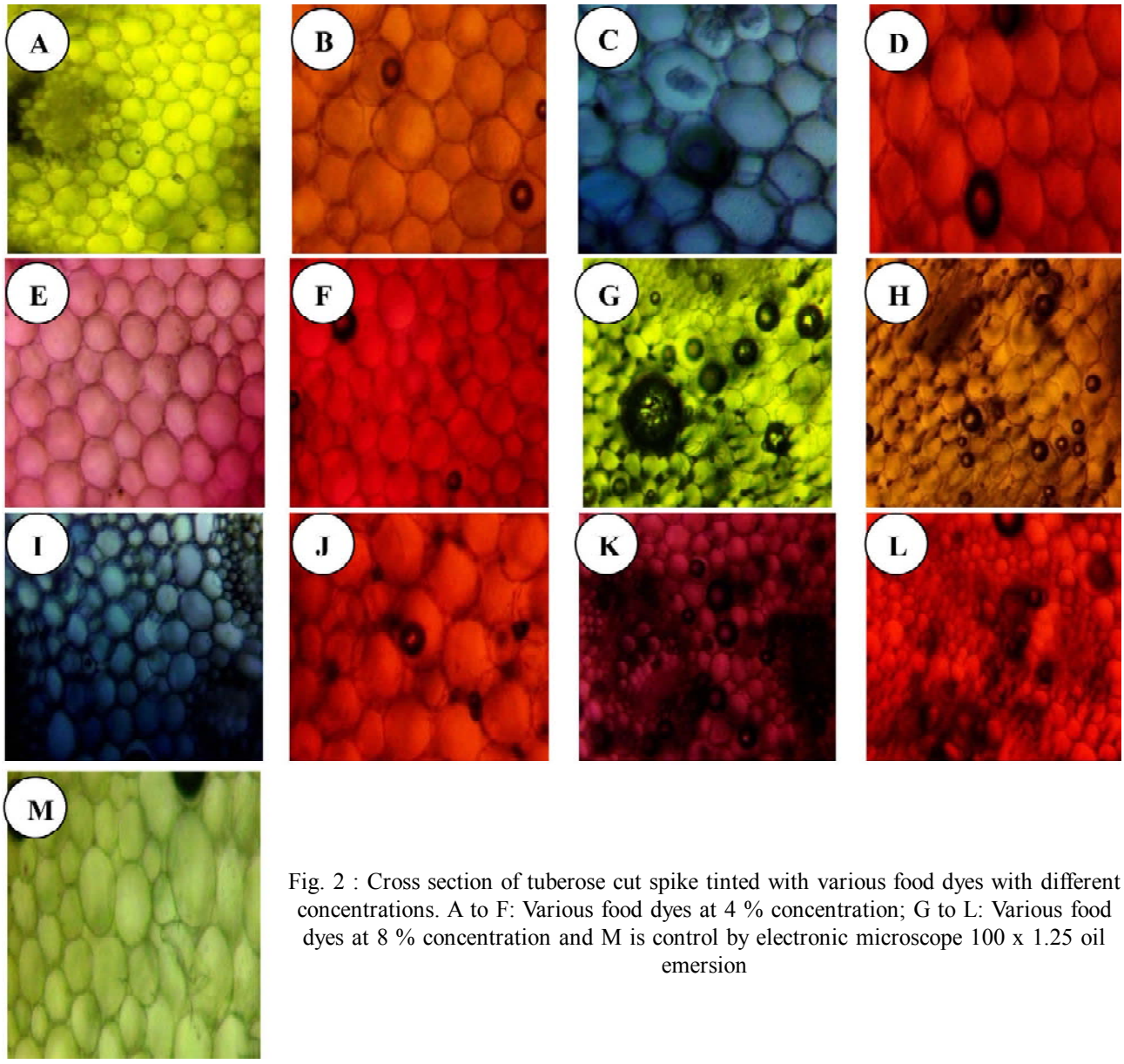

Fig. 2 : Cross section of tuberose cut spike tinted with various food dyes with different concentrations. A to F: Various food dyes at 4 % concentration; G to L: Various food dyes at 8 % concentration and M is control by electronic microscope 100 x 1.25 oil emersion

concentration 4% caused significantly less blockage than 8% (Fig. 2). Wilted florets and reduction in vase life was due to lack of nutrition since the dye particles clogged xylem and phloem vessels, disrupting the movement of water and food components. These findings are consistent with the previous studies on tuberose (Safeena et al., 2016; Kumari & Deb, 2018).

The maximum dye solution uptake (4.14 mL/spike) was recorded in spikes dipped in 4% lemon dye solution. Equally, all food colours showed more solution uptake in 4% solution in comparison to 8%. It is possible that the higher water flow and water balance associated with lower dye concentration may contribute to better uptake of dye solution. Specifically, a 3-hour immersion period resulted in highest dye solution uptake (4.77 mL/spike). Further, substantial findings were observed for interaction of food dyes and immersion period, with maximal dye solution uptake in 4% lemon yellow dye for 3 hours (5.69 mL/spike). The longer time for submerged condition in lower concentration of dye solution is responsible for more vibrant coloration of florets, as it allows for greater water circulation and water balance. The present findings corroborate the studies of Safeena et al. (2016) and Kumari and Deb (2018).

Data presented in Table 2 provides an overview of effects of different food dyes, immersion period, and their interaction on floret diameter and opening of

Table 2 : Effect of food dyes and immersion period on floret diameter and opening of florets of tuberose

Treatment	Floret diameter (cm) at 3rd day				Opening of florets (%) at 3rd day			
	1 hour (I_1)	2 hours x(I_2)	3 hours (I_3)	Mean	1 hour (I_1)	2 hours (I_2)	3 hours (I_3)	Mean
Lemon yellow 4% (C_1)	3.29	3.12	2.88	3.09	49.66	45.32	37.03	44.00
Lemon yellow 8% (C_2)	2.47	2.48	2.56	2.51	41.31	40.15	35.82	39.09
Kesar yellow 4% (C_3)	2.70	2.62	2.65	2.66	41.33	41.53	36.29	39.71
Kesar yellow 8% (C_4)	2.61	2.61	2.66	2.63	36.58	34.68	36.87	36.04
Kalakatta 4% (C_5)	2.91	2.71	2.66	2.76	39.37	38.73	36.18	38.09
Kalakatta 8% (C_6)	2.73	2.66	2.77	2.72	35.51	29.95	32.16	32.54
Orange red 4% (C_7)	2.96	2.84	2.89	2.90	48.22	43.67	36.46	42.78
Orange red 8 % (C_8)	2.91	2.84	2.80	2.85	41.08	37.42	33.93	37.48
Rose pink 4% (C_9)	2.99	2.92	2.95	2.96	41.67	35.67	33.08	36.81
Rose pink 8% (C_{10})	2.92	2.89	2.84	2.88	39.13	33.49	32.26	34.96
Raspberry red 4% (C_{11})	3.01	2.91	2.88	2.93	43.38	40.65	41.28	41.77
Raspberry red 8% (C_{12})	2.75	2.86	2.75	2.79	44.17	37.82	33.68	38.56
Mean	2.85	2.79	2.77	2.81	41.78	38.26	35.42	38.49
Control	3.08	-	-	-	48.52	-	-	-
	C	I	C x I		C	I	C x I	-
S. Em±	0.05	0.03	0.09	-	0.58	0.29	1.00	-
CD at 5%	0.15	NS	NS	-	1.62	0.18	2.80	-
Cont. v/s rest								
S. Em±	0.06	-	-	-	0.71	-	-	-
CD at 5%	0.18	-	-	-	1.99	-	-	-

florets on 3rd day. When flowers were preserved for decoration, such as in vases, flower diameter is an important quality parameter (Kumari and Deb, 2018). The diameter of the florets was noticeably affected by the concentration of the dye solutions. Florets exposed to lower concentrations of each food colour (4 %) were larger than those exposed to higher concentrations (8%) on 3rd day. Significantly, largest floret diameter (3.09 cm) was recorded in 4% lemon yellow colour over the control. However, there was no statistically significant effect of immersion duration as well as interaction of food dyes and immersion time. A sufficient supply of sucrose may have sped up respiration rate required for cell division, cell enlargement, and production of cell constituents, all of which would have contributed to larger size of florets. Kumar *et al.* (2015) have also reported analogous results in gladiolus.

Significant effect of food colouring, immersion period and its interaction as observed on meter and opening of florets (Table 2). On 3rd day, the florets treated with 4 % lemon yellow dye showed highest rate of opening (44.00 %). With comparison of 4 % and 8 % food dye solutions, it was found that 4 % solution of each food dye resulted in the most open florets. When spikes were submerged for 1 hour, the maximum opened florets (41.78%) compared to the other two immersion intervals. Maximum floret opening (49.66%) was recorded after soaking spikes 4% lemon yellow dye solution for 1 hour, demonstrating considerable influence of both food dye and immersion period. The florets opening may due to their improved carbohydrate metabolism, respiration rate, and water balance. The floret opens in part due to the availability of soluble carbohydrate and an increase in osmotic potential for growth of petal cells caused by least stem obstruction (Kumari and Deb, 2018).

On the perusal of data presented in Table 3 indicated the effect of food dyes and immersion period on physiological weight loss on 2nd and 4th days. The least physiological weight loss was recorded in the control (15.20% and 33.33%) on 2nd and 4th days, respectively, for various dyes tested. Spikes dipped in different dyes at 4% recorded least physiological weight loss on 2nd and 4th days, in comparison to cut spikes dipped in 8%. Comparatively, the physiological weight loss in cut spikes receiving 4% lemon yellow dye was lowest on 2nd (19.06%) and 4th (38.45%) day. There was significant temporal effect, with spikes losing 23.34% and 43.51% of their physiological

Table 3 : Effect of food dyes and immersion period on physiological loss of weight (%) of tuberose

Treatment	Physiological loss of weight (%)							
	2nd day				4th day			
	1 hour (I_1)	2 hours x(I_2)	3 hours (I_3)	Mean	1 hour (I_1)	2 hours (I_2)	3 hours (I_3)	Mean
Lemon yellow 4% (C_1)	16.91	19.12	21.15	19.06	34.04	38.38	42.91	38.45
Lemon yellow 8% (C_2)	20.97	24.97	26.47	24.14	37.47	43.77	46.89	42.71
Kesar yellow 4% (C_3)	24.62	25.45	28.52	26.19	40.97	45.31	49.05	45.11
Kesar yellow 8% (C_4)	26.65	29.82	31.76	29.41	46.34	55.86	58.61	53.60
Kalakatta 4% (C_5)	23.42	26.66	28.50	26.19	44.76	49.94	54.36	49.69
Kalakatta 8% (C_6)	29.44	33.14	34.76	32.45	51.06	57.95	60.29	56.43
Orange red 4% (C_7)	23.61	28.45	29.81	27.29	44.95	53.99	55.84	51.59
Orange red 8 % (C_8)	25.86	29.56	30.70	28.71	48.60	55.39	57.02	53.67
Rose pink 4% (C_9)	19.85	24.61	26.06	23.51	42.17	46.83	49.64	46.21
Rose pink 8% (C_{10})	23.29	27.36	29.39	26.68	44.90	51.78	53.64	50.10
Raspberry red 4% (C_{11})	21.66	24.02	25.36	23.68	41.83	47.49	51.25	46.86
Raspberry red 8% (C_{12})	23.79	26.94	29.68	26.80	45.02	50.35	54.23	49.87
Mean	23.34	26.68	28.51	26.18	43.51	49.75	52.81	48.69
Control	15.20	-	-	-	33.33	-	-	-
	C	I	C x I	-	C	I	C x I	-
S. Em±	0.35	0.18	0.61	-	0.49	0.25	0.85	-
CD at 5%	0.98	0.49	NS	-	1.37	0.69	NS	-
Cont. v/s rest								
S. Em±	0.43	-	-	-	0.60	-	-	-
CD at 5%	1.20	-	-	-	1.68	-	-	-

weight on 2nd and 4th day, respectively, when spikes dipped in dye for 1 hour. However, the combined effects of food colouring and immersion time did not have any appreciable impact on spike physiological leanness. There may be less impact on supply of water and food because fewer colour particles can be absorbed by spikes in 1 hour. Therefore, the physiological weight loss could be mitigated by cells potential use of water absorbed by spikes to preserve cell turgidity (Kumar *et al*., 2015).

Table 4 : Effect of food dyes and immersion period on tuberose flower colour intensity (RHS colour chart)

Treatment	1 hour (I_1)	2 hours (I_2)	3 hours (I_3)
Lemon yellow 4% (C_1)	Brilliant yellow, 7(B)	Brilliant greenish yellow, 5(A)	Vivid yellow, 9(A)
Lemon yellow 8% (C_2)	Vivid yellow, 13(A)	Brilliant greenish yellow, 6(A)	Vivid yellow, 14(A)
Kesar yellow 4% (C_3)	Strong orange, N25(C)	Vivid orange yellow, 6(A)	Vivid yellow, 14(A)
Kesar yellow 8% (C_4)	Strong orange, N25(C)	Strong orange, N25(B)	Strong orange, 25(A)
Kalakatta 4% (C_5)	Very pale purplish blue, 101(D)	Very pale purplish blue, 106(D)	Very light purplish blue, 102(D)
Kalakatta 8% (C_6)	Moderate blue, 98(B)	Light blue, 107(D)	Very pale purplish blue, 101(D)
Orange red 4% (C_7)	Strong reddish orange, 32(B)	Vivid orange, N30(D)	Strong orange, N25(B)
Orange red 8 % (C_8)	Vivid reddish orange, 32(A)	Strong reddish orange, 31(A)	Strong orange, N25(A)
Rose pink 4% (C_9)	Very pale purple, N76(C)	Grayish purplish red, N77(D)	Very pale purple, 76(C)
Rose pink 8% (C_{10})	Very light purple, 76(C)	Light purple, 77(D)	Very light purple, 76(B)
Raspberry red 4% (C_{11})	Light purplish pink, 68(D)	Deep purplish pink, 58(D)	Strong purplish red, 58(B)
Raspberry red 8% (C_{12})	Deep purplish pink, N54(B)	Vivid purplish – N57(B)	Vivid purplish red –N57(A)
Control	Pale yellow green, NN155(B)		

Fig. 1 : Colour intensity of various food dyes at 4 % and 8 % concentration for 1 h immersion time. (A) 4% lemon yellow, (B) 4% kesar yellow, (C) 4% kalakatta, (D) 4% orange red, (E) 4% rose pink, (F) 4% raspberry red, (G) 8% lemon yellow, (H) 8% kesar yellow, (I) 8% kalakatta, (J) 8% orange red, (K) 8 % rose pink and (L) 8% raspberry red

RHS colour codes of spikes influenced by various food dyes and immersion periods were recorded (Table 4). After being treated with various dyes, the white tuberose spikes took on diverse hues (Fig. 1). Preferred colour shades can be created by tinting with various food dyes. It was found that the length of the immersion correlated with the colour intensity of the flower. The results corroborate the findings of Viradia *et al.* (2015) & Sowmeya *et al.* (2017) in tuberose, rose and carnation.

Table 4 lists the RHS colour codes of spikes affected by various food dyes and immersion period. The white tuberose spikes took on a variety of colours after being dyed (Fig. 1). By adding various food dyes, one can tint spikes to desired colour tones. It was discovered that the duration of the immersion connected with the flower's colour intensity. The findings are supported by the results of Viradia et al. (2015) and Sowmeya et al. (2017) in in tuberose, rose, and carnation.

Table 5 shows the cost-effectiveness of several food dyes for creating coloured tuberoses. The two treatments that produced the highest net realisation (1.952) were 4% lemon yellow for 1 hour and 4% kesar yellow for 1 hour.

CONCLUSION

Based on the results, it can be concluded that soaking of tuberose spikes in 4% lemon yellow dye solution for 1 hour produce beautiful, natural, and pretty flowers. This is because the dye solution can make flowers more attractive in terms of colour, vase life, floret diameter, dye solution uptake, floret opening,

Table 5 : Economics of tinted tuberose cut spikes with different food dyes

Treatment	Cost of dye/500 g (Rs.)	Cost of dye solution (Rs./L)	Cost of normal spike (Rs./spike)	Cost of dye (Rs/spike)	Total cost (Rs./ spike)	Income of tinted spike (Rs./spike)	Net realization (Rs./spike)
C_1I_1	190	15.2	3	0.048	3.048	5	1.952
C_1I_2	190	15.2	3	0.054	3.054	5	1.946
C_1I_3	190	15.2	3	0.086	3.086	5	1.914
C_2I_1	190	30.4	3	0.081	3.081	5	1.919
C_2I_2	190	30.4	3	0.109	3.109	5	1.891
C_2I_3	190	30.4	3	0.156	3.156	5	1.844
C_3I_1	190	15.2	3	0.048	3.048	5	1.952
C_3I_2	190	15.2	3	0.055	3.055	5	1.945
C_3I_3	190	15.2	3	0.072	3.072	5	1.928
C_4I_1	190	30.4	3	0.097	3.097	5	1.903
C_4I_2	190	30.4	3	0.101	3.101	5	1.899
C_4I_3	190	30.4	3	0.124	3.124	5	1.876
C_5I_1	190	15.2	3	0.048	3.048	5	1.952
C_5I_2	190	15.2	3	0.055	3.055	5	1.945
C_5I_3	190	15.2	3	0.077	3.077	5	1.923
C_6I_1	190	30.4	3	0.098	3.098	5	1.902
C_6I_2	190	30.4	3	0.107	3.107	5	1.893
C_6I_3	190	30.4	3	0.135	3.135	5	1.865
C_7I_1	190	15.2	3	0.049	3.049	5	1.951
C_7I_2	190	15.2	3	0.055	3.055	5	1.945
C_7I_3	190	15.2	3	0.082	3.082	5	1.918
C_8I_1	190	30.4	3	0.092	3.092	5	1.908
C_8I_2	190	30.4	3	0.101	3.101	5	1.899
C_8I_3	190	30.4	3	0.133	3.133	5	1.867
C_9I_1	300	24.0	3	0.084	3.084	5	1.916
C_9I_2	300	24.0	3	0.099	3.099	5	1.901
C_9I_3	300	24.0	3	0.111	3.111	5	1.889
$C_{10}I_1$	300	48.0	3	0.141	3.141	5	1.859
$C_{10}I_2$	300	48.0	3	0.140	3.140	5	1.860
$C_{10}I_3$	300	48.0	3	0.222	3.222	5	1.778
$C_{11}I_1$	250	20.0	3	0.068	3.068	5	1.932
$C_{11}I_2$	250	20.0	3	0.068	3.068	5	1.932
$C_{11}I_3$	250	20.0	3	0.099	3.099	5	1.901
$C_{12}I_1$	250	40.0	3	0.110	3.110	5	1.890
$C_{12}I_2$	250	40.0	3	0.124	3.124	5	1.876
$C_{12}I_3$	250	40.0	3	0.165	3.165	5	1.835
Control	0	0	3	0	3.000	0	0

and physiological weight loss. Additionally, desired colour tones can be achieved by colouring with different food dyes including kesar yellow, kalakatta, orange red, rose pink, and raspberry red at 4% concentration for a 1 hour immersion duration. Thus, tinting could be adopted by the tuberose producers which needs less skill and fetches higher price for beautifully coloured flowers.

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Original Research Paper

In vitro susceptibility test of *Cladosporium cladosporioides* isolates from Argentinian tomato crops against commercial fungicides

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ABSTRACT

Tomato cultivation is an important agricultural activity in northeast of Argentina, and disease control is indispensable for its production. The purpose of the current study was to identify two fungi isolated from tomato plants cultivated in greenhouses, with symptoms of leaf mould disease and a strain of the genus *Cladosporium* from a culture collection, and evaluate their *in vitro* susceptibility to four commercial fungicides. Macro and microscopic examination, molecular characterisation and sequence analysis were applied for identification. Broth dilution and spread plate methods were used to determine the minimal inhibitory concentration (MIC) and minimal fungicide concentration (MFC). The active ingredients of the products were azoxystrobin+difenoconazole, trifloxystrobin+tebuconazole, chlorothalonil and metalaxyl-M+mancozeb. The results were processed using the Kruskal-Wallis method. The isolates were identified as *Cladosporium cladosporioides*$^{a-c}$; consequently, lesions found on tomato plants did not correspond to *Cladosporium fulvum*. There was a significant statistical difference between the obtained values. Qualitatively, the three strains had a similar behaviour for chlorothalonil (MIC values: 0.25 - 0.5 µg/ml, MFC values: 4 µg/ml). In all cases, tests with metalaxyl-M+mancozeb yielded higher values than those achieved for chlorothalonil (MIC values: 8 µg/ml, MFC values: 8- 32 µg/ml). trobilurin-formulated fungicides were less effective against *C. cladosporioides*$^{a-b}$ (MIC values: 16-256 µg/ml, MFC values: >64 µg/ml). *C. cladosporioides*^{c} was the most sensitive isolate. The information about the presence of a non-frequent fungus and its fungicide susceptibility, would be useful for establishing control strategies and enhance production.

Keywords: Antifungals, characterisation, *Cladosporium cladosporioides*, greenhouse, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an annual vegetable crop with a wide geographic distribution and consumption throughout the world. It is one of the most important vegetables in Argentina. In some regions, it is produced in greenhouses, which account for almost a half part of the area used for vegetables cultivation. This area is principally located in the green belt of La Plata, followed by Corrientes and other important provinces (Sinavimo, 2018; Sosa, 2013).

Producers adopt integrated disease management (IDM) to maintain healthy crops during a long production period (March-December). IDM congregates several practices maintaining low incidence levels (Obregón, 2018). However, diseases continue to cause serious economic losses (Lucas et al., 2015). Phytopathogenic fungi can cause numerous diseases in plants, such as leaf mould by *Passalora fulva*. In relation to the last microorganism mentioned, it is one of the species that commonly attacks tomato crops (Thomma et al., 2005). The province of Corrientes does not escape this reality and producers must pay special attention to this pathogen.

Initially, leaf mould infects older leaves. Yellowish spots appear on the upper leaf surface and an olive-

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green mould on the lower leaf surface. As the disease develops, the spots become darker, leaves roll up, and defoliation may occur (Fig. 1). Hence, the photosynthetic area is diminished, which affects the yield and quality of fruits. *P. fulva* grows best at high temperature and relative humidity. Its primary dissemination method is wind, and it lives as a saprophyte on crop debris. Currently, disease control methods involve the application of fungicides (preventives and curatives), and among other agricultural practices, this guarantees crops protection (Obregón, 2018).

Fig. 1 : Tomato plants with symptoms of leaf mould disease

Consequently, when climate conditions are favourable for disease development, this pathogen plays an important role in agricultural production causing big economical damage. The purpose of the current study was on identify two fungi isolates from different tomato plants cultivated in greenhouse conditions, with typical leaf mould symptoms, and one isolate from a culture collection, applying conventional and molecular methods; and to determine their *in vitro* susceptibility against commercial fungicides by broth dilution and spread plate methods.

MATERIALS AND METHODS

The identification of the three phytopathogens was made by morphological and molecular characterisation. Two of them were from the Laboratorio de Fitopatología Hortícola, INTA EEA, Bella Vista, Argentina and the other was from a culture collection of Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina. Isolates were denominated Ia, Ib and Ic, respectively. Experiments were done at Instituto de Investigaciones Científicas, Argentina, during the year 2021.

Fungicides used for *in vitro* susceptibility tests were azoxystrobin 20 g + difenoconazole 12.5 g (Syngenta®), trifloxystrobin 25 g + tebuconazole 50 g (Bayer Crop Science®), chlorothalonil 72 g (Syngenta®) and metalaxyl-M4 g + mancozeb 64 g (Syngenta®). The first three products were selected taking into account the recommendations established by Obregón (2018) for the treatment of tomato plants with leaf mould. The latter one was included due to its broad antifungal spectrum, in order to examine its efficacy for strains under study (Emara et al., 2021). The amount of active ingredients is presented per 100 g/100 ml.

Morphological characterisation

To verify the presence of a monosporic culture, moulds were sub cultured on Fungi and Yeast Agar (Laboratorios Britania S.A.). Later, each isolate was inoculated in a Petri dish with Malt Extract Agar for 14 days in dark (Piontelli-Laforet, 2011; Pitt & Hocking, 2009). For macroscopic examination, colonies description, the size, appearance, colour, presence of pigments in culture medium, border, elevation, exudate and zoning were registered. These colonies were assessed visually and with stereomicroscope. Microscopic examination was made applying the microculture technique to observe the complete mycelium structure. Microcultures were incubated for 5-7 days (Pitt & Hocking, 2009). A detailed observation and a schematised description of the fungal structures were done (Piontelli-Laforet, 2011; Winn et al., 2006).

Molecular characterisation

After 7 days of incubation, the mycelium of each isolate was used for deoxyribonucleic acid (DNA) extraction, with minor modifications. The extracted

DNA was used for polymerase chain reaction (PCR) amplification. PCR was carried out using primers which a code for a segment of the internal transcribed spacer (ITS) region of ribosomal nuclear: ITS-1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS-4 (5' TCCTCCGCTTATTGATATGC 3'). The obtained products were observed on a 1.5% agarose gel in 1x TAE buffer by electrophoresis and after running, the gel was visualised under ultraviolet light (UV) using a UV transilluminator (MLB - 21, MAESTROGEN) (Ibañez et al., 2022).

Products from amplification were sequenced with the same primers in Macrogen Inc., Korea. Sequences were aligned using MEGA 7 software (Kumar et al., 2016). Identification was performed by comparing the sequences in GenBank Nucleotide Database of the National Centre for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST).

Susceptibility tests

Broth macro dilution test was used to determine *in vitro* fungicides' minimal inhibitory concentration (MIC). M38-A standard of the Clinical Laboratory Standards Institute (CLSI) methodology was taken as basis (Balouiri et al., 2016; CLSI, 2008). Antifungal stock solutions should be prepared at least ten times the highest concentration to be tested. Stock solutions of water-soluble or water-dispersible antifungal agent were prepared in a concentration of 5120 µg/ml. For the non-soluble or non-dispersible in water fungicide the concentration was 6400 µg/ml using DMSO as solvent. Dilutions were done with Sabouraud Dextrose Broth (SDB) (Laboratorios Britania S.A.) at pH 7.0 ± 0.1, an affordable and useful medium for the cultivation of fungi. Dilutions concentration range varied from 0.03125 to 64 µg/ml for chlorothalonil, metalaxyl-M+mancozeb and trifloxystrobin+ tebuconazole and from 0.03125 to 512 µg/ml for azoxystrobin+difenoconazole.

