ISSN 0973-354X
eISSN 2582-4899

# JOURNAL OF HORTICULTURAL SCIENCES

Volume 16 December 2021 Issue 2

Society for Promotion of Horticulture
ICAR - Indian Institute of Horticultural Research, Bengaluru - 560 089

# JOURNAL OF HORTICULTURAL SCIENCES

**(Founded in 2005 by the Society for Promotion of Horticulture, Bengaluru, India)**

**Email : chiefeditor.jhs@gmail.com Webpage : https://jhs.iihr.res.in/index.php/jhs**





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**Bharathi** *et al.*, P. 241

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# JOURNAL OF HORTICULTURAL SCIENCES

**Volume 16** **Issue 2** **June 2021**


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# *In this issue…*

The new editorial team that took office in July 2021, expresses the gratitude to Society for Promotion of Horticulture for the faith in the team to carry on the editorial work in the path laid out by committed predecessors.

The rate of growth of Horticulture has been faster during the past decade than ever. This has been possible because of continuous support from research efforts in horticultural sciences. Journal of Horticultural Sciences is proud to be one of the platforms that help in dissemination of knowledge generated in horticultural science.

In recent years, awareness about effect of pollutants has motivated plant lovers to identify plant species that can help in phyto-remediation of indoor air pollutants to address the poor quality of air in cosmopolitan cities. **Shalini Jhanji and Dhatt** have reviewed the issue and suggested the plants that can help in this regard beyond aesthetics value of these plant species.

To achieve the nutritional security, horticultural crops are the main solution. Increasing the yield and quality of fruit crops is essential to achieve this. **Sheikh *et al.*** have standardized the micronutrients requirements for the Assam Lemon to obtain good yield with improved quality. **Sanjit *et al.*** reported that high density planting in banana (5000 plants per ha at 2m x 3m spacing) recorded an increased productivity in a multi-location trial under AICRP (Fruits) programme. In addition to enhanced productivity, it reduced the cost of fertilizer also. To achieve better berry size gibberellic acid is applied externally in grapes. **Satisha *et al.*** recommend that $GA_3$ at 5 ppm might be optimum for bringing about desirable changes in bunch morphology in Crimson Seedless.

Tolerance to salinity is an important criteria for selectia of the rootstocks of mango. Report by **Nimbolkar *et al.*** that seedlings Turpentine, Deorakhio, Olour and Bappakkai responded better in maintaining the mineral nutrient status in leaf and root tissues under higher level of salinity (100 mM of salt solution of NaCl+CaCl at 1:1) will help in addressing the salinity issues in mango cultivation. Similarly, salinity affects rose production. **Varalakshmi *et al.*** report that soils of rose growing areas of Rajasthan and Uttar Pradesh are poor or marginal and reclamation measures are required for cultivation of roses in these areas.

Varietal improvement is a continuous process to have superior lines of vegetable crops. Resistance gene analog polymorphism (RGAP) marker, LaRAGP63 designed by **Kaur *et al.*** could exhibit the polymorphism that co-segregated with yellow mosaic disease reaction phenotypically in the F2 population of ridge gourd. This marker will be helpful in breeding for introgression of resistance loci into the elite lines of ridge gourd. **Nitinkumar *et al.*** studied the genetic divergence in bitter gourd germ plasm based on sixteen parameters and found that the germplasm could be grouped into six clusters. The clusters that are diverse and contain genotypes with good performance can be employed in crop improvement programmes. **Varsha *et al.*** studied the general combining ability in bell pepper among the parents and specific combining ability (SCA) of crosses and identified potential hybrids for better yield. The generation mean analysis of important yield traits by **Swamini Bhoi** in bitter gourd, reveals the presence of duplicate epistasis in the traits viz. node bearing 1st female flower, fruit length, fruit diameter and yield and they observed that additive gene action may be predominant for inheritance of node bearing 1st male flower. In the transcriptome sequencing studies by **Gayathri *et al.*,** out of 10492 SSRs in okra, 34 SSR markers were identified that can be used for marker aided breeding, diversity analysis and gene discovery.

Crop improvement in flower crops is also important to exploit the genetic potential to a greater extent. **Bharathi *et al.*** have identified an advanced breeding line IIHR-4 (IC-0633777) that is highly resistant to root knot nematode and tolerant to *Alternaria polianthi* leaf blight disease under field conditions. Crop improvement in beverage crops needs encouragement to manage the biotic and abiotic stresses. **Divya *et al.*** identified F1 hybrid of coffee (S.5086) with promising performance in terms of crop yield and high field tolerance to leaf rust that has potential for commercial exploitation.

Crop health management with appropriate measures at right time not only prevents yield loss, they help in achieving the yield potential also. **Vijayakumar *et al.*** have come out with a phenophase based irrigation and fertigation schedule for the better vegetative performance of chrysanthemum and flower yield. **Yogalakshmi *et al.*** report the efficacy of *Trichoderma atroviride* isolate TA12 against tomato Fusarium wilt and identified that e 6-pentyl - 2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3-pyridazinyl) and heptadecane were the important metabolites contributing to the efficacy. Seed transmission of bean common mosaic virus-blackeye cowpea mosaic strain (BCMV-BlCM) has been a factor threatening cowpea seed health in Ghana. **Adams *et al.*** report the importance of this factor and they suggest how to devise the management strategies for this disease in Ghana. Root characters of Capsicum are important in abiotic stress tolerance. The container size and type have major effect on the root characteristics. **Raviteja *et al.*** report that bucket type container with dimension of 32 cm height 30 cm diameter with 23 kg soil media capacity was most suitable for phenotyping root characteristics. Besides, they have identified better genotypes of capsicum that possess good root characteristics.

To promote farm mechanization, suitable varieties need to identified. In this director work of **Yella Swami *et al.*** on the physio-morphological and mechanical properties of chillies for mechanical harvesting informs us that traits *viz.* fruit position, plant height, number of fruits per plant, length and diameter at shoulder of fruit and moisture content at the time of ripening are important in designing the harvesters. Attempt of **Jacob and Shobana** to harvest cashew kernels before maturity and treating them with 10% brine solution and 70 °B sugar syrup, resulted in the extension of shelf life of kernels by four more months. The quality of coffee berries is a critical factor in major coffee growing areas of Ethiopia for exporting to European countries. **Chala *et al.*** found that selective harvesting of berries followed by drying on wire mesh resulted in better quality compared to other methods. **Wani *et al.*** have standardized the limonene extraction from different Citrus species and evaluated their anti-microbial properties that may have some application in food or nutraceutical industries.

Readers may notice that this issue of JHS has research articles covering work on plantation or beverage crops. We are happy to host artides on post harvest technologies and farm mechanization related to Horticulture. The editorial team acknowledges with gratitude the interest shown by researchers belonging to diverse disciplines in publishing their work in JHS.

**S. Sriram**
*Chief Editor*

*J. Hortl. Sci.*
*Vol. 16(2) : 131-143, 2021*

***Review***

# Phytoremediation of indoor air pollutants: Harnessing the potential of plants beyond aesthetics

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## ABSTRACT

**Indoor air pollution has emerged as a major threat to human health worldwide that needs to be dealt urgently. The present review is an effort to overview the different indoor air pollutants ($CO_2$, volatile organic compounds (VOCs) like formaldehyde, benzene, nitrous oxide, trichloro-ethylene, fluorine, ammonia, radon, aldehyde, hydrocarbons etc.) their hazardous effects on human health, potential of indoor plants in their remediation and their practical utility. Besides providing oxygen to breathe, multifaceted roles of indoor plants have been well documented. Plants were used since decades for indoor decorations based on their aesthetic value, but now studies are focused on screening plant species for their efficiency in absorption of indoor air pollutants. The basis for phytoremediation is the potent efficiency of some plants to assimilate, degrade, or modify toxic pollutants into non-toxic ones. Phytoremediation seems to be the key solution to improve indoor air quality as it has many potential advantages (simple, potentially cheap, and easily implemented) in comparison to other traditional or latest methods. Breathing walls, portable air filters for rooms or whole house filtration through heating, ventilation and air conditioning systems are some of the technologies developed, to reduce indoor air pollution and improve indoor air quality but all these are costly, resource consuming and still there is question on their efficiency. Detailed account of morphological, anatomical and molecular mechanisms underlying plant leaves and leaf associated microbes in reduction of pollutants have been reviewed that could help in developing cost effective and eco friendly remediation technologies. This review gives a brief discussion about air phytoremediation to improve effectiveness of this technology in practical use.**

**Keywords**: Indoor air pollutants, herbs, phytoremediation and plants

## INTRODUCTION

Degradation in air quality has become the biggest concern and awareness regarding its maintenance and protection is increasing all over the world. There are numerous anthropogenic causes of outdoor and indoor air quality degradation *viz*., volcanic eruption, fossil fuel burning, forest fires, motor vehicle pollution, controlled burning in agriculture, fumes from paints or air sprays, waste deposits in landfills military resources such as nuclear weapons and toxic germ warfare, fertilizers, furnitures, coolants etc that leads to numerous respiratory, heart diseases and could even be carcinogenic.

Major air pollutants include particulate matters (PMs), carbon monoxide, oxides of sulphur and nitrogen, ground-level ozone ($O_3$) and volatile organic compounds (VOCs) that can cause dozens of diseases and threaten human health (Archibald *et al.*, 2017; Burns *et al.*, 2020). According to a WHO report, nearly 91% of the world's population lives in areas where the level of airborne pollutants exceeds WHO permissible limits (Health Effects Institute, 2018).

The changing lifestyle is further adding to the problem as it has limited the activities of people to indoors which forces large percent of urban population to spend most of their times, indoors. Ventilation plays a crucial role in promoting the comfort and health of occupants (Rackes and Waring, 2014). The world has

experienced unprecedented urban growth during the last three decades. Urban population is expected to increase from 55% in 2018 to 68% by 2050. Projections show that urbanization, the gradual shift in residence of the human population from rural to urban areas, combined with the overall growth of the world's population could add another 2.5 billion people to urban areas by 2050, with close to 90% of this increase taking place in Asia and Africa, according to a new United Nations data set launched (Anon., 2018b). With increasing urbanization and rising migration of people from rural to urban areas, space is the major concern. Buildings generate almost 40% of global greenhouse gases (GHG's) annually as compared to transport (23%) and industries (32%). Closed working spaces and lack of ventilation leads to accumulation of pollutants - excess amounts. Human beings spend 80-90% of their time in enclosed spaces, such as houses, office buildings, and schools with restricted air circulation (Yrieix *et al.*, 2010). In closed buildings, besides pollutants, occupants themselves are a major source of indoor air contamination. The occupants are the major source of carbon dioxide ($CO_2$) that poses threat to health at higher concentrations (Siskos *et al.*, 2001).Therefore, indoor air quality (IAQ) may be worse than outdoor air quality (Watson, 2013). According to the studies conducted, indoor air has been reported to be 12 times more polluted than outdoor air (Zabiegala, 2006). So, urban IAQ has emerged as an important international health issue that needs to be reviewed and resolved at earliest possible. Further, it demands for greener indoors with good IAQ to cater the health issues of occupants.