Seven-day old cultures of Ia, Ib and Ic, were used to prepare inoculums in a concentration between 0.4 x 10^4 and 5 x 10^4 colony forming unit per ml (CFU/ ml), aid by a Neubauer chamber. Inoculum concentration was verified by spread plate method. All macrodilution tests were incubated for 48-72 h to determine MIC (Machuca & Murguía, 2020; Machuca et al., 2015). All the tests included drug-free and fungi-free controls. Tests with non-soluble in water products also included 1% DMSO as a dilution control.

For the determination of *in vitro* minimal fungicide concentration (MFC), dilution tubes that showed a complete inhibition, the last tube with visible growth and positive control tube, were selected. Subsequently, without shaking the content of the tubes, 20 µL of each one was transferred to Sabouraud Dextrose Agar Petri dishes. Plates were incubated until positive control showed visible colonies. The MFC (µg/ml) was determined by the lower fungicide concentration that did not show any growth or when it showed less than 3 CFU (Díaz Dellavalle, 2011; Espinel-Ingroff et al., 2002). Assays were carried out in duplicate.

Statistical analysis

Statistical analysis of MIC and MFC values were done separately by Kruskal-Wallis non-parametric test, to determine the differences between them. This method was chosen because of the abnormal distribution of values and the small sample (n=3) and hypothesis are the following: (Hernández Sampieri, 2010; Spiegel & Stephens, 2009).

H_o: There is not a significant difference in the antifungal activity of the commercial fungicides against the three isolates.

H_1: There is a significant difference in the antifungal activity the commercial fungicides against the three fungi isolates.

RESULTS AND DISCUSSION

Isolates presented colonies diameters between 30 and 40 mm, being dense, flat, slightly velvety, olive-green at obverse and grey-blue at reverse. Microscopely, ramoconidia was about 11.4-45.6 µm long, not geniculate nor nodose conidiophore, oblong to limoniform conidia. For bicellular conidia, measurements of 11.4-15.2 x 3-3.8 µm were recorded; meanwhile for unicellular conidia measures were of 6.8-7 x 3.5-3.8 µm. Characteristics coincided with the key related for *Cladosporium cladosporioides* (Piontelli-Laforet, 2011; Pitt et al., 2009) (Fig. 2 & Fig. 3).

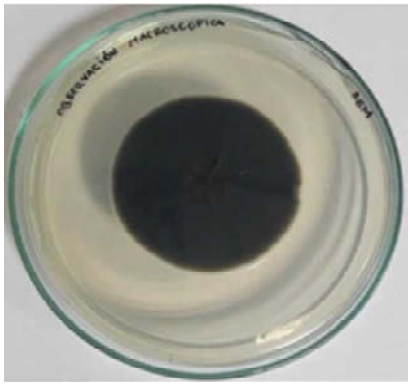

Fig. 2 : Cladosporium cladosporioides culture (malt extract agar): obverse (a) and reverse (b)

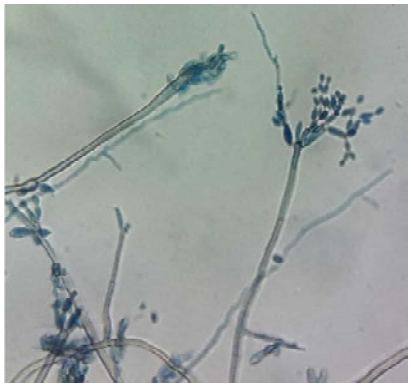

Fig. 3 : Cladosporium cladosporioides microscopic examination (400x)

Sequences of isolates Ia, Ib and Ic were identical to those recorded for *C. cladosporioides* in GenBank and were finally identified as *C. cladosporioides*. From this section on, isolates will be mentioned as *C. cladosporioides*[a], *C. cladosporioides*[b] and *C. cladosporioides*[c], respectively, with NCBI accession numbers ON585709, ON585710 and ON585708. The results for MIC and MFC are exposed in Table 1.

For *C. cladosporioides*[a] and *C. cladosporioides*[b], MIC readings were done at 48 h of incubation and *C. cladosporioides*[c] MIC reading was performed at 72 h. MFC was determined after MIC readings and were read with the same criteria.

Statistical processing was done by the application of the Kruskal-Wallis test to MIC and MFC values. To carry out MFC data processing, values that were higher than the upper limit concentration ranges were configured as lost values. The quantitative analysis of *in vitro* susceptibility of *C. cladosporioides* isolates against fungicides, showed that values for MIC ($P = 0{,}159$) and MFC ($P = 0{,}740$) were both $P > 0.05$. This means there is significant statistical difference between values.

Cladosporium cladosporioides[a-b] exhibited higher MIC values with azoxystrobin+difenoconazole and trifloxystrobin+tebuconazole. Coincidentally, these fungicides have an active ingredient of strobilurins chemical group in their composition. Chlorothalonil and metalaxyl-M+mancozeb showed lower MIC values, so *C. cladosporioides*[a-b] were more susceptible to these fungicides, being chlorothalonil the most effective and recommended. For *C. cladosporioides*[c], trifloxystrobin+tebuconazole exhibited the lowest MIC. For Metalaxyl-M+mancozeb showed the highest MIC value and was more susceptible to formulates with strobilurins. The three isolates had a similar MIC behaviour for chlorothalonil.

Regarding MFC values, the isolates showed the same behaviour for MFC values for metalaxyl-M+mancozeb were found into the concentration ranges, but they were higher than those for

Table 1 : *In vitro* susceptibility of *Cladosporium cladosporioides* isolates against fungicides, expressed in minimal inhibitory concentration (MIC) and minimal fungicide concentration (MFC) in µg/ml

Fungicide	*C. cladosporioides*[a]		*C. cladosporioides*[b]		*C. cladosporioides*[c]	
(Active ingredients per 100 g - 100 ml)	MIC	MFC	MIC	MFC	MIC	MFC
Azoxystrobin 20 g + difeconazole 12,5 g*	64	> 512	256	> 512	0.125	4
Trifloxystrobin 25 g + Tebuconazole 50 g**	32	> 64	16	> 64	0.0625	0.125
Chlorothalonil 72 g**	0.25	4	0.25	4	0.5	4
Metalaxyl-M 4 g + mancozeb 64 g**	8	8	8	16	8	32

*Concentration range tested: 0.03125-512 µg/ml, at higher concentrations the formulate precipitated

**Concentration range tested: 0.03125-64 µg/ml, at higher concentrations the formulate precipitated

chlorothalonil. Chlorothalonil was the most potent formulate. The results showed that *C. cladosporioides*[c] was the most sensitive isolate.

MFC values that were higher than the upper limit concentration ranges in *C. cladosporioides*[a-b], could be due to resistance caused by constant exposure to strobilurins, as these fungicides are frequently used to control tomato diseases (Longone & Escoriaza, 2017; Veloukas et al., 2007; Watanabe et al., 2017) found an azoxystrobin resistance of 80% or more, in *P. fulva* isolates collected from greenhouses where crops were treated with this ingredient at least two times per harvest period, for six years.

The development of resistance to strobilurins seems to be facilitated due to the high specificity of its mode of action, which results in a high risk for the appearance of resistance genotypes among populations of plant pathogens (Bartlett et al., 2002; Del Puerto Rodríguez et al., 2014; FAO, 2012). In this way, resistance generated by various pathogens was concreted faster than expected. However, the alternate or combined use of strobilurins with other fungicides proved to be a good strategy to reduce risk of resistance (Veloukas et al., 2007).

Therefore, considering the origin of the isolates used in this study, strobirulins resistance could be a factor to investigate since *C. cladosporioides*[a-b] was recently collected from greenhouses crops, where disease management is based primarily on fungicides. Whereas *C. cladosporioides*[c] was reactivated from colony fragments conserved in sterile distilled water dated from year 2007, and has not been exposed to strobilurins for fourteen years, it has shown a better *in vitro* susceptibility. In order to verify this assumption, more studies should be carried out.

CONCLUSION

As the three plant pathogens were identified as *Cladosporium cladosporioides*, it was shown that symptoms found on tomato leaves do not correspond to *P. fulva*. Since it is a different species, it would be important to study if *C. cladosporioides* accompanies *P. fulva* on tomato leaves. Finally, the current discovery provides information about the presence of an infrequent fungus in tomato crops in the region and its fungicide susceptibility, which would be useful for establishing control strategies and enhance production.

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Original Research Paper

Evaluation of screening methods for anthracnose fruit rot resistance in chilli (*Capsicum* spp.)

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ABSTRACT

Anthracnose fruit rot caused by *Colletotrichum* spp. is a serious production constraint causing severe marketable yield loss in chilli. Field evaluation of chilli accessions for resistance to *Colletotrichum* spp. depends on various factors affecting disease expression such as edaphic conditions, temperature, rainfall, humidity and other variables that are difficult to control, therefore considered less accurate. Also, high chances of cross contamination with different *Colletotrichum* species leads to inconclusive assays for specific pathogen species and isolate. To identify a stable and reliable screening method, various chilli accessions were subjected to *in vitro* pin-prick and non-wounding spray methods using a specific pathogen isolates. When chilli accessions were screened against *C. gloeosporioides* isolate '*IHRCg-1',* the *in vitro* pin- prick method showed positive correlation with the non-wounding spray method, except in the accession PBC80. The change in bioassay influenced the disease reaction pattern in the accession PBC 80, probably the pin pricks break the basal cuticle defense mechanism that was retained in spray inoculation method indicating varied resistance pattern. However, in the accession PBC 81 stable resistance pattern was observed against isolates of both species *viz*., *C. truncatum 'IIHR Ct-1'* and *C. gloeosporioides 'IIHR Cg-1'* and in the accession PBC 80 against *C. truncatum 'IIHR Ct-1'* in both the inoculation methods that depicted the expression of resistance genes during both methods of inoculation. Based on disease development pattern, the red ripe chilli expressed a variant reaction to infection by *C. truncatum* and *C. gloeosporioides*. The peak anthracnose infection at 10 DAI and 14 DAI is an accurate duration to record *'IIHR Cg-1'* and *'IIHR Ct-1'* infection, respectively on chilli ripe fruit for assaying the resistance.

Keywords: Anthracnose, chilli, pin-prick, non-wounding spray, *Colletotrichum truncatum, C. gloeosporiodes*

INTRODUCTION

Chilli (*Capsicum* spp.) is one of the most important and widely cultivated spice crops. Currently, India being the largest consumer, producer and exporter of day chillies and its products constituting approximately 42.3% of the total world production accounting for 2.05 million tonnes (FAOSTAT, 2021). In India, Andhra Pradesh produces 0.63 million tonnes of chilli from 0.23 million hectares followed by Telangana (0.55 million tonnes in 0.18 million ha) and Indian chilli exports amounting for 8581.88 crores rupees (SB, 2023).

The anthracnose fruit rot or dieback caused by *Colletotrichum* species is one of the major constraints in chilli production which affects both at pre and post-harvest stages (Saxena et al., 2016). At least 24 species of *Colletotrichum* are known to be the pathogens of chilli anthracnose disease (Mongkolporn & Taylor, 2018). Amongst them, three primary species *viz*., *Colletotrichum truncatum* (Syn. *C. capsici* Syd. Butler and Bisby), *C. gloeosporioides* and *C. scovillei* (Syn. *C. acutatum*) are very serious. All developmental stages are targeted by *C. gloeosporioides* (Sharma et al., 2005; Katoch et al., 2017), while, *C. truncatum* causes major damage at the ripe fruit stage of the plant (Saxena et al., 2014). Typical symptoms include dark spots, and sunken necrotic tissue with concentric rings of acervuli on fruits (Mistry *et al*., 2010). The disease is aggressive in major chilli growing belts causing 25-30% loss across the nation (Lakshmesha et al., 2005) which annually sums up to US$ 491.67 million (Garg et al., 2013).

The main sources of resistance to anthracnose have been identified in different accessions of *Capsicum baccatum* L. and *C. chinense* Jacq. (AVRDC, 1999

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and Kim et al., 2008a). Accurate, fast, economic and repeatable screening methodology is crucial in breeding programmes to develop resistant varieties (Galvan, 2010). Therefore, the present study evaluates the effectiveness of available chilli anthracnose fruit rot screening protocols and incubation period.

MATERIALS AND METHODS

Plant material

Six *Capsicum* spp. accessions (Table 1) were screened against virulent isolates of C. *truncatum 'IIHR Ct-1'* and *C. gloeosporioides 'IIHR Cg-1'* under field and *in vitro* conditions for anthracnose fruit rot resistance.

Table 1 : Field evaluation of chilli accessions against *Colletotrichum truncatum 'IIHR Ct-1'*

Accession	Disease Severity	Disease reaction
PBC80 (*Cb*)	1.0[c]	R
PBC81 (*Cb*)	0.8[c]	R
IHR4491 (*Cb*)	35.3[a]	HS
Solan Bharpur (*Ca*)	28.70 [a]	HS
EC382053 (*Ca*)	35.6 [a]	HS
EC399573 (*Ca*)	29.0[b]	HS

R-resistance, HS- highly susceptible

*values are *arc sine* transformed before analysis. Means the same letter are not significantly different in Duncan's multiple range test (p d" 0.05)

Isolation and identification of pathogen

A small tissue piece (5x5 mm) was taken at the edge of the infected area, washed in sterile distilled water, surface-disinfected in 70% ethanol for 30 sec and 1% (v/v) sodium hypochlorite (NaOCl) for 1 min, rinsed three times in sterile distilled water and placed on potato dextrose agar (PDA, Himedia, India) amended with cocktail of antibiotics *i.e.* streptomycin, tetracycline, ampicillin and chloramphenicol (100 µg/mL. Plates were incubated for five days at 25±1°C with a 12 h photoperiod provided by fluorescent light. The growing edges of fungal hyphae developing from the tissues were then transferred aseptically to PDA (Chowdappa et al., 2015). The pathogens were molecularly characterized using fungal ITS specific primers *viz*., ITS 1 and ITS 4 (White et al., 1990).

Inoculum preparation

The fully sporulated plates of two weeks old were loaded with sterile distilled water and conidia were gently scraped off from the plates. Spore density was made up to 10^5 spores mL^{-1} using haemocytometer (AVRDC, 1999). To reduce surface tension, Tween-20 was added to the inoculum (0.5 mlL^{-1}).

Open-field screening protocol

Field screening was performed in a randomized block design with three replications. Six accessions (Table 1) at the red ripe fruit stage were inoculated with *'IIHR Ct-1'* by spraying fresh spore inoculum of known density (Rajapakse & Ranasinghe, 2002; Susheela, 2012). The fruits were then immediately covered with a polyethene bag for four days followed by spraying of water twice a day post removal of plastic covering. Non-inoculated (spraying only with water and surfactant) was included as a control (Pedrosa et al., 2004). The symptoms were visually estimated and disease severity index (DSI) was calculated as per Montri et al., (2009) at two weeks after spraying the inoculum on fruit based on the mean percentage lesion size of fruits (Suwor et al., 2015).

In vitro assay

Fully matured red ripe fruits were surface sterilized with 1 per cent (v/v) sodium hypochloride for 5 mins. Further, twice washing with distilled water and then wiped dry with sterilized paper towels. Spore suspensions of virulent isolates of *'IIHR Ct-1'* and *'IIHR Cg-1'* were inoculated by pin prick method to infilter the inoculums (with 5 µl droplets) into fruit (Kim et al., 1989) while 5 µl droplets of sterilized water were kept as control. Fruits were then incubated at 25±1°C with a 12 h dark/light cycle in a small moist chamber (relative humidity > 90%) created by spreading layers of moistened paper towel in acrylic boxes. Anthracnose symptoms at the inoculation sites were evaluated as per AVRDC (1999). Similarly, disease severity was calculated.

RESULTS AND DISCUSSION

Detection and identification of *C. truncatum* and *C. gloeosporioides* isolates

The isolated pathogen from the naturally infected host was identified as *C. truncatum* and *C. gloeosporioides* on the basis of morphological, pathogenicity and molecular assays (Fig. 1). The ITS sequence of the *'IIHR Cg-1'* with accession MN873009 showed 99.6% similarity with accession MG282163 (Saini et al., 2017b) in NCBI-BLAST, while the accession MN873012 of *'IIHR Ct-1'* isolate exhibited 98.7%

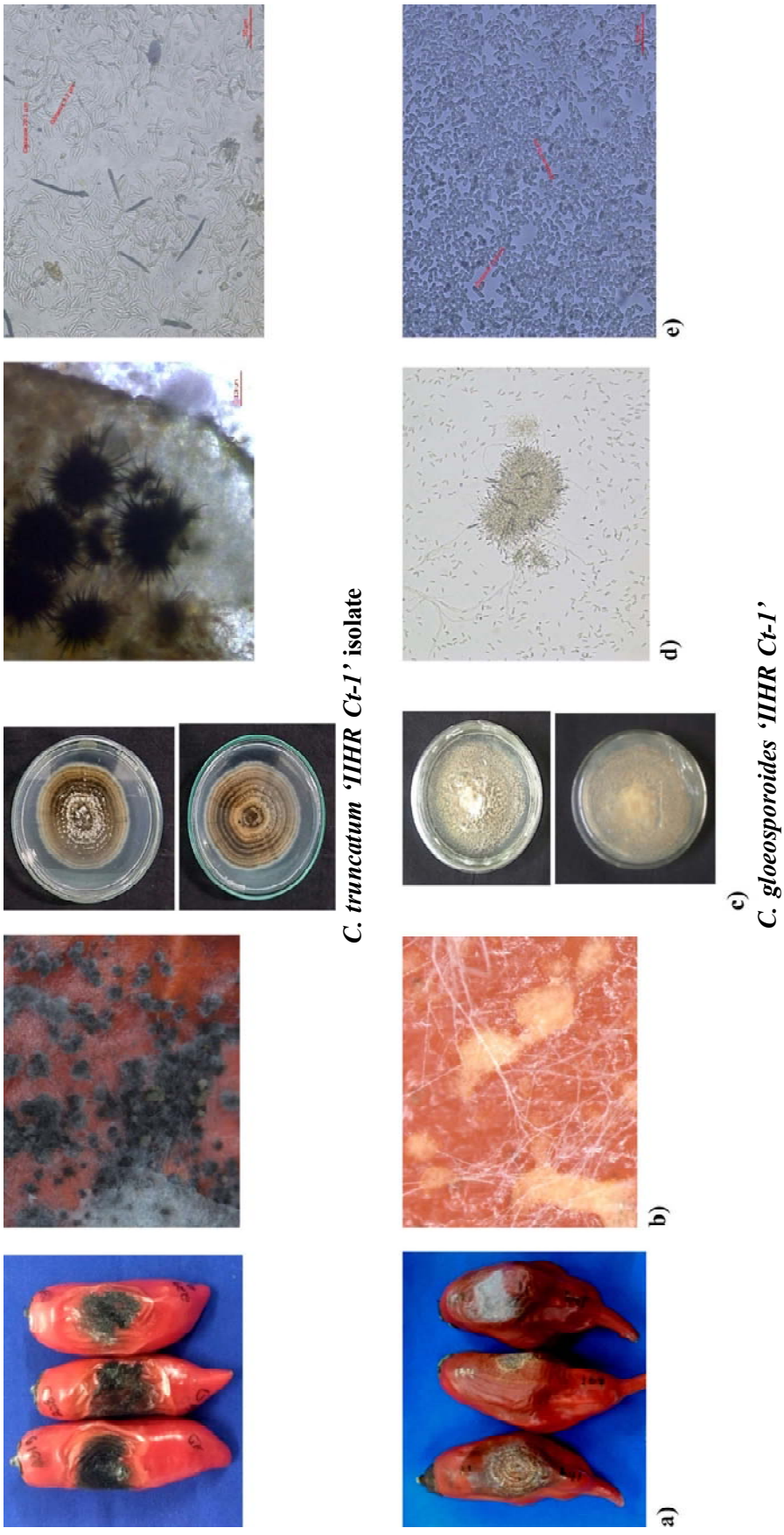

Fig. 1 : Morphological confirmation of *Colletotrichum* species, (a) Anthracnose infected fruits, (b) Stereo microscopic view mycelial and acervuli on fruit surface, (c) Upper colony and lower colony surface of culture, (d) Acervuli of *Colletotrichum* species 100 *µm*, (e) Conidia of *Colletotrichum* species. Bars 50

similarity with accession MG204566 (Saini et al., 2017a). The anthracnose species *viz.*, *C. truncatum 'IIHR Ct-1'*and *C. gloeosporioides 'IIHR Cg-1'* were able to cause infection in susceptible accessions (Table 1 & 2).

Reaction of different accessions against *C. truncatum 'IIHR Ct-1'* and *C. gloeosporioides 'IIHR Cg-1'* isolates

In artificial non-wounding method of field screening, the accessions, PBC 80 and PBC 81 conferred resistant to *'IIHR Ct-1'* showing 1.0 and 0.8 DSI, respectively, while, IHR4491 and *C. annuum* accessions were found susceptible (Table 1). Under pinprick method, the accessions *viz.*, PBC 80 and PBC 81 exhibited resistance to *'IIHR Ct-1'* which confirmed the positive correlation of both screening methods (Susheela, 2012). In the present study, PBC 81 exhibits resistant reaction with 1.80% DSI, while, PBC 80 exhibited susceptible reaction (9.57% DSI) against *'IIHR Cg-1'* in pin prick method (Table 2). This finding contradicts the report of Mongkolporn et al. (2010) that 'PBC80' exhibited resistant reaction to 11 isolates of *C. gloeosporioides* in wounding assay. However, Mahasuk et al. (2013) reported that the resistance reaction changes based on inoculation methods. The pin pricking method confirmed the presence of resistance genes and bypasses the pseudo resistance as a result cuticle and jasmonates signaling pathway that activates the pathogenic related (PR) proteins which in fact is present in the non-wounding method of inoculation (Ro et al., 2021). Therefore, the 'PBC 81' exhibited resistant to both *Colletotrichum* spp., while, 'PBC80' lacks resistance to *'IIHR Cg-1'* isolate.

Anthracnose disease progression under controlled condition

The post-inoculation period of incubation determines the progression of anthracnose disease in chilli fruit. The disease development started at 3 DAI (Fig. 2) which is in agreement with the previous studies (Garg et al., 2013; Mishra et al., 2019) and progressed further in subsequent days irrespective of the genotypes and *Colletotrichum* spp.

Depending upon the host-pathogen interaction, the peak disease progression was recorded at different days after inoculation. Despite, the peak progression of *'IIHR Cg-1'* at 14 DAI, the infection rate is at par

Table 2 : *In vitro* differential reaction of chilli genotypes against *Colletotrichum gloeosporioides 'IIHR Cg-1'* and C. *truncatum 'IIHR Ct-1'*

Accession	3 DAI			7 DAI			10 DAI			14 DAI		
	C. g 'IIHR Cg-1'	Disease reaction	*C. t* 'IIHR Ct-1'	*C. g* 'IIHR Cg-1'	Disease reaction	*C. t* 'IIHR Ct-1'	*C. g* 'IIHR Cg-1'	Disease reaction	*C. t* 'IIHR Ct-1'	*C. g* 'IIHR Cg-1'	Disease reaction	*C. t* 'IIHR Ct-1'	Disease reaction
PBC80 (*Cb*)	0.37^{e}	HR	0.10^{e}	2.94^{d}	MR	0.30^{e}	9.03^{d}	MS	0.97^{d}	9.57^{d}	MS	1.90^{e}	R
PBC81 (*Cb*)	0.03^{f}	HR	0.10^{e}	0.53^{f}	R	0.47^{f}	1.67^{f}	R	1.07^{d}	1.80^{f}	R	1.57^{e}	R
IHR4491 (*Cb*)	1.53^{c}	R	1.70^{b}	15.23^{b}	S	10.56^{a}	37.67^{a}	HS	22.57^{a}	40.67^{a}	HS	37.10^{a}	HS
Solan Bharpur (*Ca*)	4.20^{a}	MR	3.43^{a}	18.73^{a}	S	10.03^{b}	29.10^{b}	HS	12.50^{b}	31.30^{b}	HS	31.00^{b}	HS
EC382053 (*Ca*)	0.43^{d}	HR	1.53^{c}	1.80^{e}	R	6.37^{c}	5.97^{e}	MS	10.21^{c}	7.10^{e}	MS	22.80^{d}	S
EC399573 (*Ca*)	1.80^{b}	R	0.27^{d}	5.83^{c}	MS	2.70^{d}	21.40^{c}	S	9.80^{d}	22.63^{c}	S	27.33^{c}	HS

HR: highly resistant; R: resistance, MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible

*Values are *arc sine* transformed before analysis. Means the same letter are not significantly different in Duncan's multiple range test (p d" 0.05)

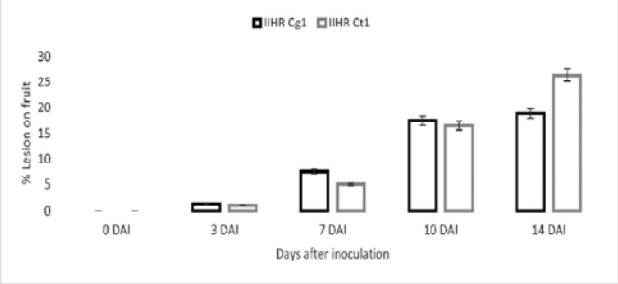

Fig. 2 : Anthracnose (*C. gloeosporioides* 'IIHR Cg-1' and *C. truncatum* 'IIHR Ct-1) progression on chilli fruit (Bar indicates standard error @ 5%)

with 10 DAI (Fig. 2) indicating 10 DAI as an appropriate to record anthracnose disease progression (Mishra et al., 2019). Alternatively, *'IIHR Ct-1'* inoculated fruits exhibited a significant difference disease progression between 10 DAI and 14 DAI (Table 2). Hence, observation for *C. truncatum* inoculated fruits were recorded at 14 DAI in agreement with Souza et al. (2019) & Ro et al. (2021).

CONCLUSION

Selection of precise screening assays is prerequisite in resistant breeding program. Both screening methods (field spraying and microinjection) substantiate equal disease reactions against the susceptible *C. baccatum* 'IHR4491' and *C. annuum* accessions. However, the wound inoculation or pin prick method is the desired bioassay for anthracnose fruit rot screening. Pin prick method provides chances of double inoculation, exhibited distinguishable symptoms, produces highly reproducible results, remove pseudo resistance and exerts the resistance gene. Additionally, the field spray method resulted in mixed species infection and thus, is not desirable for specific *Colletotrichum* spp. studies. Moreover, the experimental findings stabilized the disease reaction of the genotypes with respect to the incubation period by standardizing the incubation period at 10 DAI and 14 DAI for *C. gloeosporioides* and *C. truncatum*, respectively.

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Original Research Paper

Molecular and biological detection of impatiens necrotic spot virus (INSV) isolate from ornamental plants in Iran

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ABSTRACT

Impatiens necrotic spot virus (INSV), belong to genus Orthotospovirus, causes severe damage to greenhouse ornamental plants. INSV reported from almost every ornamental screen house in Iran. INSV general symptoms include necrotic leaf spot, chlorosis and stunting in infected ornamental host plants. A total number of 581 ornamental samples of 58 different plant species with symptoms similar to those of INSV infection were collected in 4 provinces of Iran. The results indicated an average of 20.13 per cent virus incidence. INSV infection was recorded to be 28% in Mahallat, 24.5% in Tehran, 22.1% in Mazandaran and 16.4% in Guilan provinces. No record of INSV infection on ornamental samples was obtained from Khouzistan province (Dezful). From the total of 58 ornamental species tested, 33 species recorded positive for INSV. ELISA positive samples were rechecked by RT-PCR using a set of specific primers directed to the N-gene region, which were designed to detect and characterize the virus species. The primers amplified a 777 bp product of the nucleoprotein as shown by agarose gel electrophoresis. The nucleotide sequence of amplicons was compared with related sequences, using Blast software available at NCBI GenBank, which showed highest similarity with impatiens necrotic spot virus (INSV) isolates. Accordingly, all genome components of the isolate shared 89-96.5 % nucleotide sequence identities with corresponding sequence of other Iranian and GenBank INSV isolates. The Iranian isolates were all placed in the same group and bore the most similarity to INSV isolates from the Netherlands, Italy and United States. In phylogenetic analysis based on the partial nucleotide and deduced amino acid sequences of N region, INSV from Iran appears to be closely related to the Japanese AB1099100 *Verbena* spp. isolate.

Keywords: DAS-ELISA, INSV, ornamental plants, RT-PCR

INTRODUCTION

The cultivated area of ornamental plants in Iran is approximately 4704 hectares, of which 83.7 hectares is under glass/greenhouses, 1076 hectares are wooden-plastic greenhouses, 1268 hectares are metal-plastic greenhouses, and 2276 hectares is cultivated in the open condition. 8588 producers are active in this sector and produce 1224 million cut flowers, 36 million flower pots, 137 million ornamental tree trunks and 842 million seasonal and nursery plants (Anonymous, 2018). One of the limiting factors for ornamental production is infection with various viral pathogens (Amruta et al., 2020; Daughtrey et al., 1997; Naidu et al., 2005). Impatiens necrotic spot virus-INSV- Orthotospovirus is among the most important Tospoviruses reported from ornamental plants in the world (German et al., 1992; Peters, 1998) and also in most ornamental plants growing greenhouses in central province of Tehran, Iran. Infection with viral diseases, not only reduced the quality, but also leads to leaf and flower blistering, dwarf plant, mosaic, marginal leaf chlorosis, leaf entanglement, deformity or lack of flower formation (Shahraeen & Ghotbi, 2003; Shahraeen et al., 2022). INSV is the most important harmful and predominant virus in ornamental plants in Iran and has been reported from various ornamental species in the country (Ghotbi et al., 2005; Shahraeen & Ghotbi, 2003; Pourrahim et al., 2012; Bayat & Nazerian, 2019).

Plants infected with INSV have no symptoms, in some cases, necrotic spots and necrotic rings are seen in young leaves. Symptoms vary from one plant to another and from one species to another, but in general there are symptoms such as: dwarfing, necrotic and yellow spots on the leaves, brown and black necrosis

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on the stem, circular spots, linear patterns, mosaic and vein necrosis is observed (EPPO, 1999; Peters, 1998; Ghotbi et al., 2003). This virus is transmitted by *Frankliniella occidentalis* in an unstable manner (Broadbent & Allen, 1995), which is considered as a quarantine pest in Iran, but in 2013 it was reported for the first time on ornamental plants in the Varamin region (Mehraban & Shahraeen, 2000; Shahraeen et al., 2002). Due to extensive import of ornamental plants from foreign countries, this investigation in Iran is brought in to focus. Therefore, in this study, collected ornamental samples were tested serologically and selectively by RT-PCR test.

MATERIALS AND METHODS

Serology

Enzyme linked immunosorbent assay (DAS-ELISA) test were applied on collected samples (Table 2) as described (Clark & Adames, 1977; German et al., 1992). Using INSV specific antibody antiserums provided by Dr. Winter (DSMZ/JKI-Germany). Leaf samples were extracted in the ratio of one gram of tissue with 5 mL of extraction buffer and 100 µL was added to each well (ELISA plate) at each stage. Replications of two control samples were also added. One hour after adding the substrate solution containing Paranitro-phenyl phosphate, the light absorption of the wells at 405 nm was measured using a microplate reader (Multiscan 334 Lab system, Finland).

Biological tests

Infected INSV samples were selected and leaf extracted (1:5) by cold phosphate buffer of 0.01 M containing 0.15% antioxidant 2-mercaptoethanol pH=7, were mechanically inoculated to specific indicator plants of *Chenopodium amaranticolor*, *Datura metel, Nicotiana tabacum* cv. Samson, *D. stramoniom* and *Vigna unguiculata* (Table 1). Inoculated plants were kept in greenhouse free of insect vectors, and infectivity and symptoms appearance were studied and recorded by Elisa test (Zavareh et al., 2013; Ghotbi & Shahraeen, 2022).

Table 1 : Results of biological tests on indicator plants

Indicator plant	INSV	
	local	systemic
N. tabacum cv. samson	-	CL, Mo
V. unguniculata	MNL	-
D. metel	CL, RS	LD, Mo
D. stramonium	CL, RS	CL, Mo
Ch. amaranticolor	NL	NL, RS

CL: chlorotic lesion, De: death, LD: leaf deformation, MNL: mild necrotic lesion, Mo: mosaic, NL: necrotic lesion, NT: not tested, RS: ring spot, SNL: sever necrotic lesion, VCL: vein clearing

In biological tests, *N. tabacum* cv. Samson reacted with systemic mosaic, *V. Unguiculata*, *D. Metel*, *D. stramonium* and *Ch. amaranticolor* reacted with local and systemic infection (Fig. 1a-d).

Fig. 1a : INSV infected Chrysanthemum spp. with brown necrotic and leaf malformation

Fig. 1b : Scindapsus spp. with leaf chlorosis, narrowing infected by INSV

Fig. 1c : Philodendron spp. with leaf narrowing and stunting infected by INSV

Fig. 1d : *Dianthus* spp. with yellowing and necrosis infected by INSV

Molecular diagnostic, RT-PCR

INSV coat protein gene carried out using specific primers (GeneBank, Acc. No. AB1099100) (Ghotbi & Nazerian, 2010; Ghotbi & Shahraeen, 2012). Positive INSV samples in ELISA tests was selected and total RNA extracted by commercial solution RNXTM-plus (SinaGen Company, Iran) according to the method recommended by the manufacturer. RNA extracted using INSV (Gene Bank Access No. AB1099100) specific primers (F: 5'-GTAGCATTAACATGCTGTAAATG-3'; R: 5'-GTCAAGCTTTTTG ACTCAATCTGAT-3') with two steps RT-PCR. The primers amplified a 777 bp product of the nucleoprotein as shown by agarose gel electrophoresis (Chung et al., 2006).

Amplification and sequencing

In order to determine the nucleotide sequence of the fragment for isolation of INSV from *anthurium*, 20 microliters of PCR product with specified primers (with a concentration of 50 picomol) was sent to the representative of Kiagen Biotech Korea in Iran. The fragment was sequenced and the sequences obtained from PCR products were compared with the information and sequences in the Gene Bank (NCBI) by BlastN software at the nucleotide level. The nucleotide data sequenced for the INSV virus were then multiple-aligned with virus isolates already available in the NCBI database using MEGA5 and ClustalX software, the progeny analysis was based on nucleic acid using the Neighbor-Joining method. MEGA5 software. The offspring tree was drawn in 1000 boot strap using MEGA5 bioinformatics software (Tamara et al., 2013; Nei and Kumar 2000). All branches merged with a bootstrap value of less than 70%. In this analysis, *Peanut stunt virus* (PSV) ER isolate (U15730) was used as an out-group with *cucumber mosaic virus* sequences (Poelwijk et al., 1997, Altschul et al., 1997).

Results obtained in the ELISA test (DAS-ELISA) for total of 581 ornamental samples from 58 different species with symptoms of leaf necrosis, chlorosis and dwarfism in 5 provinces of the country (Gilan 128 samples, Mazandaran 159 samples, Tehran 122 samples, Central 108 sample, Khuzestan 64 samples) indicates the contamination of 117 different samples from 5 provinces. Therefore, 20.13% of all the collected samples were infected with INSV. The highest contamination was related to central provinces. Tehran with 30 samples (24.5%), Mazandaran (22.01%) and Gilan 16.40%. Khuzestan samples were not found to be infected with INSV virus. According to the studies conducted in this research, 33 species (56.89%) of 58 ornamental species were infected with INSV virus (Table 2).

Table 2 : Result of ELISA tests for INSV infecting ornamentals samples in 5 provinces of Iran

Ornamentals	Totals from each province	INSV infected samples
Ardisia crenata (Myrsinaceae)	5 Ma*	-
Alestreomeria spp. (Alestreomeriaceae)	4 Ma	3Ma
Erica spp. (Ericacea)	6 G+7M	1G+4M
Orchis pharanopsis (Orchidaceae)	4M	-
Azali spp. (Ericaceae)	3M	-
Spathiphylum spp. (Araceae)	5G+3M	3G+1M
Sterlitzia reginae (Strelitziaceae)	5G+2M+4T+1Ma	-
Zinia elegans (Compositae)	2G+3T+1Ma	1T
Aglonema schott (Araceae)	4G+6M	-
Anthurium spp. (Araceae)	6G+4M+8T+5Ma	1G+2M
Bambusa spp. (Graminaceae)	3M	-

Begunia semperflorus (Beguniaceae)	2M+2T	-
Ficus benjamina (Moraceae)	3M+4Ma+2T	2M+1Ma
Viola spp. (Violaceae)	5T+4Ma	2T
Saintpaulia ioantha (Gesneraceae)	3T+5Ma	2T+3Ma
Pandanus veitchii (Pandaceae)	4G+4M	-
Scindapsus aureus (Araceae)	7G+3M+3T+2Ma	2G
Pilea cadieri (Urticaceae)	3Ma	2Ma
Bignonia capreolata (Bignoniaceae)	4G+3T	-
Zingiber spp. (Zingiberaceae)	2G+5M	3M
Impatiens spp. (Balsaminaceae)	2G+4M+4T+5Ma	3M+3T
Althea spp. (Malvaceae)	5G+6M+3T+3Ma+7Kh	3G+2M+1Ma
Chrysanthemum spp. (Compositae)	5G+5M+5T+10Ma	3G+3M
Dracaena Fragrans (Liliaceae)	7G+4M+3T+3Ma	4G
Diffenbachia amoena (Araceae)	4G+2M+3T	1M
Rosa spp. (Rosaceae)	9G+5M+5T+4Ma+42Kh	2G+1M+3T+2Ma
Petris cretrica (Polypodiaceae)	5M	-
Asplenium scolopendrium (Aspleniaceae)	5G+5M	-
Cupresus sempervirens (Cupressaceae)	5G	-
Salvia splendens (Labiatae)	2M+5T+4Ma	1M+2Ma
Lilium spp. (Liliaceae)	4M+4T+3Ma	-
Cycas spp. (Cycadaceae)	6M	3M
Cissus spp. (Vitaceae)	4M	2M
Syngonium podophyllum (Araceae)	3M+3T+2Ma	2M
Cheiranthus cheiri (Cruciferae)	4T+3Ma	1Ma
Sheflera arboricola (Araliaceae)	3G+2M	-
Pelargonium hortorum (Geraniaceae)	3G+3M+5T+3Ma	2Ma+1T
Pelargonium peltatum (Geraniaceae)	5G+4M+2T+3Ma	-
Aspidistra elatior (Liliaceae)	3G+4T	-
Ficus elastica (Moraceae)	2G+5M+4T+5Ma	2M
Philodendron spp. (Araceae)	2G+4M+3T+3Ma	3M+2Ma
Pinus spp. (Pinaceae)	2G	-
Bougainvillea spp. (Nyctaginaceae)	2G+3T+3Ma	2T
Camellia sinensis (Theaceae)	3M	-
Codiaeum variegatum (Euphorbiaceae)	6G+2M+3T+4Ma	3T+2G
Cordyline spp. (Liliaceae)	5M	-
Dahlia spp. (Compositae)	4G+3M+5T+8Ma	4T+2Ma
Gazania spp. (Compositae)	4G+2T+3Ma	3T+2Ma
Gladiolus spp. (Iridaceae)	5T+4Kh	2T
Lilium longiflorum (Liliaceae)	3M+4T	-
Beucarnea recurvata (Liliaceae)	5M	-
Zinia elegans (Compositae)	5T+4Ma	2Ma
Polianthes spp. (Amaryllidaceae)	11Kh	-
Dianthus spp. (Caryophyllaceae)	3T+3Ma	2T+2Ma
Phoenix Canariensis (Palmaceae)	2M	-
Chamaedorea elegans (Palmaceae)	4M	-
Calandula spp. (Compositae)	3G+5T+5Ma	2T+4Ma
Euphorbia pulcherrima (Euphorbiaceae)	4M	-
Total in number	581 128G+159M+122T+108Ma+64Kh	117 21G (%16/40)+35M (%22/0)+ 30T (%24/5)+31Ma (%28/70)

*Indicating provinces initials, M = Mazandaran, Ma = Mahallat, T = Tehran, G = Gilan, Kh = Khozestan, - = No infection found, + = Total no of infected samples

RT-PCR

RT-PCR using specific INSV primer confirmed ELISA test results. Selected ornamental samples from different provinces yielded a desired amplicon of 777bp with INSV primer. No amplification was observed from healthy plants extracts (negative control). The nucleotide sequence of amplicons was compared with related sequences, using Blast software available at NCBI GenBank (Altschul *et al*., 1997), showed highest similarity with impatiens necrotic spot virus (INSV) isolate: Imp-Neth (X66972), Sol-Italy (DQ425096-1), Imp-USA (D00914-1), Ver-Jap (AB109100-1), Beg-serb (HQ724289), Glo-USA (DQ523598), unknown (L20886). Accordingly, all genome components of the isolate shared 89-96.5% nucleotide sequence identities with corresponding sequence of other Iranian and GenBank isolates. The Iranian isolates were all placed in the same group and bore the most similarity to INSV isolates from the Netherlands, Italy and United States. In phylogenetic analysis based on the partial nucleotide and deduced amino acid sequences of the N region, the INSV from Iran appears to be closely related to the Japanese AB1099100 *Verbena* spp. isolate (Fig. 2).

Viral pathogens are one of the most economically damaging agents in the ornamental plants. The first report of the occurrence of Tospoviruses (TSWV species) in Iran was in 1996 (Bananej et al., 1996; Shahraeen & Bananej, 1995) from the hosts of Atlantic and tobacco and nightingale, button flower and cucumber from Varamin region (Tehran province). Then after, there were various reports of the occurrence INSV virus on different hosts from Iran (Pourrahim et al., 2012; Bayat & Nazerian, 2019; Ghotbi et al., 2005; Golnaraghi et al., 2001; Ghotbi & Shahraeen, 2012; Mehraban & Shahraeen, 2000). *Frankliniella occidentalis*, the potential vector of INSV reported for the first time in Iran in 2003 (Ghotbi et al., 2003) on the ornamental plants from Varamin region, thus, there remain an expectation of wider distribution of this virus in the ornamental production areas in the country (Ghotbi & Shahraeen, 2022).

INSV has a wide host range in ornamental plants of Iran (Ghotbi et al., 2005; 2003). This virus has a direct effect on the marketability by affecting the flowering and yield of ornamental plants, and is important to pay attention from an economic point of view. Cultivation

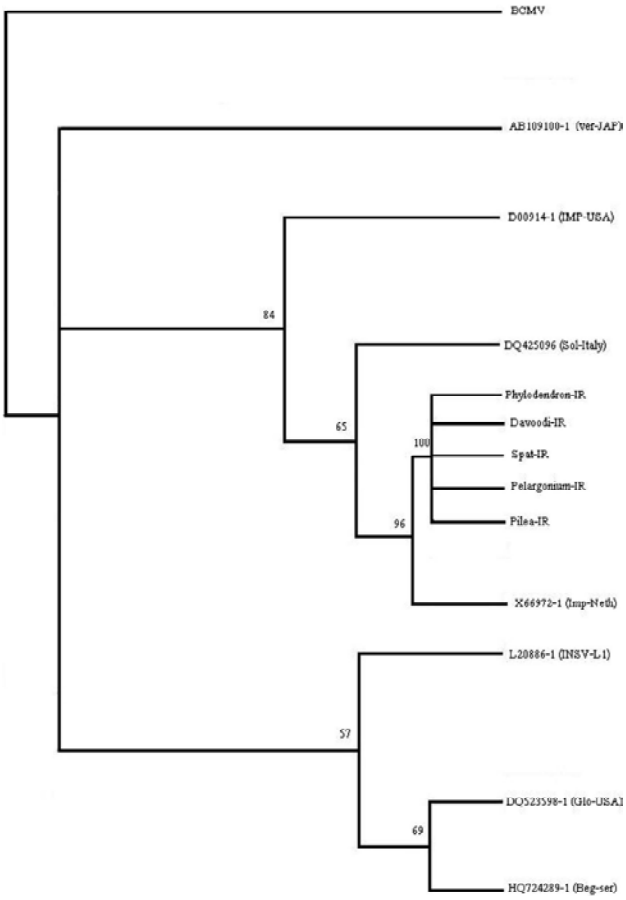

Fig. 2 : Phylogenetic tree using nucleic acid sequence of coat protein gene of INSV isolates from Anthurium using CLUSTAL X with seven other Genbank isolates. Coat protein gene sequence of BCMV was used as outgroup

of ornamental plant in Iran is both outdoor and indoor in screen-houses, unfortunately, the lack of awareness of growers about some common viral diseases of ornamental and other field crops may lead to spread and mechanical transmission of the viral agents. According to the above results, it is suggested to use healthy and virus-free imported samples as much as possible, and to use reliable methods like tissue culture for propagating ornamental plants in the country.

CONCLUSION

Regular examination of cultivation areas for infection by plant viruses, isolation and introduction of viruses identified in each area, removal of suspected weed and infected plant materials, stored in greenhouses or surrounding areas. Proper and timely use of insecticides is one of the useful strategies in controlling spread of INSV in ornamental plants.

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Original Research Paper

Standardisation of gamma irradiation dose for Sterile Insect Technique to manage South American tomato moth [*Phthorimaea* (*Tuta*) *absoluta* (Meyrick)]

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ABSTRACT

Laboratory studies were conducted to standardise optimum dose of gamma irradiation to induce sterility in males of South American tomato moth [*Phthorimaea* (*Tuta*) *absoluta* (Meyrick)], a major pest on tomato. Among the different irradiation doses tested, 150 Gy was found to be effective to induce male sterility in *P. absoluta*, where sterile males exhibited mating competitiveness, significantly reduced fecundity and hatchability in F_1 and F_2 generations. Five days old pupae were found ideal for exposing to gamma irradiation to induce male sterility in *P. absoluta* that could be used for area wide management using SIT.

Keywords : Gamma-radiation, IPM, *Phthorimaea absoluta*, sterile insect technique, tomato

INTRODUCTION

Sterile Insect Technique (SIT) is a method of pest control involving area-wide inundative release of sterile insects to inhibit reproduction in field population of same species (FAO, 2007). SIT involves mass rearing of target insect species and then exposed to different radiation (X-rays, gamma radiation) making the insect sterile. The sterile insects are then released in the field, which mate with same species of wild population resulting in infertile offspring. In order to release insects in a SIT programme, different amounts of sterility might be inflicted upon them. Moths and butterflies (Lepidoptera) exhibit several peculiar cytogenetic and cytological characteristics that distinguish them from other insects causing high resistance of ionizing radiation. These play crucial role in the mechanism of inherited sterility (IS). The characteristics include female heterogamety, holokinetic structure of chromosomes and dichotomous spermatogenesis. Thus, it is suggested to apply sub-sterilizing doses in lepidopterans as it showed better competitiveness and also transmit sterility to F_1 progeny higher than the parents.

After the first report of the incidence of *P. absoluta* from Karnataka, India (Sridhar et al., 2014), it has further spread to different tomato growing areas of the country (Hadapad & Hire, 2019) and had significantly impacted the tomato production. There are many strategies advocated for the management of *P. absoluta*. But farmers practice of overreliance on insecticides is resulting in environmental risks combined with resistance development due to its strong reproductive potential and short generation cycle (Sridhar et al., 2019). As an alternative, SIT can be used for the management of *P. absoluta* on tomato, which can be integrated with other IPM tools for its eco-friendly management. Research on SIT for lepidopteran pests is scanty in India and hence the present study was conducted to standardise the age of pupa and gamma irradiation dose required to induce male sterility in *P. absoluta*.

MATERIALS AND METHODS

Nucleus culture of *Phthorimaea absoluta* was collected from the tomato fields of ICAR-Indian Institute of Horticultural Research, Bengaluru and reared in the tomato based semi synthetic diet.

Exposing pupae of *P. absoluta* to gamma irradiation

Two and five days old pupae were exposed to five gamma irradiation at 100, 125, 150, 200 and 300 Gy doses in completely randomised block design with three replications each containing 100 male pupae and a control without any irradiation during 2021 to 2023. Extent of healthy and malformed adult emergence after irradiation of two and five days old pupae was

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recorded. The data was subjected to ANOVA, after suitable statistical transformation.

From the emerged adults, two irradiated and untreated males were mated with each of the wild female (2:1 ratio) and were transferred to wooden 'oviposition cages' (105×70×95 cm) having tomato plants (cv. Arka Rakshak), provided with honey swab (50%) as food for the moths. The caged plants were replaced with new plants at three days intervals and were examined for number of eggs laid by a female. On hatching, larvae were reared on semi-synthetic diet. Pupae were further used for biological studies to determine the effect of irradiation or to continue mass rearing. The data on different parameters like adult emergence (%), adult malformation, adult longevity, fecundity when mated with untreated female, larval period and pupal period were recorded.

Effect of gamma irradiation on F_1 and F_2 progeny's egg laying and hatching

After ascertaining the effective dose of gamma irradiation (150 Gy), in order to assess the hereditary impact of sterility on egg laying and hatching on F_1 and F_2 generations, a separate laboratory experiment was carried out with different cross combinations of FM (fertile male), IM (irradiated male), WF (fertile female, wild) and IF (irradiated female). Six replicates (petri dishes) were used for each cross combination. Various combinations included were IF x FM, WF x IM and WF x FM. In both generations, female fecundity and eggs hatched were observed and subjected to ANOVA.

RESULTS AND DISCUSSION

Effect of gamma radiation on two days old pupae of *P. absoluta*

The lowest adult emergence was observed with 300 Gy (33.34%) and highest in 100 Gy (60.12%) as against 93.34% in control. The deformity ranged from 6.7% to 22.7% within the doses and no deformity was observed under control. Longevity of adults ranged 3.03±0.11 to 5.3±0.26 days at 300 and 100 Gy, respectively, as against 7.27±0.20 days in control (Table 1). Adult emergence and longevity decreased with increased doses, while, extent of deformity enhanced. Highest adult emergence and deformity (60.12% and 22.70%) was recorded in lowest dose (100 Gy) and highest dose (300 Gy), respectively.

There was a differential response in terms of larval and pupal period of *P. absoluta* within the doses (Table 1). The larval period decreased with the increase in the doses, while, there was an increase in pupal period. Up to 58.6% pupation was observed in 150 Gy dose. The lowest larval period was observed in 300 Gy (4.67 days) and highest in 100 Gy (10.34 days) as against 11.2 days in control. Pupal period ranged 7.34 days (100 Gy) to 10.6 days (300 Gy) as against 6.34 days in control. However,

Table 1 : Effect of gamma radiation doses on two days old pupae of *P. absoluta*

Gamma irradiation (Gy)	Adult emergence* (%)	Deformity* (%)	Longevity of adult male) (days)#^	Larval period (days)#^	Pupation* (%)	Pupal period (days)#^
100	60.12	6.70	5.30	10.34	62.60	7.34
	(50.84)	(15.02)	(2.31)	(3.22)	(52.32)	(2.71)
125	57.35	13.40	5.06	9.60	60.78	7.67
	(49.23)	(21.48)	(2.25)	(3.11)	(51.23)	(2.77)
150	56.25	14.70	4.94	7.70	58.60	7.90
	(48.59)	(22.56)	(2.22)	(2.77)	(49.94)	(2.81)
200	50.67	17.40	3.5	6.20	50.67	8.60
	(45.32)	(24.67)	(1.87)	(2.51)	(55.39)	(2.94)
300	33.38	22.70	3.03	4.67	38.60	10.60
	(35.30)	(28.48)	(1.74)	(2.16)	(38.42)	(3.23)
Control	93.45	0.00	7.27	11.20	84.40	6.34
	(75.24)		(2.70)	(3.35)	(66.75)	(2.52)
CD @ 1%	2.19	0.59	0.11	1.61	1.03	3.35
CV %	1.73	1.91	1.92	0.12	0.79	0.24

^ mean of three replications; * figures in parenthesis are *arc sin* values; # figures in parenthesis are square root values

pupation ranged from 38.6% (300 Gy) to 62.6% (100 Gy) as against 84.4% in control.

Effect of gamma irradiation on five days old pupae of *P. absoluta*

Gamma irradiation of five days old pupae caused a reduction in adult emergence percentage. The adult emergence was significantly decreased as dose increased *i.e*. 66.60, 58.70, 57.34, 53.34 and 31.34% at 100, 125, 150, 200 and 300 Gy, respectively. Per cent malformed adult moths were 11.67, 13.40, 14.70, 16.43 and 18.70 with 100, 125, 150, 200 and 300 Gy (Table 2 and Fig. 1), respectively, as against no malformed adults in the control.

The lowest adult emergence was observed in irradiated doses of 300 Gy (31.34%) and highest in 100 Gy (66.6%) as against 93.34% in control (Table 2). Deformity ranged from 6.7% to 22.7% in 300 Gy and 100 Gy, respectively. Longevity of adults ranged from 3.17 days (300 Gy) to 5.6 days (100 Gy) as against 7.65 days in untreated control (Table 2).

Effect of irradiation doses on larval period, pupal period and per cent pupation

The study revealed similar results to that of two days old pupae by following decreased and increased trend of larval and pupal period with increase in doses. About 60.80% pupation was observed in 150 Gy (Table 2).