The current article gives an overview of degrading IAQ and its effect on human health; criteria for selection of indoor plants with high phytoremediation efficacy and mechanism underlying their efficacy that could stimulate more research in this area and improve its effectiveness in practical use.

## MAJOR INDOOR POLLUTANTS

Carbon dioxide is one of the major indoor pollutants that pose threat to human life. The average breath of an adult contains 35,000-50,000 ppm of $CO_2$ that gets accumulated in closed buildings (Prill, 2000). The outdoor $CO_2$ level is in a range of 350-450 ppm but the indoor $CO_2$ level is 100 times greater than outdoor $CO_2$ level, even in buildings where complaints with regard to indoor air quality are few. The $CO_2$ concentration indicates air exchange rate in buildings as $CO_2$ levels more than 1000 ppm indicate inadequate ventilation and occupants have problems like headaches, nose and throat ailments, tiredness, lack of concentration and fatigue (Bulinska *et al.*, 2014). This does not mean that low $CO_2$ levels are indicators of good indoor air quality (IAQ) as IAQ is dependent upon several other pollutants.

Besides $CO_2$, the other indoor pollutants are volatile organic compounds (VOCs) like formaldehyde, benzene, nitrous oxide, trichloro-ethylene, fluorine, ammonia, radon, aldehyde, hydrocarbons *etc*. Exposure to such chemicals leads to severe diseases like multiple chemical sensitivity, sick building syndrome allergies, asthma, headache, stroke, ischemic heart disease, chronic obstructive pulmonary disease (COPD) and lung cancer (Shinohara *et al.*, 2004).The effects of some of these air pollutants ranging from respiratory illness, cardiovascular disease to bladder and lung cancer affecting human health have been summarized by Kampa and Castanas (2008). Some indoor air pollutants, their source and health effects have been summarized in Table 1. According to the survey of World Health Organization (WHO) around 3.8 million people in a year die from the exposure to household air pollution (Anon., 2018a). Among various VOCs, formaldehyde is a main contaminant in terms of indoor air which originates from various paper products, curtains, adhesives, carpets, varnishes, permanent press-fabrics. Its concentration in new houses is often several times higher than that in older homes (Marco *et al.*, 1995).

The key solution to this problem is planting indoor plants that can survive under such adverse conditions with least maintenance and inputs. Besides acting as potential sinks of indoor pollutants, indoor plants add to the beauty and liveliness to our homes and offices and thus improve IAQ.

Indoor plants means the plants that can grow indoor i.e. their light, temperature and water requirements are low. They may be either flowering plants (Peace lily, Kalanchoe, Amaryllis, Hydrangeas, Poinsettia) or foliage plants (cactus, palm plants, fern and succulents). Besides oxygen producing ability another comprehensive research conducted by the National Aeronautics and Space Administration (NASA) has found that common household plants work as natural

air purifiers. Interestingly indoor plants can remove a notable amount of at least 87% of VOC's in 24 hours. VOC's are present in many household products, including paints, paint strippers and other solvents, wood preservatives, aerosol sprays, cleansers and disinfectants, moth repellents, hobby supplies, pesticides, dry-cleaned clothing, building materials and furnishing, office equipment including glues and adhesives, permanent markers and photographic solutions (Anon., 2021). Succulents and many indoor house plants are further advantageous as they are of small size and add continuous flush of fresh oxygen day and night. Studies have shown that people in buildings with plants like Money plant, Mother- In-Law's Tongue and Areca palm have 34% fewer respiratory problems, 54% less eye irritation and 24% fewer headaches (Anon., 2016).

## ASSESSMENT OF UTILITY OF INDOOR PLANTS

Indoor plants absorb carbon dioxide, keep the oxygen flowing and remove harmful toxins like VOCs which help to deter the illness, lower the stress levels and create a relaxed and happy ambience. This further helps in improving concentration, enhancing creativity and increasing productivity that fulfills overall well-being. The air purifying qualities of indoor plants ultimately stimulate a happier and healthier environment.

It may seem far-fetched and unconvincing that having greenery indoor can have such important effects but the scientific results and findings have proved it. Numerous indoor plants have huge capability of removing various categories of toxins from indoor air; hence foster the indoor air quality. Based on study conducted by NASA, toxins like benzene, formaldehyde, trichloroethylene, xylene, toluene and ammonia were considered and houseplants were selected to test their pollutant removal efficiency and it was found that they removed these pollutants in significant amounts (Table 2). Parallel to this, there are several other benefits of indoor plants which can be discussed under following headings:

**Air purifiers:** Succulents such as aloe vera and snake plant have excellent air purifying and toxins removal properties. The plants emit the water vapour and that in return generates the pumping action which pulls the contaminated air down to roots of plants. The succulents convert the contaminants to plant food and thus purify the air. The studies showed that many common foliage plants reduced levels of some indoor pollutants, including formaldehyde and carbon monoxide, from small, sealed test chambers. The pollution reduction was largely due to bacteria growing on the plant roots (Wolverton *et al.*, 1989).