Lowest larval period was observed in 300 Gy (4.90 days) and highest in 100 Gy (10.34 days), as against 11.34 days in control. Pupal period ranged from 7.60 days (100 Gy) to 10.70 days (300 Gy) as against 6.34 days in control. Pupation and larval period had decreased with increase in the dose of gamma radiation, where, pupation ranged from 38.67% (300 Gy) to 62.7% (100 Gy) as against 84.34% in control (Table 2).

Age and gamma radiation dose

Treating five days old *P. absoluta* pupae with 150 Gy was more desirable than two days old pupae in terms of mating competitiveness (laboratory observation), though the impacts are comparable in terms of different biological parameters. The F_1 generation of 150 Gy treated population had shown superior effect on its biological cycle, where egg laying was 78.33/ female out of which 51.33 larvae hatched, of them 31.67% entered pupation and 26.26 pupae emerged into adults, indicating that the treatment 150 Gy was highly suitable for SIT for *P. absoluta* management (Table 3). These findings are in line with Fezza et al. (2021) who observed that irradiation of older pupae

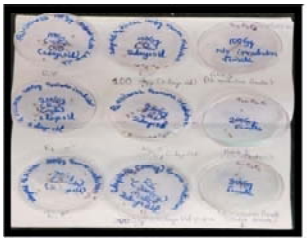
Table 2 : Effect of different gamma radiation doses on five days old *P. absoluta* male pupae

Gamma irradiation (Gy)	Adult Emergence* (%)	Deformity* (%)	Longevity of adult male) (days)#^	Larval period (days)#^	Pupation* (%)	Pupal period (days)#^
100	66.60 (54.71)	11.67 (19.96)	5.60 (2.37)	10.34 (3.21)	62.70 (52.35)	7.6 ± 0.69 (2.76)
125	58.70 (50.02)	13.40 (21.44)	4.5 (2.10)	9.34 (3.06)	61.30 (51.52)	7.34 ± 0.19 (2.71)
150	57.34 (49.23)	14.70 (22.56)	4.27 (2.06)	9.10 (3.02)	60.80 (51.25)	8.6 ± 0.34 (2.93)
200	53.34 (46.92)	16.43 (23.91)	3.76 (1.92)	6.50 (2.56)	53.50 (47.02)	9.34 ± 0.19 (3.06)
300	31.34 (34.05)	18.70 (25.61)	3.17 (1.78)	4.90 (2.22)	38.67 (38.46)	10.7 ± 0.75 (3.28)
Control	87.34 (69.18)	0.00 (0.00)	7.65 (2.77)	11.34 (3.37)	84.34 (66.73)	6.34 ± 0.25 (2.52)
CD @ 1%	1.58	1.25	0.04	0.09	1.03	0.14
CV %	1.25	4.01	0.69	1.25	0.81	1.94

^mean of three replications; * figures in parenthesis are arc sin values; # figures in parenthesis are square root values


a. Irradiated pupae in petri plates

c. Irradiated pupae in glass cages




b. Emerging adults of *P. absoluta* (100, 200 and 300 Gy)


d. Abnormal adults of *P. absoluta*

Fig. 1 : Experimental material and abnormal adults of *P. absoluta* after gamma radiation

Table 3 : Effect of gamma radiation doses on F_1 egg laying, hatching and adult emergence

Gamma irradiation (Gy)	No. of eggs# / female	No. of eggs hatched#/ female	Hatching* (%)	No. of larvae# pupated/ female	Pupation* (%)	Adult emergence* (%)
100	97.67 (9.88)	75.33 (8.68)	77.13 (61.44)	67.67 (8.23)	69.28 (56.34)	58.70 (50.01)
125	87.33 (9.35)	64.67 (8.04)	74.05 (59.39)	51.33 (7.16)	58.78 (50.06)	47.33 (43.48)
150	78.33 (8.85)	51.33 (7.16)	65.53 (54.05)	31.67 (5.63)	40.43 (39.49)	34.04 (35.69)
200	64.33 (8.02)	32.67 (5.72)	50.78 (45.45)	21.67 (4.66)	33.68 (35.47)	26.94 (31.28)
300	40.33 (6.35)	17.67 (4.20)	43.80 (41.44)	9.33 (3.05)	23.14 (28.75)	16.53 (23.99)
Control	194.67 (13.95)	163.67 (12.79)	84.08 (66.50)	155.33 (12.46)	79.79 (63.29)	70.55 (57.13)
CD @ 1%	0.14	0.18	1.35	0.15	1.06	0.65
CV %	0.71	1.14	1.21	1.06	1.15	0.79

* figures in parenthesis are arc sin values; # figures in parenthesis are square root transformed values

resulted in healthy adult with good flying ability and mating competitiveness. Various stages of *P. absoluta* and their durations observed were presented in Table 3.

Effect of gamma radiation doses on F_1 egg laying, egg hatching and emergence

Fecundity

Highest fecundity/female was observed with 100 Gy (97.67 eggs/female), followed by 125 Gy (87.33 eggs/female), 150 Gy (78.33 eggs/female), 200 Gy (64.33 eggs/female) and lowest in 300 Gy (40.33 eggs/female) as against 194.67 eggs/female in control.

Egg hatching

The highest egg hatching and hatch was observed in 100 Gy (75.33 and 77.13%), followed by 125 Gy (64.67 and 74.05%), 150 Gy (51.33 and 65.53%), 200 Gy (32.67 and 50.78%) and lowest in 300 Gy (17.67 and 43.80%) as against 84.08% hatching in control.

Pupation

Maximum pupation was observed in 100 Gy (69.28%) followed by 125 Gy (58.78%), 150 Gy (40.43%), 200 Gy (33.68%), while, lowest pupation was recorded in 300 Gy (23.14%) as against 79.79% pupation in control.

Adult emergence

The *P. absoluta* adult emergence was lowest in highest dose of 300 Gy (16.53%) and was highest in lowest dose 100 Gy (58.70%), as against 70.55% in control.

Effect of gamma radiation on egg laying in F_1 and F_2 generations

Lowest number of eggs laying and hatching was observed in the cross where irradiated male was involved in both F_1 and F_2 (Table 4).

Among the doses (100 and 300 Gy) tested, 150 Gy was significantly effective with reduced fecundity and hatchability both in F_1 and F_2 generations (Table 4). Present findings are in line with Tate et al. (2007), where F_1 generation of Cactus moths (*Cactoblastis cactorum*) were the test insect. Similar results were found with Saour & Makee (1997), Carpenter et al. (2001) and Makee & Saour (2004) with different test insects. *Labesia botrana* adults responded to increasing doses of gamma radiation with a decline in female fecundity and male fertility. Walton & Conlong (2016) reported African sugarcane borer, *Eldana saccharina*, laboratory-reared irradiated females were significantly less fertile than males and almost completely sterile at 200 Gy. Based on studies, effective irradiation dose requirement was different in various target insect species which may be due to age of pupae, laboratory culture, fitness of the insect etc.

Comparing the results of *L. botrana* male fertility with those obtained from *Cydia pomonella* (Bloem et al., 2007) and *Thaumatotibia leucotreta* (Carpenter et al., 2007), *L. botrana* was more radio-resistant than these two tortricid species (400 vs 350 Gy to obtain full sterility). However, *L. botrana* was less resistant to radiation than the potato tuber moth, *P. operculella* (Saour & Makee 1997) and *C. cactorum* (400 vs 500 Gy) (Carpenter et al., 2001).

Successful application of SIT/F_1 in European grapevine moth was achieved by Saour (2014), by selecting a irradiation dose of 150 Gy to *L. botrana* resulting in ~61% reduction in fertility of irradiated males mated with unirradiated females and only 20% of eggs hatched for F_1 males that mated with unirradiated females. In the present study also, the males irradiated with 150 Gy were competitive in mating like normal adults and sterile males resulted in reduced fecundity and hatchability in both F_1 and F_2. The effects of irradiation and IS on the reproduction were similar to those described for other insect species of Lepidoptera, *i.e.* reduced survival of larvae, the delay in the developmental time from F_1 neonate to adult, and the shift of sex ratio in favor of males in the F_1 generation.

Horner et al. (2020) also reported that the development of inherited sterility by applying SIT against the codling moth, *C. pomonella* resulting in significant reduction in its population suppression. Simmons et al. (2021) implemented the SIT to suppress populations of the European grapevine moth, *L. botrana*. These studies supported the present findings of effective reduction through SIT applications which can be a part of IPM as well.

Table 4 : Influence of 150 Gy dose of gamma irradiation on 5 days old male pupae on egg laying and hatching in F_1 and F_2 of *P. absoluta*

Cross Combination	Eggs/Female (Nos.)		Mean eggs/ Female ± SD	Hatched (Nos.)		Mean eggs hatched ± SD
	F_1	F_2	(No.)	F_1	F_2	(Nos.)
IF x WM	79.3	36.3	57.8 ±	59.3	25.9	42.6 ±16.70
WF x IM	68.5	19.6	44.1 ±	38.5	14.2	26.3±12.15
WF x FM	147.3	87.1	117.2±	139.1	84.3	111.7±27.40
SEM±	0.83	0.54	0.68	0.75	0.80	0.78
CD @ 1%	1.76	1.43	1.59	1.68	1.73	1.70
CV %	0.84	1.56	1.22	0.96	2.16	1.56

IF: irradiated females, WF: wild female, IM: irradiated males, WM: wild males

It is worth noting that the fecundity of F_1 females obtained in present study from male parents irradiated with 150 Gy and mated with unirradiated females was not significantly different from that of irradiated as also reported by Makee & Saour (1997) and Bloem et al. (2003) in F_1 progeny of *P. operculella* and *C. leucotreta*, respectively.

The SIT-based programs have been especially successful against dipteran pests. However, the SIT applicability for controlling lepidopteran pests has been challenging mainly due to their high resistance to the ionizing radiation that is used to induce sterility (Marec and Vreysen, 2019). Releasing of sub sterile *P. absoluta* males in field cages at a 15:1 substerile (200 Gy-treated pupae) to untreated male ratio caused a significant decline in larval production as compared with that control (Cagnotti et al., 2016).

In almost all SIT sterility programs against lepidopterans, both males and females are mass-reared, irradiated, and then released into the targeted area because no practical method is available to separate the adult moths by gender (Bloem et al., 2007). Moreover, the irradiated moths are released continuously from the beginning of the season, thus the possibility of crosses involving 150 Gy-irradiated males with F_1 females and F_1 males with their female counterparts could occur. The results of the study showed that the fertility of unirradiated males crossed to F_1 females did not differ significantly from that of the cross between F_1 males mated with unirradiated females, which suggests that F_1 females inherited the deleterious effects from their irradiated male parents. High values of unhatched eggs and sterility index were obtained when F_1 males were mated with either F_1 or unirradiated females and when 150 Gy-irradiated males were mated with F_1 females.

CONCLUSION

Among the different doses of gamma radiation tested against male pupae of *P. absoluta*, 150 Gy was effective for inducing sterility, where sterile males resulted in reduced fecundity and hatchability in F_1 and F_2. Five days old pupae of *P. absoluta* were more suitable for irradiation. These studies helps in evaluation of SIT under confined and area wide IPM.

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Original Research Paper

Constraints and strategies of smallholder farmers for successful protected cultivation of capsicum: A critical appraisal

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ABSTRACT

The study was framed to assess the challenges/constraints in protected cultivation of capsicum by small holder farmers in Karnataka state, India and provide strategies for sustained profitability. *Ex-post-facto* research design was followed for conducting study in three districts (Bangalore Rural, Bangalore Urban and Chickballapur) of Karnataka. From each district, 50 smallholders capsicum cultivation farmers under protected cultivation were selected through purposive random sampling, constituting 150 respondents. Garrett's ranking technique was adopted to analyse the constraints faced by the farmers in the study area. The various constraints experienced by the farmers were broadly grouped in to production constraints, market constraints, financial constraints, technological constraints, institutional constraints, weather-based constraints, health and labour constraints. These finding demonstrated urgent need to intervene towards the constraints experienced by the smallholder capsicum grows under protected cultivation, which not only ensures to get stable income but also sustain their livelihoods.

Keywords: Capsicum, constraints, protected cultivation, smallholder, strategies

INTRODUCTION

Protected cultivation plays a significant role in addressing the specific needs and challenges of agriculture in India, in the present scenario of land constraints, vagaries of climate change, prevalent pest and diseases etc. The scope of area expansion under cultivation of vegetables and flowers is very little. The only option is vertical expansion through increased productivity and cropping intensity using protected farming with environment control measures, quality seeds, fertilizers and plant protection measures (Paroda, 2013; GOH 2013). Plastic mulching, protected nursery production, use of green/polyhouses/ shade net houses for off-season production of vegetables and flowers have consistently given good results both at research farms and farmers' fields. In the recent years, increasing attention has been focused on several environmentally safe methods of pest management, including polyhouse cultivation to reduce pesticide use mainly because of growing concern over food safety issues and environmental concerns. Protected cultivation technology allows year-round off-season production of high-value low-volume vegetables, as well as virus-free quality seedlings, high-quality hybrid seeds, and the facilitation of disease resistance breeding programs.

Special schemes and programme of state government like *Krishi Bhagya* etc., and the supportive ecosystem including subsidies to the tune of 50 to 75%, led to large scale adoption of protected cultivation by smallholders also. Despite the success in polyhouse farming achieved by large scale horticultural and floricultural farmers for many years, smallholder polyhouse farming is beset by many challenges leading to the abandonment of some protected structures.

Capsicum, commonly known as bell pepper or sweet pepper, cultivated across an extensive area of 39,000 hectares with a substantial production (6,01,580 metric tons) and productivity (15.40 metric tons/ha) in 2022-23 (Indiastat, 2023). This adaptable crop excels in protected environments such as polyhouses, making it a preferred choice for cultivation. Growing capsicum under protected cultivation offers several advantages, including increased control over environmental conditions, protection from pests and diseases, and extended cropping seasons.

Consequently, it is pertinent to examine and outline the constraints and challenges faced by farmers in adopting polyhouse technology, as this understanding can help formulate strategies to overcome these hurdles.

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MATERIALS AND METHODS

The study was conducted in three districts (Bengaluru Rural, Bengaluru Urban and Chickballapur) of Karnataka state, considering the leading districts in area under protected cultivation, during 2021. In total, 150 farmers (50 farmers from each district) who had adopted polyhouse technology were selected purposefully.

Respondents were asked to rank the constraints listed according to its degree of importance so that the most crucial constraint will be ranked first. The outcome, which was in the form of ranking, was converted into per cent position by using formula:

$$\text{Percent position} = \frac{100\ (\text{Rij} - 0.5)}{\text{Nj}}$$

where, Rij = Rank given for the i^{th} variable by j^{th} respondents

Nj = Number of variables ranked by j^{th} respondents

The per cent position estimated was converted into scores with the help of Garrett's table. The scores of each individual rank corresponding to that particular constraint were added and the mean values of score were calculated. Higher mean Garrett value indicated the higher degree of constraint.

The strategies to overcome the challenges faced by farmers in adoption of polyhouse technology were pooled based on discussions with all the respondents under study. Strategies were analysed and tabulated using frequency and percentage.

RESULTS AND DISCUSSION

Production constraints

The major production constraints faced by capsicum farmers were inadequate information on scientific crop production (Table 1). Ghanghas et al. (2015) reported the lack of knowledge of latest package of practices. Hence, the farmer needs to abreast about latest information to tackle various issues in the production of capsicum.

As the crop under protected cultivation desires intensive crop management like attending to various crop needs *viz.*, from preparing soil bed to training, pruning, harvesting etc unlike under open cultivation, hence, the respondents perceived it as second major constraints under protected cultivation.

Market constraints

The major constraint perceived by the capsicum growers (Table 2) was fluctuations in market prices and demand, leading to the poor income to farmers followed by lack of specialised supply chain management practices. Rajesh & Shivalingaiah (2022) and Saravanan (2012) opined that problem of lack of suitable cold storage facilities (57.63%) and Singla et al. (2021) observed lack of market information (57.42%) as major marketing constraints.

Saini (2012) emphasized the importance of Government intervention in the price policy mechanism to prevent price fluctuation. The deficiencies in the infrastructure such as poor grading and transport facilities and cold chain management combined with market malpractices add to the risk component of farmers in India.

The primary financial challenges faced by respondent farmers were the 'high cost of initial infrastructure', followed by concerns such as the 'high cost of nutrient inputs' and the 'high cost of plant protection chemicals' (Table 3).

Financial constraints

The cost of establishment of polyhouse varies anywhere between Rs. 700 per metre to Rs. 1000/- per metre, which accounts to Rs. 35 lakhs to Rs. 40 lakhs per acre, respectively. The economic burden is heightened by the need for quality planting material, inputs, and the lack of subsidies and pricing policies, increasing the risk of cultivation. Subsidies typically cover 20 to 50% of the polyhouse erection cost, making protected cultivation financially demanding. Similar results were reported by Choudhary et al. (2022) that high cost of fertiliser, high cost of seeds as major constraints expressed by respondents.

Technological constraints

The capsicum growers expressed that availability of package of practices for cultivation of crops under polyhouse is either limited or requires lot of modification to suit their agro-ecological and socioeconomic conditions. Limited crop choice for diversification of crops under protected cultivation was also one of the major constraints perceived by the respondents (Table 4).

Farmers face challenges in obtaining the latest information and techniques for crop production under protected conditions, particularly in their regional

languages. Limited access to quality planting material at reasonable prices, dominated by a few private players, leaves farmers heavily dependent on companies. Successful polyhouse farming requires proper management and technical skills (FAO, 2013), but farmers struggle to access up-to-date information and techniques, impacting their effective use of technology. While extension staff visits significantly influence greenhouse performance, unrestricted access to unbiased technical information is essential (Omoro et al., 2015) given the limited technical support for greenhouse farming in the study region.

Institutional constraints

The major constraints perceived by the respondents were non-existence of price control mechanisms through institutional control, followed by high premium to claims ratios of insurance and absence of crop insurance for crops grown under protected cultivation. While insurance mechanisms are available for protected cultivation structures, they do not cover the crops grown in them. Choudhary et al. (2022) found that the biggest problem, reported by 60.47% of respondents, was the lack of a minimum support price (Table 5).

Institutional constraints stem from the framework and policies governing the sector, significantly affecting productivity and profitability. Inconsistent government policies, unreliable electricity supply, subsidy fluctuations, complex licensing, and regulatory hurdles create uncertainties, hindering investment and impacting operational profitability for these farmers.

Weather based constraints

The major constraints perceived by the respondents were that the structure is prone to wind damage, particularly cladding material followed by 'high summer temperatures causes pests severity and more crop loss' (Table 6). Specific climatic changes can alter yield, productivity, plant characteristics (quantitative and qualitative), and disease development (Egel & Saha 2015), which is due to the non-uniformity of microclimate inside the polyhouse (Qian et al. 2015). A single measurement cannot represent the entire polyhouse or provide complete information on the distribution of temperature and relative humidity (Korner *et al* 2007).

Health constraints

The farmers opined 'inappropriate handling of chemicals leads to ill health', 'improper disposal of chemicals causes health hazard' were some of the major health constraints perceived by them (Table 7).

Otto et al. (2017) found that 86.30% of vegetable greenhouse farmers reported pain, indicating a higher prevalence of muscular skeletal disorders (MSD) compared to traditional agricultural workers. MSDs in this context are linked to factors like physical strain, heavy lifting, and poor postures. Aging and prolonged greenhouse work contribute to tissue degeneration and chronic overload, supported by research on age and years working in greenhouses as risk factors (Yao, 2019; Leite et al., 2021).

Labour constraints

The result presented in Table 8 outlined that labour scarcity during peak seasons and denial by labours to work under protected environment due to stress and health issues, are the major labour constraints.

Finding and retaining skilled labour is difficult, leading to shortages and increased competition for experts in polyhouse operations. The seasonal nature of labour-intensive activities, such as planting and harvesting, complicates securing temporary workers during peak periods. High labour costs, especially in regions with elevated wages, affect overall profitability, requiring farmers to balance cost management with quality work. Migration trends and social factors contribute to labour shortages, as younger generations favour alternative employment opportunities. Studies by Choudhary et al. (2022) highlighted the significant impact of labour scarcity on protected cultivation.

The strategies as perceived by the smallholder capsicum farmers to increase the profitability and sustainability of capsicum under protected cultivation are presented below

To enhance capsicum profitability and sustainability in protected cultivation, farmers can implement strategies outlined in Table 9. Key measures include acquiring technical knowledge, staying updated on polyhouse practices, ensuring timely access to quality seed, reducing implementation costs, standardizing production technology, addressing marketing challenges through research and direct channels, seeking government support, managing technological constraints through training and research, mitigating

Table 1 : Production constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. Lack of scientific knowledge about crop production under protected structures	59.987	I
2. Requires intensive crop management under protected cultivation	54.953	II
3. Non-availability of required quality of seeds and planting materials of desired hybrids/varieties	50.207	III
4. Difficulty in following the recommended package of practices	46.747	IV
5. Limited and irregular power supply	46.353	V
6. Fluctuation of electrical conductivity (EC) of water under intensive crop management practices and difficult to manage this problem	43.567	VI

Table 2 : Market constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. Fluctuation in market prices and demand leading to poor income to farmers	65.78	I
2. Lack of specialized supply chain management practices	58.05	II
3. Difficulty in accessing export markets	54.22	III
4. Export of high value vegetables has drastically reduced	45.47	VI
5. Insufficient cold chain infrastructure for storage and transportation of produce	45.68	V
6. Poor payment and less adoption of business ethics in marketing of crops produced under protected environment	48.39	IV
7. Lack of exclusive markets for the crops grown under protected cultivation	38.00	VII

Table 3 : Financial constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. High cost of initial infrastructure	62.93	I
2. High cost of nutrient inputs	62.63	II
3. High cost of plant protection chemicals	59.17	III
4. High cost of seeds & planting material	52.64	IV
5. High cost of skilled labor	50.06	V
6. Complex loan procedures and high rate of interest	48.93	VI
7. Poor accessibility to subsidy and delay in release of financial assistance leading to higher debits	44.53	VIII
8. Poor financial support for maintenance and repair of protected structure	44.72	VII
9. Continuous change of guidelines by financial institutions for getting financial support for protected cultivation	35.45	IX

Table 4 : Technological constraints experienced by the smallholder capsicum growers under protected cultivation

Constraints	Mean Garret score	Rank
1. Lack of technical know-how about growing crops under protected cultivation	58.07	I
2. Limited crop choice for diversification of crops under protected cultivation	55.15	II
3. Repair and maintenance of protected structures is difficult	50.30	III
4. Availability of spare parts is difficult	44.34	VI
5. Stability of constructed protected cultivation structure towards various damages	46.17	V
6. Lack of relevant literature on production and protection in local languages	48.79	IV

Table 5 : Institutional constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. Non-existence of price control mechanisms through institutional control	54.27	I
2. High premium to claims ratios of insurance	53.83	II
3. Absence of crop insurance for crops grown under protected cultivation	53.55	III
4. There is no sustained policies and long term plans to stable growth and development of protected cultivation industry	49.25	IV
5. No specific institutions to give knowledge on Protected cultivation	47.29	V
6. Frequent change of government policies with respect to protected cultivation	42.66	VI

Table 6 : Weather based constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. Structure is prone to wind damage, particularly cladding material	61.63	I
2. High summer temperatures causes pests severity and more crop loss	51.87	II
3. Structure is prone to hailstorm damage	49.05	III
4. High rainfall causes disease intensity & crop loss	39.93	IV

Table 7 : Health constraints experienced by the smallholder capsicum growers under protected cultivation

Constraint	Mean Garret score	Rank
1. Inappropriate handling of chemicals leads to ill health	62.10	I
2. Improper disposal of chemicals causes health hazard	57.62	II
3. Use of Protected protection equipment (PPE) usage causes suffocation and difficulty to work under protected structures	41.05	III
4. Protected structures are designed to optimize the environment for plant growth, rather than for workers health	40.92	IV

Table 8 : Labour constraints experienced by the farmers

Constraint	Mean Garret score	Rank
1. Labour scarcity during peak seasons	52.90	I
2. Denial by labors to work under protected environment due to stress and health issues	49.62	II
3. Lack of availability of skilled labour for various operations	47.43	III

Table 9. Strategies to address constraints of smallholder farmers for Successful Protected Cultivation of Capsicum

Strategies to address the constraints	f	%
Production Strategies		
1. Enhancing information accessibility on capsicum cultivation	131	87.33
2. Optimizing digital platforms for information dissemination	119	79.33
3. Ensure timely availability of seeds and planting material	105	70.00
4. Addressing the high costs associated with the implementation of protected cultivation	87	58.00
5. Establishing standardized production technologies specific to protected cultivation of capsicum	65	43.33
6. Implementing measures for the management and improvement of water quality	52	34.67
Marketing Strategies		
1. Conducting market research and analysis to understand market dynamics for capsicum cultivation	112	74.67
2. Promotion of direct marketing and forward marketing of capsicum	103	68.67
3. Creating conducive ecosystem that supports and facilitates the export of capsicum	75	50.00
4. Advocating government policies that support the successful cultivation of capsicum	73	48.67
Financial Strategies		
1. Seeking government support and incentives to promote capsicum cultivation	139	92.67
2. Improving access to credit and grants for smallholder farmers engaged in capsicum cultivation	124	82.67
3. Implementing strategies to optimize costs and reduce the overall cultivation expenses	118	78.67
4. Ensuring procurement of cost-effective need-based inputs through line departments	97	64.67
Technological Strategies		
1. Conducting training and skill development programs for capsicum cultivation under protected structures	86	57.33
2. Conducting advanced research and development for varieties and planting material suitable for protected cultivation of capsicum	78	52.00
3 Fostering research and innovation in capsicum cultivation technologies	56	37.33
Institutional Strategies		
1 Advocating policies that endorse and facilitate successful cultivation	103	68.67
2 Providing insurance coverage to crops grown under protected cultivation	101	67.33
3 Implementing institutional programs providing support and incentives for capsicum cultivation	97	64.67

Weather Strategies			
1.	Designing protected structures customized to the specific agro-ecosystem and locations	105	70.00
2.	Choosing capsicum varieties resilient to abiotic stress	75	50.00
3.	Implementing measures for effective microclimate management in Capsicum	66	44.00
4.	Using wind and shelter brakes to protect capsicum crops from adverse weather conditions	60	40.00
Health Strategies			
1.	Encouraging the use of plant protection equipment for the safety of farmers in protected structures	150	100.00
2	Educating farmers on safe practices for handling chemicals	145	96.67
3	Encouraging and enforcing good agricultural practices in capsicum cultivation	129	86.00
Labour Strategies			
1	Implementing mechanization and automation to address labor scarcity	138	92.00
2	Conducting capacity development programs to enhance the skills of laborers	131	87.33

institutional issues via policy advocacy, addressing weather challenges through proper design and variety selection, and tackling health and labour constraints with good practices, protective equipment, mechanization, and skill development. This comprehensive plan aims to empower farmers and improve capsicum production, fostering increased productivity and sustainability in agriculture.

The strategies proposed by smallholder capsicum farmers aim to enhance profitability and sustainability in protected cultivation through a holistic approach. This includes improving information access for informed decision-making, ensuring timely availability of quality seed, addressing financial constraints by reducing implementation costs, and standardizing production technology. Marketing strategies focus on market research and exploring direct channels to overcome price fluctuations and enhance market access. Technological advancements and continuous learning address knowledge gaps and skill shortages, while institutional support through policy advocacy aims to positively impact regulatory frameworks. Weather-related constraints are addressed through proper structure design, resilient variety selection, and microclimate management. Health and labor challenges are tackled with good agricultural practices, protective equipment, mechanization, and skill development. These comprehensive strategies collectively work towards eliminating diverse constraints, fostering a more sustainable and profitable future for smallholder capsicum farmers in protected cultivation.

CONCLUSION

Adoption of polyhouse technology for capsicum cultivation under protected conditions in India faces multiple constraints and challenges such as inadequate information on scientific crop production, limited availability of quality seed and planting material, high costs of implementation, water quality management, market constraints, institutional constraints, weather-based constraints, health constraints, and labour constraints. To unlock full potential of protected cultivation in capsicum, a comprehensive approach is required that involves providing farmers with up-to-date knowledge, right information at right time, improving access to quality seeds and planting material, addressing the high costs of implementation through subsidies or financial assistance, developing mechanisms for price stabilization and market support, addressing institutional constraints through policy interventions, improving infrastructure and cold chain facilities, promoting crop insurance for crops grown under protected cultivation, managing weather-related risks, promoting occupational health and safety measures, and addressing labour shortages through skill development and attractive incentives.

By addressing these constraints and challenges, the potential of protected cultivation for capsicum and other high-value crops can be fully realized, leading

to increased productivity, extended cropping seasons, improved quality, reduced pesticide use, and better economic outcomes for farmers.

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Original Research Paper

Information needs of farmers on cultivation of salad cucumber *Cucumis sativus* under polyhouse

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ABSTRACT

The conventional practice of crop production is now giving its way to hi-tech farming practices. Salad cucumber (*Cucumis sativus*), is a relatively new crop in Kerala and has proven yield benefit under polyhouse conditions. The study intended to assess the information needs of farmers regarding various technical and cultivation aspects of salad cucumber production under polyhouse. Kendall's coefficient of concordance (W) and mean values were used to identify the information needs. Most of the cultivation aspects fell under either the 'highly essential' or 'moderately needed' information category. The technical aspects of construction, maintenance and repair of polyhouse were the most critical information need of farmers along with the specifics of fertilizers for polyhouse and fertigation system. Pests, diseases and nutrient deficiency problems were also the main concerns of farmers. Weed management and pollination, with low mean scores were among the least felt information need. Hence, these aspects are also found to be among essential needs. The study elucidated that an efficient information delivery system through appropriate channels is required to boost polyhouse cultivation of vegetables in Kerala.

Keywords : Hi-tech farming, information need assessment, polyhouse cultivation, salad cucumber

INTRODUCTION

Cultivation of vegetables under polyhouse has been gaining importance and popularity in the Indian farming system since two and a half decades. It lessens farmers' dependency on climate and makes the optimum use of natural resources for crop production. Compared to traditional farming, the modern technique of polyhouse farming promises a better income (Rabbi et al., 2019). Farmers can also minimise the adverse effects of weather or pest attacks on their crops. Thus, polyhouse farming can help the farmer to generate returns round the year by raising multiple crops (Franco et al., 2018).

The research and extension system in developing countries like India is gradually becoming knowledge-centred. As agriculture systems become more multifaceted, it is the need of the hour to ensure that farmers can access reliable, timely, and relevant information sources. The information must be need-based, packed, user-friendly, and disseminated in a way that is ideal for the farmer and market (Babu & Glendening, 2019).

Polyhouse is relatively a new practice in Kerala. Appraisal of the sustainability of polyhouse system after a span of time, has indicated that farmers are gradually discontinuing the cultivation under polyhouse. One of the main constraints observed was the lack of adequate knowledge regarding the protected cultivation of vegetables under polyhouse (Hena, 2017). Therefore, to provide a knowledge base to the farmers who adopted and going to adopt polyhouse in future, it was found essential to investigate the information needs of the experienced farmers.

Salad cucumber is one of the most suitable crops and popularly grown vegetable to be best cultivated under polyhouse (Lakshmi et al., 2017). An attempt was made to study the information needs of farmers in Kerala regarding salad cucumber cultivation under polyhouse.

MATERIALS AND METHODS

Based on number of polyhouses, two districts each from the north (Kozhikode, Malappuram), central (Thrissur, Ernakulam) and south (Alappuzha, Thiruvananthapuram) of Kerala were selected. A total of 371 farmers are cultivating/has already cultivated four most popular and successful crops (salad cucumber, yard long bean, amaranthus and chilli) in polyhouses of these districts. Thus, a representative

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sample of 60 polyhouse farmers (10 farmers from each district) cultivating/has already cultivated salad cucumber were selected randomly for the study.

After an extensive literature review and discussion with experts (scientists and professors from Kerala Agricultural University, officers from the Department of Agriculture and Farmers' Welfare, Kerala and 30 non-sample farmers from Palakkad district), 71 critical aspects which requires specialised knowledge regarding the cultivation of salad cucumber under polyhouse were identified. These aspects, from the construction of polyhouse to the marketing of the produce, were categorised into 16 sections to formulate the interview schedule. The selected farmers were asked to rate the items on a five-point scale from zero to four, where zero representing 'not needed' and four representing 'highly essential' information on polyhouse cultivation.

Kendall's coefficient of concordance (W) and mean values were calculated and used to identify the agreement among farmers regarding the information needs. The W-value indicates the degree of agreement among scores assigned by the respondents on different attributes (Kendall et al., 1939; Kendall & Gibbon, 1990; Hardesty & Bearden, 2004). W-values were calculated as follows.

$$W = \frac{12S}{m^2(N)(N^2-1)}; \quad 0 \leq W \leq 1$$

where,

$S : \sum d_i^2$

$d_i : R_i - \bar{A}$, where R_i is the sum of ranks assigned to item i by m respondents

m : Number of respondents

N : Number of attributes

W value close to one indicates good agreement among all the respondents. The item-wise mean scores of information needs of farmers were also assessed. Mean scores were used to determine the most important to least important information needs of farmers.

RESULTS AND DISCUSSION

The W-value closer to one indicates higher agreement among the respondents regarding that specific cultivation practice, while, a value closer to zero indicated lesser agreement. The results are presented in Table 1.

Table 1 : W values of items under each category

Items	W value
Design and construction of polyhouse	0.073**
Hi-tech seedling procurement and production	0.055*
Crop layout and design	0.177*
Disinfection of polyhouse	0.046
Micro-irrigation system	0.302**
Fertigation system	0.005
Cooling system	0.094**
Maintenance and repair of polyhouse	0.011
Pest and disease management	0.091**
Nutrient management	0.151**
Weed management	0.084**
Pollination	0.303**
Training and pruning	0.022
Harvesting of crop	0.087**
Marketing of produce	0.445**
Financial assistance	NA#

**significant at 0.01 level; *significant at 0.05 level; # not applicable as only one item was included under the category

Table 1 indicates that the category-wise W values of 11 out of 16 practices showed significant agreement among the polyhouse farmers regarding their information needs. Coefficient of concordance value of nine categories (design and construction of polyhouse, micro irrigation system, cooling system, pest and disease management, nutrient management, weed management, pollination, harvesting and marketing of produce) showed significant concordance at 1% level, whereas, cultivation practices like hi-tech seedling procurement and seedling production, crop layout and design were significant at 5% level. The significance of these categories indicates that the agreement on the information needs among the farmers was fair enough to arrange the statements (Table 2) according to their mean score. The W value of variables such as disinfection of polyhouse, fertigation system, maintenance and repair, training and pruning were non-significant at 1% as well as at 5% levels of significance. This points out that the needs of farmers differed with each other. Under the section 'financial assistance', since only one item was included, the W-value was inestimable.

The item-wise mean scores obtained was used to select the most important information needed by farmers. The mean scores were arranged in descending order under each category in order to organise the items according to the need of farmers.

Table 2 : Mean scores of different cultivation aspects

Items	Mean (M)
Design and construction of polyhouse	
Orientation of polyhouse	3.48
Selection of site for polyhouse construction	3.47
Selection of materials for construction of polyhouse	3.33
Ridge height	3.27
Gutter height	3.27
Cladding material	3.17
Hi-tech seedling procurement and production	
Crop varieties suitable for hi-tech vegetable cultivation	3.98
Source of seeds	3.95
Quality seedling production	3.88
Transplantation of seedlings	3.78
Crop layout and design	
Training of the crops	3.97
Bed preparation	3.65
Spacing of the seedlings	3.48
Planting in growbags and potting mixture	3.18
Soil analysis	3.12
Disinfection of polyhouse	
Disinfection of polyhouse	3.72
Soil sterilization	3.60
Fumigation	3.47
Micro irrigation system	
Maintenance and repair of the irrigation system	3.88
Operation of the irrigation system	3.77
Drip irrigation	3.35
Installation of irrigation system	3.20
Fertigation system	
Fertilizers suitable for fertigation	3.77
Calculation of fertilizer doses	3.75
Maintenance of soil parameters	3.73
Maintenance and repair of fertigation system	3.72
Operation of fertigation unit	3.72
Cooling system	
Maintenance and repair of fogger	1.95
Operation of fogger	1.85
Installation of fogger	1.72
Maintenance and repair of polyhouse	
Maintaining weather parameters inside polyhouse	3.77
Cleaning of cladding material of polyhouse	3.73
Changing the cladding material of polyhouse	3.67

Pest and disease management	
Identification of infestation of diseases and pests and their symptoms	3.88
Biocontrol agents against pest and diseases	3.70
Method of application of biocontrol agents	3.48
Traps used for pest control	3.40
Plant protection chemicals	3.38
Dosage of the chemicals	3.25
Soil application of chemicals	3.15
Foliar spray of the plant protection chemicals	3.13
Nutrient management	
Identifying nutrient deficiency symptoms	3.88
Toxicity symptoms	3.78
Stage and time of application of fertilizers	3.73
Rate of application of fertilizers	3.63
Bio fertilizers to be applied	3.57
Soil application of fertilizers	3.57
Type and quantity of chemical fertilizers to be applied	3.53
Foliar application of chemicals	3.38
Composting	3.07
Weed management	
Stage of weeding	2.03
Weed flora found in polyhouses	1.80
Mechanical weeding	1.73
Chemical weeding	1.48
Pollination	
Knowledge about assisted pollination	2.03
Beekeeping	0.97
Maintenance of bee hives	0.87
Stage of keeping hives	0.85
Training and pruning	
Training methods	3.43
Pruning methods	3.35
Time of training / pruning	3.33
Stage of training / pruning	3.33
Harvesting of crop	
Method of harvesting	2.68
Stage of harvesting	2.48
Harvesting time	2.35
Marketing of produce	
Market rate of vegetable	3.62
Storage	2.52
Packing	2.52
Grading	2.48
Financial Assistance	3.55

The data highlights that the most essential information needed by farmers regarding the design and construction was the orientation of the polyhouse (M = 3.48). Farmers positioned selection of site for polyhouse construction in the next place under design and construction (M=3.47). Information about the right construction materials and their sources are very important as far as polyhouse construction is concerned. Due to this, they placed it as the next most important item under design and construction aspects with a mean value 3.33. The farmers considered the information regarding the cladding material which includes the quality and other specifications is also very important with a calculated mean score of 3.17 out of four.

Farmers opined that the information on hi-tech seedling production or procurement of good quality seedlings as essential. As self-pollinated and hybrid seeds were not commonly available to most of the farmers and were not aware of the availability, they marked the item 'seed source' as an important information requirement (M=3.95). Even some of the experienced farmers were unfamiliar with the practice of transplantation in a crop like salad cucumber in the polyhouse. They found it as an important and basic need. Hence score of 3.78 out of four.

The most important information under crop layout and design was on training of the crops, as farmers realised the need of specialised structures to grow salad/sweet/ English cucumber under a polyhouse (M=3.97) 3.65. Most of the farmers who had less experience in salad cucumber cultivation required information on bed preparation and spacing of seedlings and thus, they assigned it with a mean score of 3.65 and 3.48, respectively. Method of cultivating the crop in growbags was also placed as an important information requirement among crop layout and design with the mean score 3.18. The data from farmers indicate that the information regarding soil analysis was essential for farmers (scores 3.12).

Farmers experienced that the micro atmosphere in polyhouses is in such a way that, the pests and disease control is difficult if proper care is not taken (Choudhary et al., 2022). Therefore, they felt the information on disinfection of polyhouse is very essential and they placed disinfection, soil sterilisation and fumigation at high ranks (scores of 3.72, 3.60 and 3.47 respectively).

Micro-irrigation is an inevitable part in crop cultivation under greenhouse (Rathod & Shaikh, 2023). The information on maintenance and repair and operation of irrigation system was very essential for farmers and they have placed it in the first two positions (mean scores of 3.88 and 3.77, respectively). The mean scores indicated that farmers found the information a much needed one (M=3.20). Some of the farmers were aware of the general information on drip irrigation and remaining farmers needed the information (score 3.35).

Most of the farmers were not familiar with operation and maintenance of the fertigation unit (score above 3.70). Farmers required information about fertilizers suitable for fertigation followed by calculation of fertilizer doses (scores 3.77 and 3.75, respectively). Maintenance of soil parameters was positioned next with mean score 3.73. Operation, maintenance, and repair of the fertigation unit were found equally important to farmers (M=3.72).

Majority of farmers did not feel the need of fogger or any cooling equipment inside polyhouse (score less than two), which indicated that the information was least important for the farmers. The items under this were installation, operation, and maintenance and repair of the fogger with M values were 1.72, 1.85 and 1.95, respectively.

The knowledge regarding the maintenance and repair of polyhouse was very crucial according to the opinion of farmers. Algal growth resulting in the restriction of availability of sunlight to plants, leading to yield reduction is a major problem reported by polyhouse farmers in Kerala. The adverse climatic conditions such as heavy rainfall, wind, and high humidity result in damage of the polyhouse besides creating difficulties in maintaining weather parameters inside polyhouse. So, the farmers felt the importance of proper cleaning and maintenance of polyhouse. The items listed under the category were maintenance of weather parameters inside the polyhouse, cleaning of cladding material, and frequency of changing of the cladding material (scores 3.77, 3.73 and 3.67, respectively).

According to the farmers, in protected cultivation, the closed atmosphere and microclimate make pest and disease management a difficult task compared to that of open cultivation (Choudhary et al., 2022). The priority was given to items like symptoms of disease and pest infestation (M=3.88) as early diagnosis was

the most important factor to ward off the spread of pests and diseases. Many of the farmers had initially practiced organic methods of pest control. So, they were interested to know about different biocontrol agents (M=3.70) and the methods to apply them (M=3.48). Therefore, the polyhouse farmers marked items such as chemicals used, dosage of pesticides, soil, and foliar application of chemicals as highly crucial information (scores 3.38, 3.25, 3.15 and 3.13, respectively).

Under the aspects regarding nutrient management, polyhouse farmers needed more information on identifying the symptoms of nutrient deficiency and toxicity (mean value 3.88 and 3.78, respectively). They needed more insight on the stage and time of application as well as rate of application of fertilizers (scores 3.73 and 3.63, respectively). It was also reflected from the scores that, soil application and foliar application of chemicals were much needed information (scores 3.57 and 3.38, respectively). The items, composting and biofertilizer application had high mean scores (3.07 and 3.57, respectively).

As per the response from most of the polyhouse farmers, weed management was one of the least needed information by the polyhouse farmers. They opined that the weed control was possible without much difficulty and was not different from weeding in open fields and hence, very few farmers pointed it as a needed information (M=1.80). A few more farmers needed the information on the stage of weeding (M=2.03). Being in a confined area, farmers felt manual weed control is easier in polyhouses when compared to open cultivation.

It is observed that, majority of the farmers used self-pollinated vegetable varieties inside polyhouse. Only a very few farmers reported the requirement of information on the natural pollination aided by honeybees and other insects (values less than one). A slightly higher number of farmers needed information on hand pollination and other artificial crossing methods which can be possible under greenhouse (M=2.03).

The farmers said that training and pruning are critical aspects while cultivating salad cucumber under protected conditions. The respective mean scores of training methods, pruning methods, time of training and pruning and correct stage of training and pruning were 3.43, 3.35, 3.33 and 3.33, respectively. Skill trainings are absolutely essential to take advantage of right stage and methods of training and pruning.

Generally, it is observed that farmers under the study were familiar with harvesting procedures. But in protected cultivation, vertical growth of the plant makes the procedure a bit difficult. Hence, some farmers opined that, the process will be easier if they get more information on harvesting methods (M=2.68). Salad cucumber cultivation is comparatively a new practice in Kerala according to the farmers' opinion, and hence, some of the farmers were unaware of the correct stage of harvesting (M=2.48). Some farmers who were new to vegetable farming, required information on the time and method of harvest of salad cucumber under polyhouse (M=2.35).

Another key aspect regarding the cultivation of salad cucumber is about its market price. The main information farmers required regarding the marketing was about the market rate of salad cucumber (value 3.62). Apart from this, all aspects of marketing such as storage, grading, packing and transportation was moderately required for polyhouse farmers as they were familiar with these practices.

Initial investment to construct polyhouse is so high and most of the farmers reported that they cannot afford that. They pointed out that without any subsidies, loans or government schemes, polyhouse construction was impossible for them. So, they mentioned that the updated information on financial assistance should be made available by the concerned authorities, without which polyhouse cultivation is not economically feasible (high score 3.55), indicate that the need is very essential.

CONCLUSION

The study analysed the information needs of farmers about salad cucumber cultivation under polyhouse in Kerala. As the farmers are novice and facing many constraints on crop production through this technology, it can be observed from the results that the farmers pointed out their high information needs on almost all the aspects related to the crop cultivation. They found the information regarding design and layout, training of the crop, disinfection and maintenance of the polyhouse, marketing of the crop, pest and disease management, and financial assistance as most essential among all (high score >3.0).

It is concluded from the study that there is a felt need of providing accurate, reliable and need based information among the polyhouse farmers in Kerala. An elaborate study on the constraints faced by the polyhouse farmers has to be conducted to understand the field level problems faced by the farmers and to bridge the gap between farmers and technology. As the number of active polyhouses are declining in the state of Kerala, the needs of the farmers should be a serious concern. Proper measures should be taken to disseminate required information through trainings, media and tools most suitable for the situation and can be easily disseminated within least time in order to encourage polyhouse cultivation in the region.

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Original Research Paper

An alternate statistical method for dealing outliers in perennial crop experiment

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ABSTRACT

A statistical method based on Robust ANOVA to handle outliers induced high coefficient of variation (CV) in pooled (2011-2018) analysis of long-term Mango cv. Totapuri rootstock trail was suggested. Based on the results, it was concluded that the rootstock treatment T3: Olour (average yield over the period 2011 to 2018 as 57.21 kg/tree) as the best. Precision gained as estimated by reduction in CV (%) was in the range of 11.01 % to 78.9 %. SAS IML codes were built-in for analysis. Hence, this study calls for employing robust ANOVA approach in testing the significance of evaluated treatments in a designed perennial crop experiment with high CV that would have reduced the sensitivity of testing the significance of treatment differences otherwise.

Keywords: Mango, Outliers, Robust ANOVA

INTRODUCTION

Classical analysis of variance (ANOVA) approach to compare the significance of set of treatments in a perennial crop field experiment is mainly based on the requirement of certain assumptions for the ANOVA model. The major hindrance to this is the presence of outlier(s) among the replicated values. Outliers in any of the replications (of any treatment) lead to failure of normality assumption. Presence of such aberrant values may finally leads to non-significance/on par results coupled with high coefficient of variation (CV), especially in perennial fruit crops spaced very widely in the open field such as mango.

One way out is to identify such an outlier(s) and delete them to have a possible comparison among treatments. However, deleting the outlying replication is not recommended because its deletion leads to violation of basic principle designs of experiment (i.e. randomization) and from experimenter point of view every observation carries some information that should be exploited. This aspect is very much pertinent especially when we deal with perennial trees, as the number of replicated values for a treatment kept at a bare minimum. To address this problem, a method based on Robust ANOVA is suggested and its efficacy is studied using primary data on yield related traits with a view to identify best treatment. Robust ANOVA techniques are designed to be less sensitive to outliers, which are data points that deviate significantly from the rest of the data. It improves the reliability of the analysis by reducing the influence of outliers (Paul & Bhar, 2011; Venugopalan & Manjunath, 2019).

MATERIALS AND METHODS

Eight root stocks treatments such as T1: Totapuri, T2: Vellaikulumban, T3: Olour, T4: Peach, T5: Kensington, T6: Mylepelian, T7: Nekkare and T8: Turpentine were selected for the study and evaluated in RCBD, with three replications, at an experimental plot of Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during the period 2010-2018 was considered. Primary data recorded on three important characters of Mango cv. Totapuri that were showing high CV of more than 20% almost consistently throughout the experiment period viz., fruit yield per tree in kilograms, average weight of individual fruit and number of fruits harvested per tree, while all other characters studied were showing less than 18% CV consistently, for eight rootstocks treatments. Both classical and Robust ANOVA were employed to identify best rootstock treatment for each of the traits.

a) Classical two-way analysis of variance :

The two-way ANOVA (Federer, 1975) model that describes the response variable with treatment and block effect is given by

$$Y = \mu + \alpha_i + \beta_j + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where α_i= effect of ith treatment, β_j=effect of jth block, ε=random error

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When the experiment is conducted over seasons or years or places pooled ANOVA or combined analysis of data is done after the analysis of individual experiments. Before going for the pooled analysis the data is tested for homogeneity of error variance using Bartlett's Chi-square test. If the chi-square test result is significant, we go for pooled ANOVA.

Bartlett's Chi-square test

Null hypothesis of Bartlett chi-square test H0: The variances in the different groups are equal against the alternate hypothesis H1: The variances in the different groups un- equal, indicating heterogeneity of variances.

A. When p =2

$$\chi^2 = \frac{S_{e_1}^2}{S_{e_2}^2} \sim F_{(n1,\, n2)} \text{ .....(2)}$$

Where $S_{e_1}^2$ and $S_{e_2}^2$ are mean square error for two years or seasons or places.

B. When p >2

$$\chi^2_{p-1} = \frac{\sum n_i \log S_e^{-2} - \sum n_i \log \hat{e}_i^2}{1+\frac{1}{3(p-1)}\left(\sum\frac{1}{n} - \frac{1}{\sum n_i}\right)} \sim \chi^2_{p-1}, \text{ .....(3)}$$

where $S_e^{-2} = \frac{\sum n_i S_{e_i}^2}{\sum n_i}$, $S_{e_i}^2$ is the mean sum of square of ith year with n_i df

When the calculated value is more than the critical value the test is significant, which means null hypothesis is accepted and pooled ANOVA is performed. If the chi – square result is not significant, indicating the heterogeneity of error variance and to stabilize the error variance, appropriate transformation is chosen, then pooled ANOVA could be performed.

b) Robust Analysis of variance :

Robust M-estimation approach instead of minimizing the sum of squared residuals in the classical ANOVA based approach, minimizes the sum of a less rapidly increasing function of the residuals ($\rho(e_i)$), as given below (Paul & Bhar,2011; Venugopalan & Manjunath, 2019).

$$Min\sum_{i=1}^{n}\rho\left(y_i - \sum x_{ij}\beta_j\right) = Min\sum_{i=1}^{n}\rho(e_i) \text{ .....(4)}$$

The solution is not scale equi-variant, and thus the residuals must be standardized by a robust estimate of their scale $\hat{\sigma}_e$ which is estimated simultaneously. As in the case of M-estimates of location, the median absolute deviation (MAD) is often used. Taking the derivative of above equation and solving, produces the score function

$$\sum_{i=1}^{n}\Psi\left(y_i - \sum x_{ij}\beta_j / \hat{\sigma}\right)x_{ik} = \sum_{i=1}^{n}\Psi\left(e_i / \hat{\sigma}_e\right)x_i = 0 \text{ .....(5)}$$

Where $\hat{\sigma} = median\left|e_i - median\left(e_i\right)\right| / 0.6745$

With $\Psi = \rho'$. There is now a system of k+1 equations, for which Ψ is replaced by appropriate weights that decrease as the size of the residual increases

$$\sum_{i=1}^{n} w_i\left(e_i / \hat{\sigma}_e\right)x_i = \sum_{i=1}^{n}\frac{x_{ij}\{\psi[(y_i - x_i'\beta)/s]/(y_i - x_i'\beta)/s\}/(y_i - x_i'\beta)}{s} = 0 \text{ .....(6)}$$

→ j=0.1,.....,k

As

$$\sum_{i=1}^{n} x_{ij} w_{i0}\left(y_i - x_i'\beta\right) = 0 \text{ .....(7)}$$

→ j=0.1,.....,k

where

$$w_{i0} = \begin{cases} \dfrac{\psi\left[\left(y_i - x_i'\hat{\beta}_0\right)/s\right]}{\left(y_i - x_i'\hat{\beta}_0\right)/s} & \text{if } y_i \neq x_i'\hat{\beta}_0 \\ 1 & \text{if } y_i = x_i'\hat{\beta}_0 \end{cases}$$

Hence by matrix notation $X'W_0X\beta = X'W_0y$

where W_0 is n × n diagonal matrix of weights then one step estimator is -

$$\hat{\beta} = (X'W_0X)^{-1}X'W_0y \text{ .....(7)}$$

Robust criterion functions

Criterion	$\rho(z)$	$\psi(z)$	$w(z)$	Range
Least squares	½ z^2	z	1.0	$\|z\| < \infty$
Huber's function	½ z^2	z	1.0	$\|z\| \le t$
	$\|z\|t - 1/2t^2$	t sign(z)	$t/\|z\|$	$\|z\| > t$

Here $\rho(z)$ is the function of residual, $\psi(z)$ is the derivative of and w (z) is the weight function (Huber, 1973). SAS codes using SAS V 9.3 were generated for both the estimation procedures and used for analysis (SAS V 9.3, 2012).

Comparison of classical ANOVA vs Robust ANOVA

Efficacy of set of treatments in both the approaches are tested by computing the p-value (a measure of strength of the inference drawn) and the coefficient of variation (Gomez and Gomez,1988). The results are presented in Tables 2-4.