Plants grown in potting soil have been rated for their relative removal rate of toxins, such as formaldehyde. For this compound, Boston fern can remove 1863 µg/h, Bamboo palm 1350 µg/h, Janet Craig dracaena 1328 µg/h, English ivy 1120 µg/h, peace lily 939µg/h, areca palm and corn plant 938 µg/h. All the details of how plants clean such air, and how to use them, are in the classic paperback book "*How to Grow Fresh Air*" by the researcher B.C. Wolverton.

Living walls of plants have become more common in buildings, including modular units that one can even install in a home. A new technology of breathing walls introduces a method for improving the indoor air quality, and this technology is called breathing walls. The concept of a breathing wall is to purify the internal air flowing out through the walls and to reduce the concentration of indoor air pollutants. This draws a steady stream of filtered air through the walls and into the buildings all the times, providing exceptionally clean ventilation to occupants (Zhai, 2016). A company in Sydney (Australia) has partnered with the University of Technology to quantify the positive effects of what they term "breathing walls" to remove carbon dioxide and volatile organic compounds from interior air. The U.S. researchers Fisk and Rosenfeld of the Berkeley National Laboratory have quantified a $58 billion annual savings from sick-building illness with the use of plants (Anon., 2007).

**Improvement in indoor humidity level:** The indoor plants regulate the humidity level which is an important factor influencing indoor weather. The studies conducted by Agricultural University in Norway revealed that indoor plants at home regulate the humidity levels inside the house as plants release moisture in form of vapour, and this increased moisture improves the sore throat, allergies, cold and dry cough and even some skin diseases (Fjeld, 2000).

The foliage plants can raise relative humidity to healthier and more comfortable levels in interior spaces. The relative humidity of the air inside buildings is often below the range of 30% to 60% recommended for human comfort, especially when buildings are

being heated. When the indoor relative humidity is too low, colds are more frequent and wood dries and cracks. In this study, when plants were present, less than 2% of the space was occupied by the plants, the relative humidity was raised from 25% without plants to 30% with plants. The enhancing effect of indoor plants on relative humidity raised the concern that indoor plants might result in too much increase in relative humidity. But this is unlikely to occur as when the relative humidity rises, the rate of water loss from the plant slows due to decreased concentration gradient between plant tissue and atmosphere (Lohr, 2010)

**Continuous oxygen supply:** Unlike most of the plants, succulents do not release carbon dioxide at night instead they have specialized built in mechanism which maintains the continuous supply of oxygen.

**Improve our focus:** Number of studies has been conducted on both students as well the workers which have proved that attentiveness, concentration and brain capabilities increase when plants were kept in their room. The University of Michigan conducted a study which found memory retention is improved as much as 20% when plants are present in room (Anon., 2008).

**Increase pain tolerance:** The plants in our vicinity have capability to decrease our sensitivity to pain. The Horticulture Therapy Research was conducted by University of Kansas which showed that patients needed less medication when they had plants in their room. One more study was conducted to examine people's ability of perception to pain in presence or absence of plants. About 71% of subjects in room with plants had their overall physical health above excellent whereas this per cent decline to 56% in the conditions when there were no plants around (Lohr and Pearson-Mims, 2000).

**Increase working productivity:** Mental fatigue has been shown to be reduced by plants. Indoor plants have potential to increase the productivity per working person. The studies conducted in Texas, Washington State and England revealed that the employees with plants in their environments were 12% more productive than those working in environment without exposure to interior plants.

Students in dorm room with the view of nature and plants had better and increased productivity than those without it, assuring the role of plants in increasing the potential of the subject for performance. (Lohr, 2010)

**Improve memory**: Another important aspect is memory which is influenced by plants. A study was conducted in the University of Michigan, which revealed that there are numerous benefits of interacting with nature. They reported that interaction for just an hour with nature could increase the memory retention (Berman *et al.*, 2008).

**Help to prevent diseases**: The positive effect of plants on health and disease prevention has been proved from several years. The presence of plants on working desk in office improved the health of subject as a study conducted in the University of Norway revealed that there was 60% decrease in sickness rates with plants in office (Fjeld, 2000).

**Speed up the healing process**: Researchers who have assessed the impact of nature/plants on human health have suggested that people-plant interactions provide physiological stress reduction within minutes along with faster physical recovery from stress that further improves emotional and cognitive health (Kaplan, 2001; Chang and Chen, 2005).

The healing process through plants was also supported by Texas A & M University. They recommended that patients who interacted with plants, engaged in gardening have faster recovery rate and less downtime in post-surgery patients. The soothing effects of ornamental flowers and plants are so great that simply having daily views of flowers and other ornamental plants in landscaped areas outside patient recovery room can also significantly speed up recovery time (Hall and Dickson, 2011).

**Reduce stress:** The study was conducted that stress reducing responses also occur when people are in a room with a few containerized interior plants, even when their attention is not drawn to the plants (Lohr *et al.*, 1996). A study revealed that people given a task on computer had higher systolic blood pressure in a room without any plant as compared to people doing same task in a room with plants (Lohr, 2010).

**Reduce noise:** The studies have shown that plants also reduce the indoor noise levels, making it a pleasant environment. For instance, a hedge of small indoor plant in workspace can reduce the noise levels up to five decibels. Plants absorb the sound and create a calming effect. A study from London South Bank University showed that there is a positive effect on noise reduction from large plants placed in corners of the rooms (Perry, 2018)

**Table 1. Common indoor air pollutants and their effects on human health**

| Pollutant | Sources | Health Impact | References |
|---|---|---|---|
| Particulate Matter (PM) | Outdoor environment, cooking, combustion activities (burning of candles, use of fireplaces, heaters, stoves, fireplaces and chimneys, cigarette smoking), cleaning activities | Premature death in people with heart or lung disease, nonfatal heart attacks, irregular heartbeat, aggravated asthma, decreased lung function | Hamanaka *et al*. (2018)<br>Miller *et al.* (2012)<br>Brook *et al.* (2010) |
| Volatile organic carbons (VOCs) | Paints, stains, varnishes, salvents, pesticides, adhesives, wood preservatives, waxes, palishes, cleansers, lubricants, sealants, dyes, air fresheners, fuels, plastics, printers, tobacco products, perfumes, dry-cleaned clothing, building materials | Eye, nose and throat irritation - Headaches, loss of coordination and nausea - Damage to liver, kidney and central nervous system - Some organics can cause cancer | Tang *et al.* (2015)<br>Brickus *et al.* (1998)<br>Weschler *et al.* (2012) |
| NitricOxide ($NO_2$) | Gas-fueled cooking and heating appliances | Enhanced asthmatic reactions - Respiratory damage leading to respiratory symptom | Bernstein *et al.* (2008) |
| Ozone ($O_3$) | Outdoor sources, photocopying, air purifying, disinfecting devices | DNA & lung damage, asthma, decreased respiratory functions | Salonen *et al.* (2018)<br>Huang *et al.* (2019) |
| Sulphur di oxide ($SO_2$) | Cooking stoves; fireplaces; outdoor air | Impairment of respiratory function - Asthma, chronic obstructive pulmonary disease (COPD), and cardiovascular diseases | Seow *et al.* (2016) |
| Carbon oxides (Cox) | Cooking stoves; tobacco smoking; fireplaces; generators and other gasaline powered equipment; outdoor air equipment | Outdoor air fatigue, chest pain, impaired vision, reduced brain function | Raub *et al*. (2000) |
| Heavy Metals | Outdoor sources, fuel-consumption products, incense burning, smoking and building materials | Cancers, brain damage, Mutagenic and carcinogenic effects: respiratory illnesses, cardiovascular deaths | Hamanaka *et al*. (2018)<br>Rashed *et al.* (2008)<br>Madany *et al.* (1994) |
| Aerosals | Tobacco smoke, building materials, consumer products, incense burning, cleaning and cooking | Cardiovascular diseases, respiratory diseases, allergies, lung cancer, irritation and discomfort | Koivisto *et al*. (2019)<br>Oh *et al*. (2019)<br>Kulmala *et al*. (1999) |
| Radon | Soil gas, building materials, and tap water Outdoor air | Lung cancer | Bruno *et al.* (1983) |
| Pesticides | Termiticides, insecticides, rodenticides, fungicides, disinfectants and herbicides - Building materials: carpet, textiles, and cushioned furniture - Outdoor environment | Irritation to eye, nose and throat; Damage to central nervous system and kidney; Increased risk of cancer | Halt *et al.* (2017)<br>Colt *et al.* (2004)<br>Hwang *et al.* (2008) |
| Biological allergens | House dust, pets, cockroaches, mald/dampness, pallens originating from animals, insects, mites, and plants | Asthma and allergies Respiratory infections, sensitization, respiratory allergic diseases and wheezing | Baldacci *et al*. (2015) |
| Micro-organism | Bacteria, viruses, and fungi are carried by people, animals, and soil and plants | Fever, digestive problems, infectious diseases, chronic respiratory illness | Anonymous *et al*. (1988) |

**Improves perception:** Plants improve the rate of perception as revealed in a study of Opryland Hotel in Nashville. The occupancy rate of that hotel was higher than national average. A scientific study was conducted and the main factor accounting for this was their high investment over $1 million on interior plants, as a matter of fact it was the largest investment on indoor plants in country (Perry, 2018).