RESULTS AND DISCUSSION

For the entire study we took eight root stock treatments for 3 characters such as yield / tree, fruit weight, number of trees during 2011-2017 respectively. The results of both classical and robust ANOVA methods for three characters studied are presented in Tables 2-4. Individual year based assessment of significant treatments as revealed by the respective P-values of the treatment along with CV values are computed and presented.

It may be observed that for the character Yield/tree, except for 2017-18, all the treatments are *on par* to each other (Table 2), as the respective p-value exceeded 0.05. However, for the trait average fruit weight, all the individual year based classical ANOVA resulted in *on par* results (Table 3),as the respective p-value exceeded 0.05. Further, for the trait number of fruits, except for 2017-18, all the treatments are on par to each other (Table 4), as the respective p-value exceeded 0.05. However, in most of the analysis, the value of coefficient of variation exceeded 20%, a cut of value desired for any field based experimental study.

Since, there is significance of results among the treatments in some of the years of experiment, before going for the pooled analysis, the data is tested for homogeneity of error variance using Bartlett's Chi-square test. In our study the preliminary results showed heterogeneity in error variance, hence transformed the original values using logarithmic transformation and the proceeded for pooled ANOVA. Perusal of the results presented in Table 1 justified for proceeding to pooled analysis of variance as the computed x^2 values, for the all the three traits supported for the presence of heterogeneous error variance.

Accordingly, the results of pooled ANOVA for all the three traits are presented in the last row of first two columns of Table 2-4. Similar trends as observed in individual year based analysis was also observed in pooled ANOVA based results, leading to inability for identifying the best rootstock treatment. This may probably due to the presence of outliers in one or two replications across treatments in some of the individual year based analysis. Outliers are values that are unusually far from the main concentration of data points. These extreme observations can skew and mislead the statistical analysis, leading to inaccurate conclusions if not properly addressed or accounted for. Accordingly, robust ANOVA method was employed and the results are presented in Table 2-4.

Perusal of the results of robust ANOVA for the trait yield / tree revealed a significant difference among all the treatments tested during all the years and also for the pooled data, since the probability value being less than 0.05. There is a considerable reduction in the value of coefficient of variation in most of the cases to lesser than 20%, with the pooled data resulting in CV value as 14.16%. The precision gained (as computed as the reduction in CV due robust ANOVA over classical ANOVA) due to the robust ANOVA approach for individual year based and pooled analysis is presented in the penultimate column of Table 2. It was observed that the precision gained by this approach was high as 11.53%. The remain other two traits (Table 3 and 4) with the precision gained being around 60.37 and 34.97% respectively. A DMRT based post-hoc test was adopted to suggest the best treatment for all the three traits individually. The results presented in the last column of the respective tables revealed that the Olour rootstock for Totapuri scion (T3) as the best for both the traits, yield / tree and average fruit weight, however the rootstock treatment Turpentine for Totapuri (T8) is the best for number of fruits, and was on par with T3.

Table 1 : Results of Bartlett's test for individual traits

Character	χ^2 Cal
Yield / tree	2.77**
Average fruit weight	8.61**
Number of fruits	4.23**

** significance at p<0.05

Table 2 : Comparison of regular ANOVA and Robust ANOVA methods for yield/tree

Year	Classical Pooled ANOVA			Robust Pooled ANOVA			Reduction in CV (%) precision gained	Best treatment (as per robust pooled)
	P-value Treatment	P-value Treat vs. Year	CV (%)	P-value Treatment	P-value Treat vs. Year	CV (%)		
2011	0.77	NA	34.59	0.77	NA	34.60	11.54	-
2012	0.04	NA	16.79	0.03	NA	16.75	8.22	-
2013	0.40	NA	14.06	0.34	NA	14.09	7.47	-
2014	0.57	NA	10.51	0.57	NA	10.46	0.46	-
2015	0.41	NA	11.21	0.408	NA	11.23	10.71	-
2016	0.43	NA	8.65	0.432	NA	8.71	7.75	-
2017	0.006	NA	9.58	0.0008	NA	10.57	7.09	-
Pooled	0.001	0.682	44.70	0.001	0.68	14.160	68.32	T_3

Table 3 : Comparison of regular ANOVA and Robust ANOVA methods for average fruit weight

Year	Classical Pooled ANOVA			Robust Pooled ANOVA			Reduction in CV (%) precision gained	Best treatment (as per robust pooled)
	P-value Treatment	P-value Treat vs. Year	CV (%)	P-value Treatment	P-value Treat vs. Year	CV (%)		
2011	0.22	NA	2.82	0.218	NA	2.75	2.54	-
2012	0.36	NA	1.26	0.364	NA	1.49	13.34	-
2013	0.123	NA	1.24	0.126	NA	0.49	60.37	-
2014	0.14	NA	1.30	0.139	NA	1.32	21.75	-
2015	0.17	NA	1.26	0.174	NA	1.12	11.22	-
2016	0.81	NA	1.26	0.808	NA	1.40	4.23	-
2017	0.53	NA	1.86	0.49	NA	1.15	37.86	-
Pooled	0.178	0.213	1.79	0.18	0.21	1.68	5.81	T_3

Table 4 : Comparison of regular ANOVA and Robust ANOVA methods for number of fruits

Year	Classical Pooled ANOVA			Robust Pooled ANOVA			Reduction in CV (%) Precision gained	Best treatment (as per robust pooled)
	P-value Treatment	P-value Treat vs. Year	CV (%)	P-value Treatment	P-value Treat vs. Year	CV (%)		
2011	0.836	NA	15.52	0.84	NA	25.01	34.97	-
2012	0.026	NA	12.95	0.026	NA	12.81	1.10	-
2013	0.369	NA	9.87	0.34	NA	11.17	10.88	-
2014	0.746	NA	7.00	0.75	NA	7.56	21.96	-
2015	0.279	NA	9.10	0.28	NA	8.58	5.72	-
2016	0.477	NA	6.10	0.43	NA	8.71	1.79	-
2017	0.530	NA	6.58	0.510	NA	11.50	16.38	-
Pooled	0.001	0.613	11.07	0.001	0.613	11.09	0.72	T_8

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Short Communication

Morpho-biochemical characterization of a unique avocado (*Persia americana* Mill.) accession PA-026 (IC0644455)

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ABSTRACT

A unique avocado accession PA-026 (IC0644455) bearing yellow colour fruits was identified and evaluated for morphological and biochemical parameters. The accessions PA-026 characterise as yellow coloured pulp, young shoots and leaf midribs, fruit weight (398.3 g), pulp weight (255.38 g), seed weight (92.35 g) and peel thickness (1.43 mm). The biochemical profiling showed that, it has high carotenoid content (7.17 mg/100 g), total phenols (102.24 mg GAE/100 g), FRAP activity (87.32 AEAC/100 g) and high β-carotene (3.85 µg/g) followed by α-carotene (1.03 µg/g), while, fatty acid profile showed presence of five fatty acids, among which oleic acid (52.11%) and palmitic acid (41.56%) were most dominant. In conclusion, avocado accession PA-026 was found unique with respect to yellow fruit, pulp colour, and high carotenoid content especially β-carotene, which could be used to improve the carotenoids content in avocado through breeding.

INTRODUCTION

The avocado is one of the important emerging fruit crops, widely used in cosmetic and food industries for preparation of different products. It belongs to the family Lauraceae and originated in Mexico and Central America (Dreher & Davenport, 2013). Avocado includes approximately 150 species, among which Guatemalan (*Persea americana* var. *guatemalensis* Williams), Mexican (*Persea americana* var. *drymifolia* Blake), and West Indian (*Persea americana* var. *americana* Mill) (Bergh & Ellstrand, 1986) races are very important from horticultural point of view. It is a good source of bioactive compounds and minerals especially lipids, fibers, carotenoids and potassium (Sinyinda & Gramshaw, 1998). Avocado is commercially grown in more than 80 countries (FAO, 2019). Presently, in India it is grown in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Maharashtra, Sikkim and to some extent in Uttarakhand, Himachal Pradesh and Arunachal Pradesh. The wide variability in fruit size, shape, colour and other pulp traits has been reported (Tripathi et al., 2022). Avocado is gaining popularity among growers and consumers due to the presence of enormous health benefits and high market price. Keeping this in mind, a survey was conducted to identify superior and unique avocado genotypes in hotspots *i.e.* Coorg, Wayand, Salem, Dindigul districts of South India. A unique avocado accession 'PA-026' with dark yellow pulp colour fruits was identified and evaluated for morphological and biochemical parameters.

About 20 years old avocado accession PA-026 was characterise for morphological and biochemical parameters, with three replications at ICAR-Indian Institute of Horticultural Research, Bengaluru, and Central Horticultural Experiment Station, Chettalli during 2019 to 2021. The observations on tree, leaf, fruit and seed traits were recorded as per descriptors of Bioversity International for avocado (IPGRI, 1995). The morphological characters *viz.*, leaf length (cm), leaf width (cm), fruit weight (cm), fruit length (cm), fruit width (cm), seed weight (g), seed length (cm), pulp weight (g) and peel thickness (mm) were recorded systematically.

Biochemical traits such as total antioxidants, total phenols (mg GAE/100g), total carotenoids (mg/100 g), oil content (%), dry matter content (%), total soluble solids (°B) and crude fibre (%) were also recorded. The moisture content was estimated as per Ranganna

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(1986). The ferric-reducing antioxidant potential was studied (Benzie & Strain, 1996), and the absorbance was recorded at 593 nm. The total phenols were estimated according to Singleton & Rossi (1965) with slight modifications and readings were recorded at a wavelength of 700 nm. Total carotenoids were estimated by using spectrophotometric method developed by Lichtenthaler (1987) and observations were recorded at 470 nm in UV/VIS spectrometer. The pulp oil was extracted using a Soxhlet apparatus as per Rangana (1986) with slight modifications, and total crude fibre content was analyzed (Maynard, 1970).

The fatty acid profiling of avocado pulp oil was carried out using Shimadzu GCMS-TQ8040 equipped with an ion trap triple quad mass spectrometer with electron ionization mode 0.1 kV with the scan mass range of 45-500 m/z at a sampling speed of 1666. An auto sampler AOC-20i and a capillary column Rxi-5MS were used. The total fatty acid production was calculated by the sum of all GC peak areas in the chromatogram and expressing individual compounds as relative per cent areas. The compounds were identified by comparing the retention index, which was determined using homologous series of n-alkanes (C5 to C32) as a standard. They compared the spectra using spectral libraries available in Wiley/ NIST-2015.

Carotenoids profiling was carried out by using UPLC method as given by Serino et al. (2009), with slight modifications. The PDA detector was equipped with an Acquity-UPLC BEH-C18 column (1.7 µm, 2.1 × 50 mm) and a BEH-C18 (1.7 µm, 2.1 ×5 mm) guard column was used. The individual carotenoids were identified on the basis of their diode array spectral characteristics, retention time and relative elution order in comparison to those of the standards and quantified as β-carotene equivalent. The fifteen fruits and leaves were used for recording morphological characters and three replications were used for all biochemical analysis.

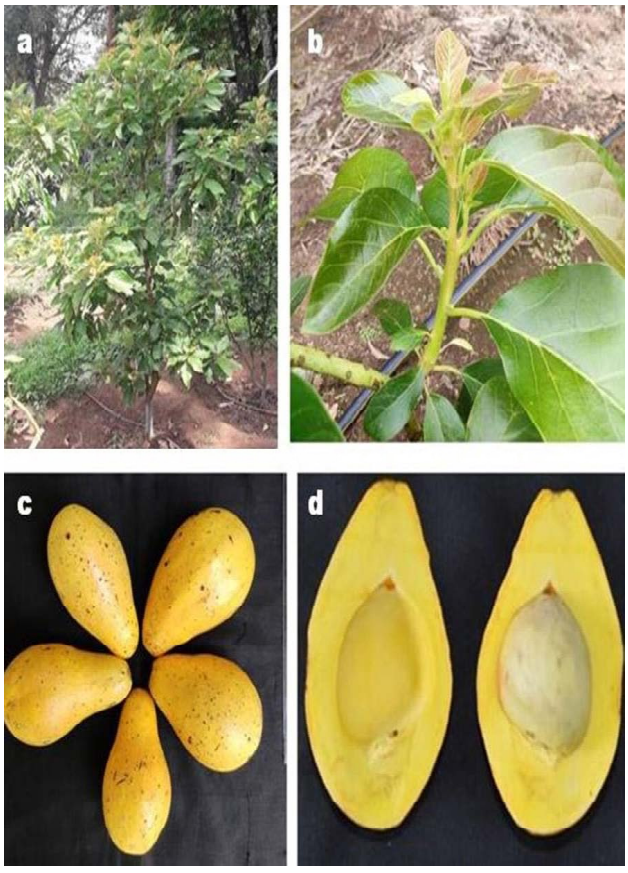
The morphological characters such as leaf, fruit, pulp, seed and pulp quality of accession PA-026 was studied using Bioversity International descriptors (Table 1). The plant showed a rough trunk surface, roundish leaf shape, acute and obtuse leaf base. These leaf traits are not distinct from regular once, and similar leaf traits were also reported (Ranjitha et al., 2021) in different accessions of avocado.

Table 1 : Characterization of avocado accession PA-026 (IC0644455) as per Bioversity International descriptors

Trait	Descriptor
Trunk surface	Rough
Leaf shape	Roundish
Leaf apex shape	Acute
Leaf base shape	Obtuse
Fruit shape	Narrowly obovate
Fruit size uniformity	Intermediate
Fruit base shape	Inflated
Fruit apex shape	Slightly depressed
Fruit apex position	Slightly depressed
Ridges on fruit	Partial
Gloss of fruit peel	Strong
Fruit peel surface	Smooth
Fruit peel colour	Yellow
Pedicel position of fruit	Asymmetrical
Pedicel shape	Cylindrical
Adherence of peel to pulp	Slight
Pulp texture	Buttery
Sweetness of pulp	Intermediate
Bitterness of pulp	Low
Nut taste of pulp	Intermediate
Fibre in pulp	Intermediate
General taste of pulp	Fair
Seed shape	Broadly ovate
Seed position in fruit	Apical
Free space of the seed cavity	Space on seed apex
Cotyledon surface	Intermediate
Seed coat	Seed not free, coat not attached to the pulp

The fruit shape was narrowly obovate, intermediate size, uniform, fruit base shape inflated with slightly depressed fruit apex, firm glossy peel with partial ridges and smooth peel surface. The occurrence of obviate, rhomboid, pyriform and ellipsoid fruit shapes indicate the existence of the West Indian race (Abraham et al., 2018). The most interesting characteristic of this accession was immature yellow colour fruit peel (Muralidhara et al., 2023) which was not reported earlier (Fig. 1). The pedicel position was asymmetrical with cylindrical shape. The dominance of the conical shape of pedicel was observed in

Indonesian and Tanzanian accessions compared to cylindrical shape (Abraham et al., 2018; Juma et al., 2020).



a. Plant, b. Yellow coloured twigs and midribs of leaves, c. Fruits, d. cut fruit

Fig. 1 : Characteritic features of acovado accessions PA-026

The pulp of PA-026 was buttery in texture, low bitterness, intermediate nutty taste and fair pulp quality. The seed shape was broadly ovate and placed at apical portion of the fruit, however, broadly ovate shapes were more dominant to Indonesian and Tanzanian types (Abraham et al., 2018; Juma et al., 2020). The seed coat is not free and not attached to the pulp with intermediate cotyledon surface.

On the perusal of data presented in Table 2 showed that PA-026 recorded medium sized leaves with medium fruit weight (398.3 g), pulp (>60%) with peel (12.6%). Oliveira et al. (2013) reported wide variation in pulp weight in Brazilian accessions. The accession had medium thick peel (1.43 mm), thereby enhances the shelf life of fruit and provides tolerance to anthracnose and scab infections.

Table 2 : Morphological and biochemical characteristics of avocado accession PA-026 (IC0644455)

Trait	Mean
Leaf length (cm)	11.9
Leaf width (cm)	7.13
Pedicel diameter (cm)	0.9
Fruit weight (g)	398.3
Fruit length (cm)	13.2
Fruit width (cm)	7.9
Pulp weight (g)	255.38
Pulp per cent (%)	64.25
Peel weight (g)	50.6
Peel per cent (%)	12.6
Peel thickness (mm)	1.43
Seed weight (g)	92.35
Seed per cent (%)	23.15
Seed length (cm)	6.33
Seed diameter (cm)	5.23
Length of seed cavity (cm)	7.03
Width of seed cavity	5.3
Average yield (kg/tree)	99.83
CUPRAC activity (µmol Trolox/100 g)	0.25
FRAP activity mg (AEAC/100 g)	87.32
DPPH activity mg (AEAC/100 g)	53.83
Total phenols mg (GAE/100 g)	102.24
Total carotenoids (mg/100 g)	7.17
TSS (^{o}Brix)	8.2
Moisture content (%)	81.54
Dry matter content (%)	18.47
Oil content in dry pulp (%)	44.5
Oil content in fresh pulp (%)	8.23
Crude fiber (%)	12.5

In the biochemical characterization, accession PA-026 showed good amount of FRAP activity (87.32 AEAC/ 100 g), total phenols content (102.24 GAE/100 g), total carotenoids (7.17 mg/100 g) and crude fibre (12.5%) in fresh pulp (Table 2). The lower FRAP activity was observed by Wang et al. (2012) in the avocado pulp (2.93 µmol Fe^{2}+ /g FW). The high carotenoids content is mainly due to the presence of dark yellow pulp colour.

The oil content in fresh pulp was medium (8.23%), which was lower than the Hass (15.4 g/100) (USDA, 2011) and Fuerte (12.55%) varieties (Rodriguez-Carpena et al., 2011). The avocado oil is highly digestible and contains mainly unsaturated fatty acids, primarily oleic acid (Gomez-Lopez, 1999). The fatty acid profiling of yellow avocado accession showed five major fatty acids (Table 3). The accession showed high content of oleic acid (52.11%) followed by palmitic acid (41.56%) and palmetoleic acid (4.32%). Galvo et al. (2014) reported 60.79% monounsaturated fatty acids (MUFAs) content and MUFA/SFA ratio (1.31) in var. Fortuna.

Avocado is good source of carotenoids which are fat-soluble in nature (Cortes-Herrera et al., 2019), and profiling of carotenoids showed nine compounds *i.e.* lutein, neoxanthin, violoxanthin, α-carotene, β-carotene, all trans lutein 5,6 epoxide, neochrome, chrysanthemaxan and an unidentified compound (Table 4). Lu et al. (2009) reported lutein (6.02 µg/g) as major compound in Hass variety pulp and Jacobo-Velazquez & Hernandez-Brenes (2012) identified lutein (4.02 µg/g) as major compound in avocado paste.

In this study, accession PA-026 showed maximum values for β-carotene (3.85 µg/g) followed by α-carotene (1.03 µg/g), all trans lutein 5, 6 epoxide (0.61 µg/g) and neoxanthin (0.59 µg/g). In conclusion, the avocado accession PA-026 showed unique traits such as yellow pulp colour, high carotenoid content and β-carotene, which can be exploited in avocado improvement.

Table 3 : Fatty acid profile of avocado accession PA-026 (IC0644455)

Fatty acids	Content (%)
Palmitic acid (C16:0)	41.56
Stearic acid (C18:0)	1.56
Palmetoleic acid (C16:1)	4.32
Oleic acid (C18:1)	52.11
Linoleic acid (C18:2)	0.47
Linolenic acid (C18:3)	0
SFA	43.11
MUFA	56.43
PUFA	0.47
UFA	56.9
MUFA/SFA	1.31
PUFA/SFA	0.01
UFA/SFA	1.33

SFA-saturated fatty acids, MUFA-mono unsaturated fatty acids, PUFA-poly unsaturated fatty acids, UFA-unsaturated fatty acids

Table 4 : Carotenoids profile of avocado accession PA-026 (IC0644455)

Carotenoids	R. Time (min)	λ_{max} (nm)	Quantity (µg/g)
Lutein (µg/g)	0.74	420, 440, 472	0.42
Neoxanthin (µg/g)	0.94	398, 420, 448	0.59
Violoxanthin (µg/g)	1.24-1.33	400, 424, 452	0.53
α-carotene (µg/g)	1.55	415, 438, 467	1.03
β-carotene (µg/g)	1.71	428, 449, 474	3.85
All trans lutein 5,6 epoxide (µg/g)	2.72	415, 440, 469	0.61
X (µg/g)	3.23-3.5	420, 443, 469	0.55
Neochrome (µg/g)	4.38	399, 421, 446	0.44
Chrysanthemaxanthin (µg/g)	5.21	415, 439, 446	0.15

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Short Communication

Growing sweet potatoes [*Ipomoea batatas* (L.) Lam.)] for their greens and the impact on storage roots

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ABSTRACT

Sweet potato greens are an underused but highly nutritious vegetable that grows well in urban environments and could help alleviate food insecurity and related health problems. Therefore, trials were conducted in field rows and a green roof with seven varieties of sweet potatoes to determine whether 1) they differed in their production of greens and 2) harvesting greens influenced yield or nutrients of storage roots. There was no difference in the mass of sweet potatoes greens harvested among the varieties in either production system. Harvesting greens severely reduced the harvested mass of storage roots, although it increased the content of eight minerals in storage roots, including boron, calcium, copper, iron, phosphorous, potassium, sulfur, and zinc. Urban farmers may have to decide whether harvesting greens or storage roots are their primary objective if harvesting the former limits the latter. Future research should explore the timing of harvesting greens and the amount taken to see if different methods allow for a high yield of storage roots that are high in nutrients.

Keywords: Extensive green roof, field row, *Ipomoea batatas*, mineral, nutrient, variety trial, yield

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is native to tropical areas of the America, but has become an important staple food crop elsewhere, such as Asia and Africa (Duke, 1983; Reynolds et al., 2015). Sweet potato can be cultivated over a range of climatic conditions, exhibiting tolerance to drought and heat (Laurie et al., 2013), which makes it a resilient crop. The storage roots are good sources of multiple vitamins, minerals, carbohydrates, dietary fiber, phenolic compounds, and antioxidants (Neela and Fanta, 2019) and they can be stored for extended periods, so they can promote nutritious eating and help human populations where malnourishment is a problem (Motsa et al., 2015; Low et al., 2020). Sweet potato greens are also edible and consumed as a vegetable in parts of Asia and Africa, although they are underused worldwide. Similar to other leafy greens, such as spinach, sweet potato greens are highly nutritious and rich in vitamin B, β-carotene, iron, calcium, zinc, protein, and polyphenols (Pace et al., 1985; Yoshimoto et al., 2003; Alam, 2021). Sweet potato greens have received attention in recent years because their environmental tolerance make them an option in areas where fresh food may be scarce and their nutritional content may provide various health benefits, including protection from cancer, liver damage, inflammation, diabetes, and bacterial infection (Nguyen et al., 2021). An extract from sweet potato greens is also a folk remedy for various maladies such as asthma, bug bites, burns, diarrhea, fever, nausea, stomach distress, tumors, and anemia (Osime et al., 2008).

Sweet potato greens are not widely consumed in the United States of America (USA) and its plants are not as commonly grown in some urban areas in the USA because of the relatively low economic value of storage roots compared to high land and operating costs for urban farms. However, the USA is home to large Asian and African populations that may desire sweet potato greens. Marketing storage roots and greens to these populations, and expanding consumption of greens by other racial and ethnic groups, may increase viability of this crop in urban areas. Furthermore, many urban populations are food insecure due to food apartheid. For example, food insecurity impacts 16% of the population in Washington, DC, USA (District of Columbia Office of Planning, 2020). Food insecurity leads to a variety of negative health outcomes, which can lower overall health and limit daily activities

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(Gunderson and Ziliak, 2015). Therefore, crops that are high in nutrients and suitable for cultivation in urban areas may help promote positive health outcomes by reducing food insecurity (Jeffery and Richardson, 2021; Luthria et al., 2021).

As the worldwide human population becomes more urbanized and urban agriculture becomes more commonplace, there is a need for urban crop trials that explore sustainable production methods (Cerozi et al., 2022) and diverse production systems (Richardson & Arlotta, 2021, 2022; Richardson et al., 2022). Within Washington, DC, there is space available at ground level and particularly on flat roofs in poorer areas of the city for urban agriculture to be implemented (Taylor et al., 2021). Therefore, the primary objective was to test whether production of greens differed across seven varieties of sweet potatoes in ground-level field rows and on a green roof in Washington, DC. A secondary objective was to test whether harvesting the greens influenced the production or nutrients of storage roots in field rows.

Systems and sweet potato cultivars

Two cropping systems were used at two locations: (1) the 1858 m^2 green roof at the University of the District of Columbia's (UDC) Van Ness Campus and (2) UDC's 58 ha Firebird Farm (Beltsville, MD, USA). Sweet potatoes grew in these systems from 2017 to 2018 to collect data and adjust methodology but report data solely from 2018 because the variable methods prevent comparisons across years. Slips of seven varieties of sweet potatoes *viz.*, Beauregard, Bunch Porto Rico, Georgia Jets, Ginseng, Hernandez, O'Henry, and White Hamon (Southern Exposure Seed Exchange, Mineral, VA, USA).

UDC's green roof planter boxes (hereafter 'Green Roof Planters')

Twenty-eight planter boxes that each had a surface area of 0.9 m^2 and depth of 46 cm were used. Green roof planters were positioned around the roof's periphery and filled to a depth of approximately 30 cm with rooflite® semi-intensive green roof media (Skyland USA, Landenberg, PA, USA), which had an average pH of 7.4, soil organic matter (loss on ignition %) of 33%, calcium of 581 mg/kg, magnesium of 48 mg/kg, and potassium of 41 mg/kg. The boxes were only partially filled to prevent exceeding the weight-bearing limit of the roof. A total of four sets of seven boxes were along three edges of the roof: two on the south, one on the west, and one on the north side. The seven varieties of potatoes were randomly assigned to the seven boxes in each set, with each box containing three slips of the same variety planted 61 cm apart. This created a randomized complete block design with each block being a set of seven boxes and each box being a replicate (n = 4). Drip irrigation was used as needed to supplement rainwater. Plants were fertilized once due to a suspected iron deficiency with 1 teaspoon of DTPA iron chelate (CropKing, Inc, Lodi, OH, USA) mixed into 3.8 L water. Backpack sprayer was used to apply the fertilizer evenly across leaves and soil within all planter boxes. No other fertilizers or amendments were added.