## AMELIORATIVE POTENTIAL OF INDOOR PLANTS TO IMPROVE INDOOR AIR QUALITY

The studies conducted by NASA back in 1980s, demonstrated that plants have potential to ameliorate airborne pollutants. Susanto *et al.* (2021) provided evidence-based insight into usefulness of indoor plants as an alternative way for indoor air remediation. Several studies demonstrated efficient phytoremediation of specific indoor air pollutants through the use of specific plants. The plants *Osmunda japonica, Davalliamariesii, Selaginella tamariscina, Polypodium formosanum,Lavandula* spp.*, Pteris dispar, Pteris multifida, Pelargonium* spp.*, Aloe vera,* and *Epipremnum aureum* were reported to be efficient in removing formaldehyde from indoor air (Kim *et al.*, 2010). Liu *et al*. (2007) reported that *Crassula portulacea, Hydrangea macrophylla, Cymbidium "*Golden elf*", Syngonium podophyllum, Euphorbia milii, Sansevieria trifasciata, Chlorophytum comosum, Dracenas anderiana, Hedera helix, and Clitori aternatea, with Chlorophytum comosum* have high benzene removal efficiency. Phytoremediation of toluene may be achieved through *Schefflera elegantisima, Philodendron spp. "*Sunlight*," and Hedera helix* (Kim *et al.*, 2011). *Zamioculcas zamiifolia* was potent in remediating xylene (Sriprapat *et al.*, 2013) and ethylbenzene to some extent (Toabaita *et al.*, 2016). *Hoya carnosa, Hemigraphis alternata, Fittonia argyroneura and Asparagus densiflorus* efficiently remove VOCs like benzene, toluene, octane, TCE, and a-pinene and *Ficus benjamina*octane and a-pinene. Wood *et al.* (2006) documented that the popular indoor pot plant *Dracena deremenis* "Janet Craig" as an excellent species for the removal of VOCs. Similar results for removal of VOCs *viz*., benzene, ethylbenzene, xylene, styrene, formaldehyde, acetaldehyde, and toluene was recorded for *Ficus* spp. (Hong *et al*., 2017). The efficiency of *Nephrolepis obliterate* in reducing indoor formaldehyde levels was found up to 100% (Teiri *et al*., 2018). Aydogan and Montoya (2011) revealed the efficacy of plants such as *Hedera helix*, *Chrysanthemum morifolium*, *Dieffenbachia compacta*, and *Epipremnum aureum* in reducing formaldehyde levels up to 90% within 24 hours. Indoor plants are not only potent to reduce VOCs, CO and $CO_2$ levels but also particulate matter (Panyametheekul *et al.*, 2018).

Several other studies also have shown the use of potted plants as a mechanical system for phytoremediation of several indoor air pollutants. Latest technologies have provided biofiltration walls or green walls or large filtration systems but potted plants are most effective in terms of phytoremediation capacity, maintenance and cost (Agarwal *et al*., 2019). Recent advancements in indoor air phytoremediation technologies, botanical biofiltration systems could more efficiently reduce the concentrations of indoor air pollutants through action of active airflow in plant growing medium, along with vertically aligned plants that leads to higher leaf area density per unit of floor space. Despite of clear potential of these latest systems, still research needs to be focused on potential and cost effectiveness for proper selection of plants and their functional integration in buildings (Petit *et al*., 2018). The use of indoor plants as phytoremediation technology in comparison to latest technologies is slow but environment friendly air-purification strategy i.e. financially affordable with minimal energy consumption (Susanto *et al*., 2021).

## STRATEGIES FOR SELECTION OF INDOOR PLANTS

Selection of plants for indoors is based on criteria such as aesthetic features, good survival and low maintenance. Generally evergreen plant species with broad leaves and inhabitants of understorey of large canopies of tropical and sub-tropical climates have been selected as indoor foliage plants as they have been adapted to photosynthesize under low light intensities and grow profusely (Anderson *et al*., 1987). The plants that are adapted to shade have large leaf area and reduced stomatal aperture leading to removal of pollutants through adsorption rather than absorption (Gommer *et al*., 2013). This selection criteria of shade adaption for indoor plants need to be supplemented with morphological (i.e.,) leaf shape, size, and hairiness), anatomical (i.e.,) composition of epidermis

**Table 2. Indoor Plants and their indoor pollutant removal efficacy**

| Indoor Plant | Indoor pollutants | | | | | |
|---|---|---|---|---|---|---|
| | Benzene | Formaldehyde | Trichloroethylene | Xylene | Toluene | Ammonia |
| Peace Lilly | | | | | | |
| Parlour Palm | | | | | | |
| Lady's Palm | | | | | | |
| Florist's chrysanthemum | | | | | | |
| English Ivy | | | | | | × |
| Bamboo palm | | | | | | × |
| Variegated Snake palm | | | | | | × |
| Red-edged dracaena | | | | | | × |
| Cornstalk dracaena | | | | | | × |
| Janet Craig | | | | | | × |
| Warneckei | | | | | | × |
| Flamingo lily | × | | × | | | |
| Dwarf Date palm | × | | × | | | × |
| Areca palm | × | | × | | | × |
| Boston fern | × | | × | | | × |
| Kimberely queen fern | × | | × | | | × |
| Spider plant | × | | × | | | × |
| Weeping fig | × | | × | | | × |
| *Dendrobium* orchid | × | | × | | | × |
| King of Hearts | × | | × | | | × |
| Moth orchids | × | | × | | | × |
| Chinese evergreen | | | × | × | × | × |
| Aloe vera | | | × | × | × | × |
| Heartleaf philodendron | × | | × | × | × | × |
| Selloum philodendron, | × | | × | × | × | × |
| Elephant ear philodendron | × | | × | × | × | × |
| Rubber plant | × | | × | × | × | × |

(Source: NASA Clean Air Study)

and mesophyll layers, and stomatal density and size) and physiological ($CO_2$ assimilation rate and activity of detoxifying enzymes) properties that determine their air phytoremediation potential. Advanced omics technologies (genomics, proteomics, and metabolomics) could be used to understand the biochemical mechanism of indoor air pollutant degradation. The detailed unraveling of metabolic pathways, genes and enzymes involved in catabolism of pollutants will enable the determination of biomarkers for phenotyping appropriate plant species for improving IAQ.

The selection of plants for indoor should be done very carefully with scientific approach as some plants may have negative impact on human health too. Some ornamental plants produce allergic pollens and/or release harmful VOCs; their surfaces may harbor pathogenic microbes and/or pests (Carinanos and Casares-Porcel, 2011). Thus, ornamental shade-loving plants with more foliage (leafy part), less pollen, and short blooming period could be selected to reduce indoor air pollution. Low grade plastic pots used for planting can also produce VOCs; hence, alternatives to plastic pots for indoor planting besides appropriate plants have to be considered.

One of the best tools for selecting plants for indoor is the Air Pollution Tolerance Index (APTI). APTI considers biochemical properties of leaves such as ascorbic acid; relative water content, total chlorophyll and leaf extract pH. These properties affect the value of the plant's tolerance to air pollutants. For example, under water stress, the content of chlorophyll induces reactive oxygen species in the chloroplast. The high value of ascorbic acid is one of the strategies to prevent oxidative damage to the thylakoid membranes under water stress conditions (Bandehali *et al*., 2021).

## HOW PLANTS REMEDIATE INDOOR AIR POLLUTANTS

The plants during photosynthesis simultaneously take up $CO_2$ and release $O_2$ and during transpiration, release water vapour through stomata on their leaf surface (Smith and Pitt, 2011). Thus, the potential of plants to improve IAQ depends upon the capacity of leaves to exchange gases and pollutants from indoor air through stomata. Schreck *et al.* (2012) also reported that the points of entry of metal-enriched particles after their deposition on the leaf surface, could be the cuticle and stomata. This capacity is further limited by physical constraints pertaining to stomatal and mesophyll resistance. The size of stomatal pore varies with variation in environmental conditions *viz*., light, temperature, humidity -and cascades of signaling through plant hormones especially abscisic acid. Besides stomatal absorption, pollutants can get adsorbed on the external surfaces of plants or soil-root interface and thus get removed. The process of absorbing lipophilic semi volatile compounds is achieved through leaf surface adsorption, where atmospheric resistance serves as a major limiting factor (Wei *et al.*, 2017). This type of removal depends upon the total surface area and anatomical, morphological and chemical features of the plant surface along with characteristics of the soil substrate (Irga *et al.*, 2013). The adsorption of pollutants especially lipophilic VOCs, such as benzene, on plant surface is dependent upon type and density of trichomes (Li *et al.*, 2018), cuticular wax deposition and lipid composition of epidermal membrane (Gawronska and Bakera, 2015). The potential of plants to ameliorate indoor pollutants was earlier based on simplistic approaches but more accurate experimentation through simulation of foliage to indoor air pollutants not only confirmed the earlier reports (Fares *et al*., 2015) but also revealed that the amount of pollutants absorbed through stomata is 30-100 times more than adsorbed on the plant surface or non-stomatal deposition (Tani *et al*., 2009). After entering into plant leaf either through absorption or adsorption, pollutants are translocated to shoots and roots for metabolic degradation through oxidases or hydrolases and then conjugation with different metabolic compounds (sugars, amino acid, organic acids, and peptides) to form bioproducts. These products are either re expelled (into air or as root exudates into soil) or used as carbon and energy sources (Oikawa and Lerdau, 2013).

In addition to air phytoremediation through absorption and adsorption of pollutants by plant surface, another least explored aspect is phylloremediation i.e. remediation through habituated microbes either on leaf surface or endophytes by biodegrading or transforming pollutants into less or nontoxic molecules (Sandhu *et al.*, 2007). Leaves are the primary photosynthetic organs with dorsiventral symmetry and play pivotal roles in supporting phyllosphere microbes (Bringel and Couee, 2015). Several reports documented that both

plant leaves and leaf-associated microbes mitigated air pollutants, such as azalea leaves and the leaf-associated *Pseudomonas putida* in reducing VOCs (De Kempeneer *et al.*, 2004) and poplar leaves and the leaf-associated *Methylobacterium* sp. decreased xenobiotic compounds (Van Aken *et al.*, 2004).

Thus, the different mechanisms underlying the phytoremediation potential of plants for indoor pollutants are microbial degradation through rhizospheric microorganisms, phytoextraction i.e. plant-liquid extraction, plant-gas extraction i.e. stomatal uptake, enzymatic catalysis inside tissues and plant transpiration & evaporation from leaves (Sharma *et al*., 2019)

## CONCLUSIONS AND FUTURE PERSPECTIVE

The complexities in the source, chemical nature, effects and stability of air pollutants pose a great challenge for standardizing technologies for their remediation. Further, different pollutants are prevailing at different rates in different microenvironments which demands for location specific remediation for pollutants individually or in groups. The selection of plants suitable for indoor phytoremediation should follow unambiguous scientific criteria that reflect their capacity to sequestrate air borne pollutants, instead of only taking into consideration their aesthetic features. The plant-soil-microbe system through metabolizing, sequestering or degrading air pollutants improve indoor air quality. Limited information on number and group of plant species indicating their potential and suitability for removing air pollutants entails uncertainties. The studies should be planned to concentrate on developing plant by editing genomes through DNA modifications to over express or insert genes coding for detoxifying enzymes. The integration of smart sensor networks and computerized technologies with highly performing indoor plant species could provide great opportunities to improve IAQ through eco-sustainable and cost-effective techniques. Recent advancements in indoor air phytoremediation technologies, botanical biofiltration systems could more efficiently filter indoor air but still research needs to be focused on potential and cost effectiveness for their functional integration in buildings. Further research is needed to develop, test and confirm their effectiveness and safety before they can be.