Firebird farm field rows (hereafter 'field rows')

Five slips of each sweet potato variety was planted in each of two tilled field rows using a completely randomized design, with each slip being a replicate (n = 5). The slips were spaced 30.5 cm apart and watered with a manually operated drip tape system as needed. One of these rows was designated as the "clipped" row because vines and leaves were harvested, whereas the other was the "control" row because no vines and leaves were harvested. The loam soil had an average pH of 6.9, soil organic matter (loss on ignition %) of 12%, calcium of 2,766 mg/kg, magnesium of 152 mg/kg, and potassium of 174 mg/kg. No fertilizers or amendments were added.

Plant productivity and minerals

Sweet potato greens were harvested three times from all plants in green roof planters (17-18 August, 21-22 September, 23 October) and the clipped field row (10-12 August, 15-22 September, 16-22 October). Greens were not harvested from the control field row in order to ascertain differences in production and mineral content of storage roots when greens were clipped versus unclipped. Leaves were harvested by cutting all vines from a plant at 61 cm from the base and removing all leaves (including petioles) from detached vines. Marketable leaves were weighed separately from non-marketable leaves. Marketable leaves had feeding damage, discoloration, and disease on one quarter of the leaf or less. The green roof had multiple plants per replicate, some of which died, so we divided the total mass from all harvested leaves by the number of plants and harvests to calculate mass on a per-plant, per-harvest basis.

Storage roots were harvested in field rows the week of 22 October by hand with shovels and weighed marketable storage roots (*i.e.* free from rot) separately from non-marketable storage roots. From this harvest, samples of 'Ginseng' and 'Hernandez' were collected in the control and clipped field rows for analysis of mineral nutrients. These two varieties were selected because they produced enough storage roots of different sizes in both field rows to allow for adequate replication. For each variety and field row, one medium and one large storage root were analyzed from each of three replicates for a total of six samples per variety and field row. Storage roots were rinsed to remove debris and then sliced, freeze-dried, ground, and stored them at -80°C in sealed cryotubes. Samples were shipped on ice to New Age Laboratories (South Haven, MI, USA) where content of 11 mineral nutrients, including boron, calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulfur, and zinc, was determined by inductively coupled plasma optimal emission spectrometry (AOAC International 2012). Results are presented on a dry matter basis.

Statistical analyses

The differences were analysed in mass of greens across varieties within a cropping system with separate general linear models (PROC GLM; SAS Institute 2020). Differences in the mass of storage roots across treatments (*i.e.* control versus clipped) in field rows were analyzed with a general linear model. Square-root transformation was used on the data to meet assumptions of normality prior to analysis. Means for non-transformed data are presented in the results. Differences in minerals in storage roots across treatments were also analyzed with separate general linear models. Since it was unable to collect a third sample of large storage roots for 'Hernandez' in the control row, we included storage root size as a variable in the general linear models. However, the content of minerals in storage roots was not influenced by their size (all p values > 0.12), so lacking one replicate of large storage roots would not alter the overall results. The Tukey–Kramer means separation test was used for all analyses to determine which means differed ($p < 0.05$).

There was no difference in the mass of sweet potatoes greens among varieties in the green roof planters ($F = 0.68$, $DF = 6$, $p = 0.67$) or the field rows ($F = 1.41$, $DF = 6$, $p = 0.25$). The mean yield harvested from each plant during each harvest was 70.7 ± 3.7 g and 299 ± 17.6 g in green roof planters and field rows, respectively. If the primary purpose is to grow sweet potato plants to harvest greens, then other characteristics of the plant, such as habit, taste, and nutrient content, may dictate which variety to select rather than yield. However, there is some evidence that other varieties that we largely did not use in our trial could differ in their yield of leaves (Anabire, 2021), so future research investigating more varieties in common urban and rural production systems is needed.

Varieties differed in the mass of storage roots they produced (Table 1). The variety Bunch Porto Rico recorded highest production of storage roots followed by Georgia Jets, Hernandez, and O'Henry in the control field row, yielded approximately 3 to 5.6 times more mass of storage roots than 'White Hamon,' which was the lowest producer. The average mass of storage roots across all varieties in the control row was nearly 4.8 times more than when greens were clipped (Table 1), indicating that harvesting greens severely reduced the production of storage roots. Previous research found that harvesting sweet potato leaves mostly did not reduce production of storage roots (Anabire, 2021). The incongruence between present results is likely due to the harvesting method. Vines were harvested in addition to leaves and removed most of the aboveground biomass each time harvested, whereas, Anabire (2021) removed only a small portion of the young, aerial leaves. The immature leaves are the most frequently consumed, but all leaves and stems are edible and can be prepared in multiple ways. Harvesting leaves repeatedly from sweet potato plants has been shown to decrease the nutrient content of leaves in the second and third harvests (Pace et al., 1988). The present results taken together with these other studies suggest that a grower needs to consider their priorities before developing a harvesting strategy for sweet potato greens and storage roots. Maximizing yield of storage roots may require that only a small portion of leaves be removed once, or a larger quantity removed late in the development of storage roots. This harvesting strategy may also maximize nutrient content of leaves (Pace et al., 1988; Suárez et al., 2020), although leaf quality declines very late in the season, so nutrient content might, too. Alternatively, maximizing yield of leaves and harvesting efficiency may require repeated harvests of most of the aboveground biomass.

Table 1 : Mean mass (g) of sweet potato storage roots across seven varieties and two treatments

Variety	Treatment	
	Clipped	Control
Beauregard	137.3[a]	868.5[bc]
Bunch Porto Rico	352.0[a]	1130.2[ab]
Georgia Jets	332.5[a]	2004.4[a]
Ginseng	294.5[a]	748.5[bc]
Hernandez	220.9[a]	1254.6[ab]
O'Henry	287.2[a]	1377.3[ab]
White Hamon	1.7 b	356.9[c]
Treatment mean	232.3	1105.8

clipped = vines and leaves were harvested; control = vines and leaves were not harvested.

Means with different letters within a column are different (Tukey–Kramer means separation test, $p < 0.05$). Treatment means are also different ($F = 160.6$, $df = 1$, $p < 0.01$).

The amount of eight minerals in storage roots differed across treatments, with higher levels of boron, calcium, copper, iron, phosphorous, potassium, sulfur, and zinc in plants where greens had been clipped (Table 2). The difference in calcium was especially large, with the amount of calcium in storage roots almost double when greens were clipped than in storage roots from the control field row. The mechanism that resulted in higher amounts of some minerals in storage roots when greens were clipped is unknown. Perhaps since storage roots are reserves of carbohydrates, minerals, and vitamins that enable plant growth, the higher minerals in storage roots when greens were harvested could be due to the fact that there were times the plants had very little aboveground biomass to which to direct these reserves of energy. Increases in nutrients in storage roots are a positive development of removing greens, but a much lower yield of storage roots is a negative tradeoff.

Table 2 : Mean of minerals mg/kg that differed in storage roots of sweet potatoes across two treatments

Mineral	Treatment		*p*
	Clipped	Control	
Boron	9.7[a]	7.4[b]	<0.01
Calcium	3886[a]	1862[b]	<0.01
Copper	9.6[a]	5.9[ab]	<0.01
Iron	82.0[a]	42.0[b]	<0.01
Magnesium	1052	954	0.28
Manganese	44.7	30.2	0.05
Phosphorous	2529[a]	1984[b]	<0.01
Potassium	18,298[a]	16,417[b]	0.02
Sodium	488	518	0.85
Sulfur	1122[a]	857[b]	<0.01
Zinc	10.4[a]	6.5[b]	<0.01

clipped = vines and leaves were harvested; control = vines and leaves were not harvested.

Means with different letters within a row are different (Tukey–Kramer means separation test, $p < 0.05$).

The sweet potato varieties did not influence the yield of greens. Also, harvesting greens severely reduced the harvested mass of storage roots, but increased the content of eight minerals in storage roots. Urban farmers may have to decide whether harvesting greens or storage roots is their primary objective if harvesting the former limits the latter. Future research should explore the timing of harvesting greens and the amount taken to see if different methods allow for a good harvest of storage roots that are high in nutrients and also elucidate the mechanism that results in increased mineral concentration in storage roots when greens are harvested. Overall, sweet potato greens are an underused vegetable that grows well in urban areas and are highly nutritious, so there is a need for more trials in urban environments that maximizes its production, economic value, and nutritional content.

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Short Communication

Studies on crossability in cashew (*Anacardium occidentale* L.) genotypes

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ABSTRACT

Successful hybridization primarily depends on the crossability of the parents involved as well as development of the F_1 hybrids and their derivatives. In the present study, 12 crosses were attempted to study the crossability relationship among cashew genotypes. Among the crosses, the highest crossability was recorded in the cross H-303 x VTH 711/4 (17.16%), whereas, high rate of abscission of young fruits was observed in crosses involving NRCC Selection -2 as female parent. The genotype VTH 711/4 has shown substantially high per cent of crossability and better nut set with different genotypes of cashew.

Keywords: Cashew, crossability, hybridization, genotypes

The cashew (*Anacardium occidentale* L.), is an economically important fruit crop which has been witnessing steady internal demand in addition to its export potential in the recent years and hence, it is imperative to increase production and productivity. Its cultivation is now being extended to non-traditional areas like Bastar plateau and Northern hills of Chhattisgarh (Ramteke et al., 2020). Besides production management, the other feasible approaches of achieving this is to intensify crop improvement programmes and development of high yielding varieties needs regular attention as the risk of production is increasing under the scenario of climate change which has association with biotic and abiotic factors (Saroj and Mohana, 2016). Broadening the genetic base of existing germplasm by hybridization and systematic exploitation of heterosis could pave the way for overcoming the problem of low productivity (Masawe, 1994). Bolder cashew kernels fetch premium price in the international markets. Hence, emphasis is being given to develop new improved cashew varieties with bold nut size and high yield potential. Currently, the concept of high density planting using dwarf genotypes with compact canopy is gaining more acceptances in cashew cultivation.

Crossability is thus a pre-requisite for generating genetic variability and also to transfer genes in the form of hybrids. An understanding of crossability relationship among the species had been helpful not only in choosing methods for producing F_1 hybrids, but also useful in tracing phylogenic relationship among the related species. Successful hybridization primarily depends on the intercrossing potential/ crossability of the parents involved as well as development of the hybrid embryos including fertility of the F_1 hybrids and their derivatives (Sethi et al., 2016). Hybrid vigour is best manifested in crosses involving parents with greater genetic diversity. Hybridization work carried out at Vengurla, Bapatla and Vridhachalam also confirms expression of hybrid vigour in cashew (Saroj and Mohana, 2016). Therefore, an attempt was made to develop and identify suitable parents for crossing, and heterotic hybrids with high nut yield potential and semi-tallness suitable for agro-climatic conditions of south Chhattisgarh region.

The experiment was conducted under All India Co-ordinated Research Project on Cashew, S.G. College of Agriculture and Research Station, Indira Gandhi Agricultural University, Jagdalpur, Chhattisgarh during 2020. The basic experimental materials comprised of eight cashew genotypes *viz.*, V-4, VTH 711/4, H-303, CARS-7, VRI-3, VRI-1, CARS-8 and NRCC Selection-2, which were used as parents in hybridization, with an objective to transfer economic traits like bold nut, cluster bearing and compact canopy in popular cultivars/promising genotypes/strains of the region. Paper-roll method of hybridization was used for pollination among the

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Table 1 : The parental lines of cashew used in the study

Parental line	Source	Character
V-4	RFRS, Vengurle	Cluster bearing, medium size nut, high shelling percentage
VTH 711/4	ICAR-DCR, Puttur	Jumbo nuts
CARS-7	SG CARS, Jagdalpur	Cluster bearing, medium size nut, semi spreading canopy, early bearing
VRI-3	RRS, Vridhachalam	Compact canopy, medium size nut, late bearing, responsive to pruning
VRI-1	RRS, Vridhachalam	Compact canopy, medium size nut, early bearing, responsive to pruning
H-303	RFRS, Vengurle	Cluster bearing, medium size nut, high shelling percentage, compact canopy
CARS-8	SG CARS, Jagdalpur	Jumbo nuts, good shelling percentage
NRCC Sel. 2	ICAR-DCR, Puttur	Medium size nut, high shelling percentage, compact canopy, responsive to pruning

desired parent as suggested by Bhat et al. (1998). The observations on crosses attempted are successful crosses, nuts produced, crossability and other related parameters were recorded as suggested by Basavraja et al. (2018). Intervarietal hybridization was attempted through direct crosses to study the extent of crossing, pollen fertility, seed viability, hybrid lethality and hybrid breakdown per cent in hybrids. The analysis of variance was performed considering mean values to compare the results. To test whether the mean difference of crossability, simple t-test was performed. The data were analyzed using OPSTAT software (Sheron et al., 1998). The details of parents used are given in Table 1.

Total twelve cross combinations were made utilizing eight genotypes from different research stations and desirable crosses for characters like bold nut, cluster bearing and compact canopy were obtained. Before attempting crosses, the emasculated flower twigs were sprayed with plain water followed by spray of 2-3 per cent of glucose water to retain maximum flower and then pollinated with the desired male flowers.

The highest emasculated flower drop was recorded in cross VRI-1 x VTH 711/4 (57.77 %) followed by NRCC Sel. 2 x V-4 (56.01%)) and VRI-3 x V-4 (46.29%). The highest per cent of successful crosses were obtained in NRCC Sel. 2 x V-4 (61.11%) followed by NRCC Sel. 2 x CARS-8 (51.61%), VRI-3 x V-4 (48.27%), V-4 x H-303 (44.23%) and CARS-8 x H-303 (44.00%), whereas, the lowest successful crosses were recorded in V-4 x VTH 711/4 (25.42%), VRI-1 x V-4 (29.52%) and VTH 711/4 x H-303 (31.14%) (Table 2).

In the present study, the highest crossability was recorded in the crosses H-303 x VTH 711/4 (17.16%), VRI-1 x VTH 711/4 (10.52 %), VRI-3 x V-4 (10.34 %) and CARS-7 x VTH 711/4 (9.61%), which, were considered as successful cross combination as well as diverse compatible and adapted parents or combiners (Table 3). This suggests that VTH 711/4 may be considered as ideal parent for transfer of useful genes for the character like bold nut in genotypes to broaden the genetic base of intervarietal hybrids. The similar results were also reported by Sethi et al. (2016).

Moderate crossability was recorded in V-4 x H-303 (7.69 %), V-4 x VTH 711/4 (7.14%) and VTH 711/4 x H-303 (6.55%), whereas, low crossability was observed in NRCC Sel. 2 x CARS-8 (3.22%), CARS-8 x H-303 (5.13%), NRCC Sel. 2 x V-4 (5.55%), VRI-1 x V-4 (5.71%) and H-303 x CARS-8 (6.12%), which may imply that parents of these cross combinations might be originated from diverse secondary gene pool. These cultivars might have cross compatibility problems.

The high rate of abscission of young fruits between 3 to 30 days after pollination and low nut set was observed in crosses of NRCC Sel. 2 x V-4, NRCC Sel. 2 x CARS-8, VRI-3 x V-4 are suggestive for the presence of some genetic and compatibility barriers.

Table 2 : Number of crosses attempted, successful crosses, nut produced and other related parameters in cashew

Cross combination	Emasculated flowers (Nos.)	Emasculated flowers drop (Nos.)	Emasculated flower drop (%)	Crosses attempted (Nos.)	Successful crosses (Nos.)	Successful crosses (%)	Nut set at pea stage	Nuts obtained (Nos.)
V-4 x VTH 711/4	81	25	30.86	56	15	25.42	7	4
VTH 711/4 x H-303	89	28	31.46	61	19	31.14	6	4
H-303 x CARS-8	63	14	22.22	49	17	34.69	5	3
CARS-8 x H-303	72	22	30.55	50	22	44.00	7	4
VRI-1 x VTH 711/4	45	26	57.77	19	8	42.20	5	2
CARS-7 x VTH 711/4	90	38	42.22	52	20	38.46	9	5
H-303 x VTH 711/4	178	44	24.72	134	57	42.53	38	23
NRCC Sel. 2 x V-4	41	23	56.01	18	11	61.11	8	1
V-4 x H-303	70	18	25.71	52	23	44.23	10	4
NRCC Sel. 2 x CARS-8	49	18	36.74	31	16	51.61	6	1
VRI-3 x V-4	54	25	46.29	29	14	48.27	9	3
VRI-1 x V-4	141	36	25.53	105	31	29.52	11	6
Mean	81.08	26.42	35.84	54.66	21.08	41.10	10.08	5.00
SE ±	11.28	2.45	3.36	9.15	3.54	2.78	2.48	1.62
SD	39.08	8.51	11.65	32.63	12.25	9.62	8.61	5.61
CV (%)	48.19	9.31	9.37	59.69	58.11	92.58	85.42	112.20
Skewness	1.33	0.63	0.72	1.21	1.96	0.27	2.77	2.66
Kurtosis	0.83	-0.52	-0.81	0.69	3.35	-0.42	6.21	5.87

Table 3 : Crossability, germination and hybrid lethality in crosses of cashew

Cross combination	Crossability (%)	Seed sown (Nos.)	Seeds germinated (Nos.)	Germination (%)	Plants died (Nos.)	Hybrid lethality (%)
V-4 x VTH 711/4	7.14	4	3	75.00	0	0
VTH 711/4 x H-303	6.55	4	4	100.00	0	0
H-303 x CARS-8	6.12	3	2	66.66	0	0
CARS-8 x H-303	5.13	4	3	75.00	0	0
VRI-1 x VTH 711/4	10.52	2	2	100.00	0	0
CARS-7 x VTH 711/4	9.61	5	5	100.00	1	20.00
H-303 x VTH 711/4	17.16	23	21	91.03	1	4.76
NRCC Sel. 2 x V-4	5.55	1	1	100.00	0	0
V-4 x H-303	7.69	4	4	100.00	0	0
NRCC Sel. 2 x CARS-8	3.22	1	1	100.00	0	0
VRI-3 x V-4	10.34	3	3	100.00	0	0
VRI-1 x V-4	5.71	6	5	83.33	0	0
Mean	7.89	5.00	4.50	90.92	0.17	2.06
SE ±	1.01	1.62	1.48	3.46	0.11	1.61
SD	3.50	5.61	5.14	12.01	0.37	5.56
CV (%)	44.36	112.20	114.22	13.20	217.64	266.99
Skewness	1.13	2.66	2.70	0.84	1.79	2.76
Kurtosis	1.47	5.87	5.90	-0.89	1.20	6.02

The failure of endosperm nuclei to divide or the delayed endosperm nuclear divisions is responsible for abortion of embryo and the subsequent abscission of young fruits. The failure of embryo to reach maturity might be the probable cause of the production of shriveled seeds from these crosses. These results are in accordance with the earlier reports (Manoj & George, 1993; Sethi et al., 2019; Eradasappa et al., 2020).

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Short Communication

Differential performance of *Dianella tasmanica and Pleomele reflexa* under coloured shade nets

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ABSTRACT

Cut greens are emerging as an important constituent of the floricultural industry as they add freshness and colour to floral designs. An experiment was conducted to evaluate the effect of different coloured shade nets (black, blue, green, red, white and no shade) at 50% shade intensity on growth and quality parameters of *Dianella tasmanica* and *Pleomele reflexa.* Both species significantly responded to the change in colour of shade nets. In *D. tasmanica*, maximum plant height (85.66 cm), stem diameter (8.63 mm) and leaf length (68.33 cm) were recorded under red shade net, while, plant spread (63.03 cm) and leaf breadth (4.06 cm) under black shade net, however, leaf thickness (0.57 cm) and vase life (24.33 days) under white shade net. In *P. reflexa*, maximum plant height (63.66 cm), stem diameter (9.76 mm), leaf length (17.50 cm), leaf thickness (0.93 cm) and SPAD index (60.10) were recorded under red shade net, whereas, plant spread (36.34 cm) and leaf breadth (3.00 cm) under black shade net, and vase life (25.66 days) under white shade net. Thus, red coloured shade nets could be preferred commercially for better performance of *D. tasmanica* and *P. reflexa*.

Keywords: Coloured shade net, cut stem, *Dianella tasmanica*, *Pleomele reflexa*, vase life

INTRODUCTION

Cultivation of ornamental plants is considered to be the advanced form of agriculture. Cut greens/foliage impart freshness and colour to the floral designs. They are largely used as fillers or accents in bouquet making and flower arrangement and hence, constitute an important component of floricultural industry (Reid & Jiang, 2012). The plants with dark green, green or variegated broad leaves with long shelf life are used in floriculture industry as cut foliages. The production of foliage plants in both national and international market plays a lead role in income generation (Abou El-Ghait et al., 2012). The species *Dianella tasmanica* (Tasmanian Flax-lily) and *Pleomele reflexa* (Song of India) are popular ornamental plants, both in home landscaping and as cut greens. They can be used as a specimen plant, accent, or pruned to create a border and considered as houseplants as they prefer bright, filtered light without direct sun exposure and could tolerate infrequent watering.

Most of the foliage plants are native to tropical and sub-tropical regions and hence their cultivation requires moderate temperature and high humidity. These conditions can be met throughout the year by using shade nets. The shade nets are lightweight knitted polyethylene fabric that protect plants from extreme climatic conditions *viz.*, scorching sun or chilling breezes. The colored shade nets characteristic of changing the spectral quality of radiation, thus allowing manipulation of growth and development of plants. Plants respond to changes in the spectrum of electromagnetic radiation through alterations in morphology and physiological functions that result in adaptation to different environmental conditions. They can protect the plants from hail and excessive radiation, and help in the absorption of various spectral lights, which are good for quality production (Perez et al., 2006). The coloured shade net approach has been evaluated in ornamentals (Nissim-Levi et al., 2008). Keeping this in view, an experiment was conducted to study the effect of coloured shade nets on plant growth and leaf production in commercially important cut foliage species, *D. tasmanica* and *P. reflexa.*

A pot experiment was conducted at the research farm of the Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana during 2017. The 8" pots were filled with potting mixture comprising of soil and well rotten FYM in the ratio

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of 2:1. Six months old plants of *D. tasmanica* and *P. reflexa* were planted under 5 coloured shade nets *viz*., black, blue, green, red and white with 50% shade levels and control (without shade net), in completely randomized design, replicated thrice with five pots in each replication. After establishment of plants, pots were irrigated on alternate days and nitrogen in the form of urea was applied at the rate of 1 g/pot at monthly intervals.

Observations on plant height, plant spread, number of cut stems, chlorophyll content and vase life were recorded after one year during March 2018 The relative leaf chlorophyll content was determined with a portable chlorophyll meter (Minolta SPAD 502) as SPAD index. Vase life was recorded by keeping stems in vases containing distilled water. The vase life studies were conducted under ambient conditions (maximum and minimum temperature 26.8 ^{f}C and 12.5 ^{f}C, respectively and relative humidity 62.15%). Vase life was considered to be terminated when cut stems showed signs of wilting or root initials. The data were statistically analyzed with the help of software statistix 10 programme.

In *D. tasmanica*, plant height and stem diameter were recorded maximum under red shade net (85.66 cm and 8.63 mm, respectively), which was significantly at par other coloured shade nets except for green shade net and control (Table 1). Similar results were observed in *P. reflexa* (Table 2), however, minimum plant height was recorded in the plants grown under open conditions. The increase in plant height under shade nets could be attributed to lower light intensity under shade nets as compared to control that led to better auxin transport causing cell elongation below the apical meristem. The plants under low light intensity have more apical dominant than grown under high light intensity (Godi et al., 2018). Increase in plant height under red shade net may be attributed to the reduced R: FR ratio and deficiency of blue light (Myrthong & Sudhdevi, 2016). Similar results were also obtained by Gaurav et al. (2016a) in *Draceana* and Myrthong and Sudhdevi (2016) in *Nephrolepis.*

Table 1 : Effect of coloured shade nets on different parameters in *Dianella tasmanica*

Shade net	Plant height (cm)	Plant spread (cm)	Stem diameter (mm)	No. of cut stems	Leaf length (cm)	Leaf breadth (cm)	Leaf thickness (mm)	Chlorophyll content (SPAD index)	Vase life (days)
Black	81.00	63.03	8.28	8.00	64.66	4.06	0.56	45.13	22.66
Blue	84.00	59.27	7.70	7.33	65.66	3.60	0.54	31.23	23.66
Green	69.33	51.92	7.16	6.00	43.33	3.20	0.53	43.43	23.00
Red	85.66	54.06	8.63	9.33	68.33	3.60	0.51	33.46	19.00
White	84.66	51.18	7.86	7.33	65.66	3.80	0.57	42.76	24.33
Control	55.00	46.83	7.32	5.33	36.66	2.76	0.49	36.46	24.00
C.D. (P=0.05)	14.94	4.32	NS	NS	6.65	0.31	0.08	NS	3.31

Table 2 : Effect of coloured shade nets on different parameters in *Pleomele reflexa*

Shade net	Plant height (cm)	Plant spread (cm)	Stem diameter (mm)	No. of cut stems	Leaf length (cm)	Leaf breadth (cm)	Leaf thickness (mm)	Chlorophyll content (SPAD index)	Vase life (days)
Black	62.00	36.34	7.36	5.32	18.06	3.00	0.78	41.96	24.33
Blue	61.00	29.45	8.20	4.66	15.50	2.73	0.80	49.06	24.00
Green	58.66	32.33	8.50	7.66	16.66	2.96	0.65	46.23	24.66
Red	63.66	31.51	9.76	4.93	17.50	2.86	0.93	60.10	23.00
White	61.00	30.29	6.80	5.67	15.60	2.90	0.86	45.06	25.66
Control	45.66	27.93	6.56	6.33	14.53	2.63	0.69	38.63	25.00
C.D. (P=0.05)	11.78	6.87	2.03	NS	1.19	0.30	0.13	16.81	1.33

In *D. tasmanica*, plant spread was recorded maximum under black shade net (63.03 cm), which was significantly at par with blue shade net (Table 1). Similar results were recorded for *P. reflexa*, while, minimum values were recorded under open field conditions (Table 2). Myrthong and Sudhdevi (2016) also reported higher plant spread in *Nephrolepis exaltata* and *Asparagus* cultivated under red and black coloured nets. The effect on physiological processes is displayed through morphological parameters *viz.*, plant height, spread etc. (Naveena & Thamaraiselvi, 2020).

In *D. tasmanica*, maximum cut stems (9.33/plant/year) were produced under red coloured shade net, whereas, minimum was produced under open field conditions (5.33/plant/year) (Table 1). Gaurav et al. (2016a) also reported that *Dracaena* plants grown under red shade-nets produced more number of leaves. Naveena et al. (2019b) affirmed that although leaf mass of *Philodendron xanadu* was not influenced under black, blue, grey or red netting but the number of leaves were more under red netting and least under blue netting. In contrast, in *P. reflexa*, the number of cut stem did not vary significantly under different shade nets. The variation in number of cut stems could be explained as a cascade of mechanism between light intensity, photosynthesis and growth hormones which results in varied growth in terms of height, spread or nodes.

Longest leaves (68.33 cm) were produced in *D. tasmanica* under red shade net, which was significantly at par with all other coloured shade nets except for green and open field conditions (Table 1). Similar results were recorded for *P. reflexa* (Table 2). Oren et al. (2003) also reported that the red net markedly enhanced vegetative growth rate and vigour of *Pittosporum variegatum*. In *D. tasmanica,* black coloured shade net recorded maximum leaf breadth (4.06 cm), whereas, it was recorded minimum (2.76 cm) under open field conditions (Table 1). Similar results were recorded for *P. reflexa* (Table 2). All shade nets in both species led to more leaf breadth than open field as shade nets altered the microclimate making it better in terms of reduced temperature, relative humidity, wind speed and light intensity (Medany et al., 2009).

In *D. tasmanica*, leaf thickness was recorded maximum (0.93 mm) in red coloured shade net which was statistically at par with white shade net (Table 1), however, in *P. reflexa*, it was recorded maximum under white shade (Table 2). The utilization of coloured shade nets provide more optimal growth conditions than open field that led to more growth and enhanced vegetative parameters (Zare et al., 2019).

The intensity of leaf colour is closely correlated with the quality of the cut foliage. A close correlation exists between SPAD values and chlorophyll content in plants. *D. tasmanica*, chlorophyll content was found non-significant under different coloured shade nets (Table 1). In *P. reflexa*, it was recorded significantly maximum under red coloured shade nets and minimum under control (Table 2). Gaurav et al. (2016a) also reported that *Dracaena* plants grown under red and white shade-nets exhibited better leaf chlorophyll content. The better performance of plants in terms of SPAD index under shade nets could be accounted to their indirect exposure to sun that led to increased production of chlorophyll to capture the diffuse radiation. This could be an adaptation to produce carbohydrates required for their growth and development.

Vase life was recorded maximum in the cut stems harvested from the plants grown under white coloured shade net (24.33 days), however, it was statistically at par with all the treatments except for red coloured shade net (Table 1). Similar results were recorded in *P. reflexa* (Table 2). The improved vase life of cut foliage grown under white shade nets could be attributed to better protection of leaves from high light intensity. Improved vase life under white shade nets have been reported in fronds of *Nephrolepis cordifolia* (Khyber et al., 2019). Most of the cut stems under all treatments strike roots in the water during their vase life ranging from 19 to 24 days in *D. tasmanica* and 23 to 25 days in *P. reflexa*. The rooting was considered as one of the criteria to terminate vase life. Naveena et al. (2019a) also reported improved vase life of foliage plants (*Asparagu*s and *Nephrolepis*) grown under different shade nets.

After twelve months of planting, lamina colour of all plants in *D. tasmanica*, showed yellow green-group (yellow green group, 147A) except the plants grown under red shade net (green group, 137B (Table 3), however, in *P. reflexa,* showed same group of colour (yellow green group, 147A) except the plants under blue shade nets (green group, 146A).

Table 3 : Effect of coloured shade nets on foliage colour in *Dianella tasmanica* and *Pleomele reflexa*

Shade net	*Dianella tasmanica*		*Dracaena reflexa*	
	Lamina	Margin	Lamina	Margin
Black	Yellow green group, 147A	Yellow group, 4D	Yellow green group, 147A	Yellow group, 3D
Blue	Yellow green group, 147A	Yellow group, 4D	Green group, 146A	Yellow group, 4C
Green	Yellow green group, 147A	Yellow group, 4D	Yellow green group, 147A	Yellow group, 2D
Red	Green group, 137B	Yellow group, 4D	Yellow green group, 146A	Yellow group, 4C
White	Yellow green group, 147A	Yellow group, 4D	Yellow green group, 147A	Yellow group, 3D
Control	Yellow green group, 146A	Yellow group, 1D	Yellow green group, 146A	Yellow group, 4B

The results showed significant variation for different parameters in both the species under varied colour of shade nets. Differential response of different foliage plants varied with the colour of shade net. Gaurav et al. (2016b) also recommended red or white coloured shade nets to replace commercially prevalent green shade nets for cut green production in Cordyline.

Keeping an insight into commercially significant parameters of both cut foliage species, it could be inferred that *Dianella tasmanica* should be grown under red shade net, whereas, green shade net was found to be superior for commercial cultivation of *Pleomele reflexa*.

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Short Communication

Changes in sugars in organs of *Phalaenopsis* florets during different flowering stages of intact plant inflorescences

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ABSTRACT

Phalaenopsis flowers possess extraordinary longevity. However, the changes of sugars, including glucose, fructose and sucrose, in organs of floret during different flowering stages of inflorescences attached to a plant have not been reported. To accomplish this, the sugars level in different floret organs were studied at 4 different stages (1. half open, 2. bloom 1 month, 3. bloom 2 months, and 4. wilting). Glucose and fructose were the major soluble sugars in the sepal, petal, labellum, pedicel, and remainder (including the column, anther cap, pollinia, and stigma) of a floret, but their levels decreased from stages 1 to 4. However, the amount of sucrose increased significantly at stage 4 in the sepal, petal, pedicel, and remainder, with the exception that the labellum remained constant throughout all stages. These results demonstrate that glucose and fructose are the major solutes that contribute to floret opening and blooming, and sucrose is salvaged and exported before floret senescence for opening other florets on the same inflorescence. Meanwhile, labellum possesses different sugar metabolism from other organs of *Phalaenopsis* floret.

Keywords : Floret, flowering stages, *Phalaenopsis*, sugars

INTRODUCTION

Phalaenopsis flowers are popular worldwide as they feature a variety of shapes, sizes, colors, and have a long life, reaching up to 3 months (Halevy et al., 1996). Flowers require energy and turgor pressure for opening and blooming. Sugars are the primary energy source in plants (Gibson, 2004). Sugars also act as osmoticum to reduce water potential and provide turgor pressure for flower opening and blooming (Shu et al., 2010). To date, few studies have examined the changes in endogenous sugars in the *Phalaenopsis* flower during different flowering stages.

Among the soluble sugars, sucrose is the most frequently used carbon source in cut flowers, such as *Gerbera jamesonil* (Wani et al., 2012), *Dendrobium* (Ratchanee et al., 2013), and *Lilium* (Majidian et al., 2014). In cut *Dendrobium* inflorescences, sucrose feeding had no effect on the sugar concentrations in the tepals of open flowers, whereas it increased the sugar concentrations in the column and labellum (Ratchanee et al., 2013). These results imply that flower organs differ in terms of their sucrose metabolism. Trivellini et al. (2011) indicated that sugar concentrations in *Hibiscus rosa-sinensis* L. flowers, an ephemeral flower that opens and wilts within 1 day, exhibit a multifarious spatiotemporal partition during development and senescence.

The *Phalaenopsis* floret structure consists of a sepal, petal, labellum, column, anther cap, pollinia, stigma, and pedicle, which connects the floret to the flower stalk (O'Neill et al., 1993). Because the column, anther cap, pollinia, and stigma cannot be easily and quickly dissected, they were collected and denominated as the "remainder" in this study. Moreover, the floret phenotype development was divided into the following stages: (1) half open, (2) bloom 1 month, (3) bloom 2 months, and (4) wilting (Fig. 1). This study offers insights as to how the transitional changes of sugars within the various organs of a floret during different flowering stages on the inflorescences of intact plants.

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Fig. 1 : *Phalaenopsis aphrodite* flower developmental stages
Stage 1, half open (A); stage 2, bloom 1 month (B); stage 3, bloom 2 months (C); stage 4, wilting (D). Bar = 1 cm.

Glucose, fructose, and sucrose were the major free soluble sugars in the floral organs of *Phalaenopsis*. The highest glucose content found for the sepal, petal, labellum, remainder, and pedicel were 9.90, 10.82, 10.36, 9.88, and 6.11 mg g^{-1} fresh weight (FW), respectively (Fig. 2A). These high values all appeared at stage 1 and proceeded to exhibit a decreasing trend until stage 4. Similarly, the highest fructose content of the sepal, petal, labellum, remainder, and pedicel (observed at stage 1) were 6.39, 6.93, 5.89, 5.90, and 4.01 mg g^{-1} FW, respectively (Fig. 2B). These too exhibited a decreasing trend until stage 4. Meanwhile, the amount of glucose was always higher than that of fructose in these organs at all four stages.

Conversely, the sucrose content exhibited a decreasing trend until stage 3, then increased in stage 4, at which the highest sucrose contents were 3.45, 3.28, 5.63, and 4.28 mg g^{-1} FW, respectively, in the sepal, petal, remainder, and pedicel (Fig. 2C). However, the sucrose content of the labellum remained constant at each stage. Moreover, at stage 4, sucrose accounted for 68%, 70%, 62%, 46%, and 60% of the total sugar

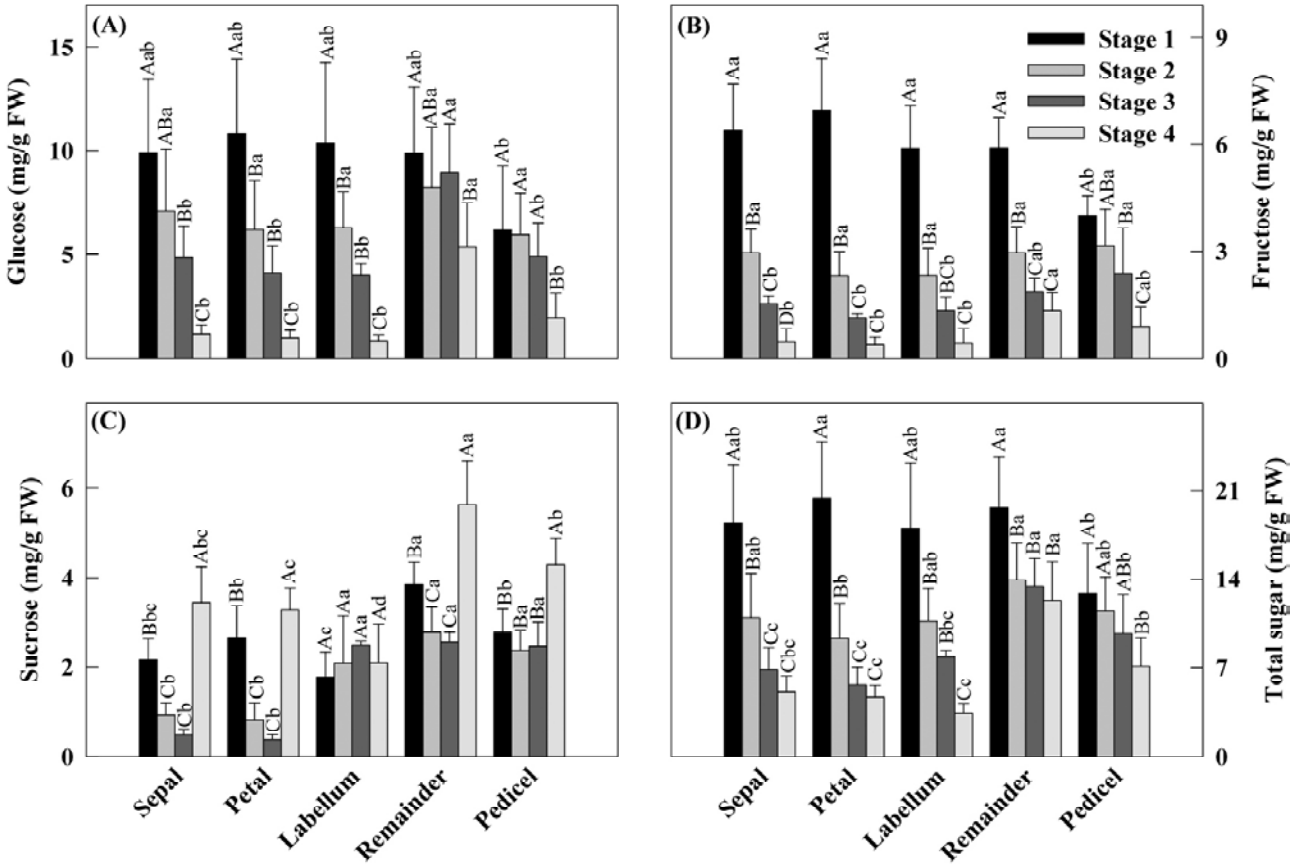

Fig. 2 : Changes in free sugars (mg/g FW) in various organs of Phalaenopsis aphrodite florets during different flowering stages of intact plant inflorescences.

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content (the sum of glucose, fructose, and sucrose) in the sepal, petal, labellum, remainder, and pedicel, respectively. Note that sucrose contributed less than 60% only in the remainder.

Fig. 2D shows the total sugar content in different organs declined gradually from stage 1 to stage 4, however, the decline rates in the sepal, petal, and labellum differed from those in the remainder and pedicel. In particular, compared with the total sugar content at stage 1, those at stages 2, 3, and 4 were 59%, 37%, and 26% in the sepal; 46%, 27%, and 23% in the petal; 59%, 44%, and 19% in the labellum; 71%, 68%, and 63% in the remainder; and 89%, 75%, and 55% in the pedicel, respectively. Thus, the decreasing trends of total sugar in the remainder and pedicel were less significant than those in the other organs.

All values are presented as the means ± SE of six individual florets. The different capital letters show statistically significant differences according to least significant difference (LSD) test ($P \leq 0.05$) between the different stages of the same organ. The different lowercase letters show statistically significant differences according to LSD test ($P \leq 0.05$) between the different organs in the same stage.

Although a dramatic decrease in sugar concentration during flower senescence is a universal phenomenon, various species, including cultivars, undergo different changes. In the senescence flower of *Dianthus*, there is a reducing sugar decline in *D. chinensis*; however, reducing sugars remained almost constant from flower opening to senescence in *D. barbatus* (Dar et al., 2015). In the corolla of *Digitalis purpurea*, the glucose content declines more rapidly than the fructose content, resulting in fructose being the major reducing sugar during senescence; meanwhile, sucrose cannot be detected in the flowers (Stead & Moore, 1977). Conversely, in cut flowers of *Lilium*, nearly identical amounts of fructose and glucose are present within the lily tepals during flower bud development (Majidian et al., 2014). Figure 2 shows the glucose, fructose, and total sugar contents all decreased from stage 1 to stage 4, and the average level of glucose was always higher than that of fructose in various floral organs of *Phalaenopsis*.

In contrast to hexoses, the sucrose content significantly increased 7.3-, 8.9-, 2.6-, and 1.7-fold from stage 3 to stage 4 in the sepal, petal, remainder, and pedicel, respectively. However, the content remained constant in the labellum at all four stages. Moreover, at stage 4, sucrose was the major sugar in all organs, accounting for > 60% of the total sugar content, except in the remainder, wherein accounted only for 46%. Based on these results, labellum and remainder possess discrepant sucrose metabolisms, and the increase in sucrose contents in the sepal and petal may act as a crucial indicator in the floret senescence of *Phalaenopsis*. In fact, sucrose content also significantly increases in the wilting petals of *H. rosa-sinensis* (Trivellini et al., 2011). Bieleski (1995) demonstrated that sucrose synthesis occurs during senescence in the daylily petal, and sucrose is the principal sugar in phloem exudate. In *gladiolus* (Yamane et al., 1993) and *Dendrobium* (Ketsa & Wongs-aree, 1995) inflorescence, the wilting floret remobilizes carbohydrates to younger buds on the same inflorescence. The structure of the *Phalaenopsis* floret is the basis of contact between the sepal, petal, and labellum with the remainder, which directly connects to the pedicel. Therefore, it is reasonable to infer that the sucrose in the sepal and petal during the wilting stage is transported through the remainder into the pedicel to salvage the carbon source before floret senescence, thereby opening other florets on the same inflorescence.

In conclusion, the present study is the first to report the spatiotemporal changes in sugars in various organs of *Phalaenopsis* florets from the half-open to wilting stages of intact plant inflorescence. Glucose and fructose contribute to floret opening; however, sucrose may be transported to the pedicel before floret senescence to salvage for opening the other florets on the same inflorescence. More detailed assessments of the distinct mechanisms of sucrose metabolism on various floral organs would offer better insights into the biochemical aspects used to control the senescence of florets that are attached to *Phalaenopsis* inflorescence.

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Short Communication

Comparison of essential oil content and composition in two German chamomile (*Matricaria chamomilla* L.) genotypes

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ABSTRACT

Two German chamomile genotypes (wild and domestic) were investigated for essential oil content and composition. Wild and domestic chamomile flowers were collected from Noor-Abad and Karaj regions and dried before essential oil extraction using distillation method. Essential oil components were identified by analytical gas-chromatography and mass spectrometry. Results revealed that essential oil efficiency in domestic and wild chamomile was recorded as 0.87 and 0.77%, respectively. Amongst fifteen different components identified in essential oil samples, (E)-â-farnesene, á -bisabolol oxide A and B and chamazulene were found to be the major components with frequency of 12.86, 31.86, 5.24 and 6.16% in domestic species and 8.83, 2.52, 1.81 and 55.606% in wild species. Irrespective of slight reduction in essential oil content and its components, wild German chamomile genotype can be used as valuable source in future domestication programmes.

Keywords: á-bisabolol oxide A, á-bisabolol oxide B, chamomile, chamazulene.

INTRODUCTION

German chamomile (*Matricaria chamomilla* L.) is a medicinal plant belongs to the family Asteraceae. Chamomile flowers are used as medicinal herb, cosmetic agent, herbal tea and aromatherapy ingredient (Omidbaigi, 2000). *M. chamomilla* (Syn: *M. recutita*) is known as German chamomile, European chamomile, wild chamomile or standard chamomile in Iran. *M. chamomilla* is an annual herbaceous plant with branched and erect stems which grows to a height of 10–70 cm (Ghanavati, 2007). The main active ingredients in *Matricaria* species include farnesene, α-bisabolol, chamazulene, flavonoids including apigenin, quercetin, patuletin and luteolin and coumarin (Azizi, 2006). Chamazulene and α-bisabolol have anti-bacterial, anti-inflammatory and anti-spasmolytic properties and are used in gastrointestinal drugs (Anne et al., 2001). In addition, it has been confirmed that chamomile extraction is effective in relaxing the nervous system and reducing paroxysm (Ghanavati, 2007). Chamazulene have anti-inflammatory and antifungal properties and are widely used in cosmetic products (Rahmai et al., 2009).

Unfortunately, in Iran, due to lack of knowledge on genetic resources and genes function in medicinal plants, there are no appropriate breeding programmes. Therefore, identification and characterization of desirable genes in different varieties and species can help researchers to take the appropriate steps to make their results more accessible to other researchers. Taviana (2001) assessed the genetic diversity in 13 German chamomile for flower yield and essential oil properties. D'Andrea (2002) investigated genetic diversity in diploids and tetraploids cultivars based on morphological attributes, flower yield and essential oil components. According to Cirecelav et al. (1993), essential oil components in the different chamomile biotypes were significantly different.

Medicinal plant growth and development is controlled by genetic factors, climate conditions, soil properties, geographical categorization and field management. Each of these factors and also interaction between them can significantly affect essential oil quality and quantity in medicinal plants. Most of the current researches refer to chemical analyses and determination of bioactive properties of wild stands and there are not many studies that can be used for comparison between wild and domestic species. Although, chamomile is one of the most important medicinal plants in global markets, it is not a commercial plant in Iran and also there is not enough information on its wild populations. Therefore, in this

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study, two different German chamomile genotypes (wild and domestic) were compared for essential oil and its composition.

In order to compare two German chamomile genotypes (wild and domestic) in term of essential oil quantity and quality, wild and domestic chamomile flowers were collected in 2020 from natural habitat at Noor-Abad region in Fars province and a commercial field located in Karaj, respectively. The samples were confirmed at Yasooj University Central Herbarium.

To determine essential oil content, flower samples (100 g) were dried under shade, powdered using electric blender, replicated thrice during each harvest, was used to extract essential oil using Clevenger-type apparatus for 2 h. Finally, the aqueous essential oil was dehydrated by sodium sulfate (Zeinali et al., 2008) to calculate essential oil efficiency (%). Essential oil components were identified by analytical gas-chromatography and mass spectrometry. Obtained spectrums were compared with standard spectrums and the relative percentage of each component was calculated using area under the curve and area normalization method (Kapoor et al., 2008).

The essential oil extract was diluted with acetone and injected into the gas-chromatograph coupled with the mass spectrometer. The compounds were detected and identified by comparing their retention times and indices with those in the mass-spectral library maintained by the National Institute of Standards and Technology (NIST 11.0), Wiley MS data system (Wiley, Chichester, UK), and previous literature. Inhibition indexes were calculated using normal hydrocarbons (9-25 °C) under similar thermal conditions. Further identification was made by matching the mass spectral fragmentation patterns of different compounds with corresponding data (Adams and Wiley 7.0 library) and other published mass spectra (Adams, 2007).

A Thermo-UFM Gas-Chromatograph (Model 9A) with Hp-5 column was used. The column used was 10 m × 0.1 mm with 0.4 mm film thickness. The temperature programme was initial temperature 60ÚC for 3 min; increase to 285ÚC at a rate of 5.8ÚC per min, injector and detector (FID) temperatures were 280ÚC. Helium was used as the carrier gas for GC/FID analysis with the pressure of 3 kg per cm^2. Percentage was calculated by electronic integration of FID peak areas without the use of response factor correlation.

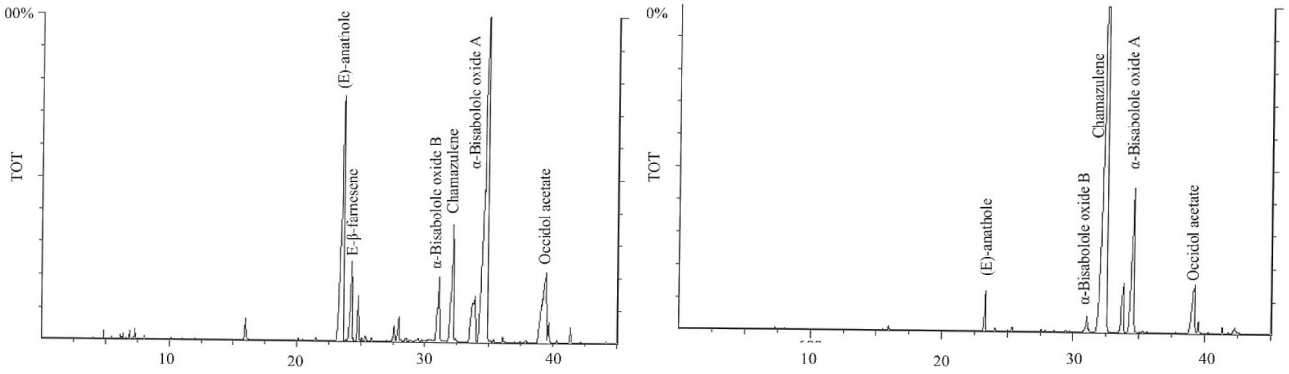
A Varian 3400 GC/MS connected to ion trap Mass Spectrophotometer was used. The used column was DB-5 (30 m × 0.25 mm, film thickness of 0.25 mm). Similar temperature program was used; however final column temperature was 250ÚC. Injector temperature was set at 260ÚC. Helium gas was used as the carrier gas with the flow rate of 31.5 cm per second. Ionization voltage was 70 eV, scanning time was one second, and mass range analyzed was 40-340 amu (Jalali et al., 2008). Identification of the essential oil components was accomplished by comparing their retention times, indices, and mass spectra with authentic standards. Percentage evaluation of the oil components was accomplished by assessing the area normalization.

The results indicated that domestic genotype (0.87%) contain more essential oil than wild genotype (0.77%). Moreover, essential oil constituents in two different German chamomile genotypes were determined using GC-MS. A total of fifteen different compounds were identified in chamomile samples (Table 1). These compounds were commonly found in essential oil extracted from chamomile flower heads with different percentages depending on genotypes. Qualitative and quantitative differences were observed, when a comparison was made between two different German chamomile genotypes (Fig. 1).

Chamomile essential oil properties like other plants is controlled by genetics factors, however, climate conditions and interaction between plant and environment affect essential oil properties (Ebadi et al., 2008). Considering the high essential oil content (0.77%) in wild genotype, wild genotype can be used in future breeding programs although compared to a domestic genotype, the essential oil content was less. It has been reported that essential oil percentage in domestic genotypes like Soroksari (0.9%) was significantly higher than wild genotypes such as Shiraz and Damavand (0.1%) (Omidbaigi, 1999). The results demonstrated that chamazulene content (55.606 %) in wild genotype was significantly higher than domestic genotype which indicates the higher ability of wild genotype in the synthesis of chamazulene. Therefore, wild genotype can be used in domestication programmes as a valuable source of chamazulene. Chamazulene content is affected by growth conditions.

Table 1 : Essential oil compositions in domestic and wild genotype of *Matricaria chamomilla*

Essential oil component	Retention indices	Domestic genotype	Wild genotype
Sabinene	984	0.263	——
á-Terpinene	1044	0.049	——
ñ-Cymene	1049	0.139	——
1,8-Cineole	1068	0.393	0.391
Artemisia ketone	1091	0.117	0.149
(E)- Anethole	1310	20.569	1.323
(E)-ß-Farnesene	1465	12.863	8.839
Germacrene D	1533	1.173	0.729
Bicyclo germacrene	1566	0.577	0.256
(E)-Nerolidol	1650	1.050	——
á-Bisabolol oxide B	1706	5.248	1.818
á-Bisabolone oxide A	1712	1.337	——
Chamazulene	1736	6.166	55.606
á-Bisabolol oxide A	1821	41.862	21.521
Occidol acetate	1955	7.279	7.370
Unknown compounds	——	8.194	1.998
Total identified (%)	——	91.806	98.002
Essential oil content (%)	——	0.87	0.77



Wild genotype Domestic genotype

Fig. 1 : Chromatograms for essential oil composition

α-bisabolol oxide A content in wild genotype (21.52%) was found to be less than domestic genotype (31.86%). Similar results were observed as to α-bisabolol oxide B, 5.24% in domestic genotype compared with 1.81% in wild genotype. E- ß-farnesene is another component found in chamomile essential oil which was found to be higher in domestic genotype compare to wild genotype. Jalali et al. (2008) have found 5 different components in *M. recutita* flower essential oil, the major compounds included α-bisabolen oxide A (63.6%), α-bisabolol oxide A (15.4%) and chamazulene (10.6%).

According to the obtained results, irrespective of slight reduction in essential oil content and some essential oil components, wild German chamomile genotype can be used as valuable source in future domestication programmes. However, further research is needed in the field of medicinal plant biochemistry and breeding to release appropriate cultivars and chemo types.

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