Studies on indoor air phytoremediation technologies is a multidisciplinary approach that demands collaboration among researchers from different fields at regional, national, and international levels so that there is a paradigm shift in the way in which plants will become functional entity instead of just a decorative tool.

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***(Received on 02.06.2021, Revised on 20.07.2021 and Accepted on 30.07.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 144-151, 2021*

***Original Research Paper***

# Response of fruit yield and quality to foliar application of micro-nutrients in lemon [*Citrus limon* (L.) Burm.] cv. Assam lemon

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## ABSTRACT

**Assam lemon [*Citrus limon* (L.) Burm.], an indigenous lemon cultivar of Assam, is widely cultivated in warm southern slopes of the Himalayas in North-Eastern India. Since this cultivar of lemon is having a prominent trait of bearing fruits in several flushes throughout the year, it is essential to provide sufficient nutrition for obtaining optimum yield with good quality fruits. In the current experiment, a randomized block design having twelve treatments with three replications was followed to find out the response of lemon fruit yield and quality to foliar application of micronutrients during the year 2019. Among all, the treatment $ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%) gave the best performance in improving the yield and quality of fruits. The highest number of fruits per plant at the time of harvesting (73), yield per plant (11.5 kg), fruit fresh weight (158 g), fruit length (9.60 cm), fruit diameter (5.80 cm), juice content (152 mL/fruit), TSS (6.40 °B), ascorbic acid (49.10 mg/100g), total sugar (6.30%), reducing sugar (3.90%), non-reducing sugar (2.40%) with lowest titratable acidity (3.13%) were obtained which revealed that the yield and fruit quality of lemon depends on the application of different micronutrients.**

**Keywords:** Assam lemon, fruit quality, micronutrients, nutrition and yield

## INTRODUCTION

Citrus is considered as one of the most important fruits widely cultivated in different parts of the world. It belongs to the family Rutaceae. It is very famous for its juice and pulp all over the world. Among the citrus cultivar, Assam lemon [*Citrus limon* (L.) Burm.] is an indigenous lemon cultivar of Assam and is grown all over the North-Eastern region. As this plant is having a prominent trait of bearing fruits in several flushes throughout the year, it is essential to provide sufficient nutrition for obtaining optimum yield with good quality fruits. Micronutrients like zinc, iron, boron and copper are not only essential but they are equally significant like other macronutrients, in spite of their requirement in minute quantities. They also play a vital role in the various enzymatic activities and synthesis. Their acute deficiencies are sometimes incurable in nature (Kumar, 2002). Zinc is required for the synthesis of tryptophan which is the precursor of indole acetic acid synthesis resulting in the growth and development of tissues. Iron is also an important micronutrient necessary for the citrus plants. It has been reported that the application of iron sulphates as foliar spray reduces the leaf chlorosis and consequently increase the yield (Devi *et al.,* 1997). It also helps in a significant increase of fruit yield, fruit volume, ascorbic acid content and leaf iron content in citrus (Aboutalebi and Hassanzadeh, 2013). Similarly, boron also plays a vital role in the growth behaviour and productivity of citrus fruits. It increases the phenolic compound production in the plant system that is responsible for the polar transport of auxin. This increases the auxin activity resulting in increasing the vegetative growth of citrus trees (Gurjar *et al.,* 2015). Boron also helps in pollen grain germination and

elongation of pollen tubes that results in increasing the fruit set percentage and ultimately increase in yield (Abd-Allah, 2006). As a micronutrient, copper plays a significant role in plants. It is involved in stimulation activities for lignification of the cell wall of plants, and photosynthesis, and acts as an electron carrier in the plant system (Somasundaram *et al.,* 2011). It is reported that copper helps in production of sugar compounds and leads to more accumulation of total soluble solids (TSS) in the fruit juice (Singh *et al.,* 2018).

Since citrus is a micronutrient loving crop, the more precise management of nutrition is required to meet the nutrient demand for its growth and development. Thus, fulfilling the nutritional requirement is very important for economically profitable citrus fruit production. Assam lemon being a heavy and regular bearing crop which bears throughout the year has to be supplied with adequate nutrients to ensure the yield and quality of the harvest. Most of the time citrus growers are not giving proper emphasis to application of micronutrients, as they are required in minute quantities. This leads to a drastic decrease in the growth and development of plants and it also affects the yield and quality of fruits. So, the current experiment was conducted to study the effect of micronutrients on fruit yield and quality of Assam lemon in the North-Eastern region of India.

## MATERIAL AND METHODS

The experiment was laid out with the objectives of evaluating and standardizing the impact of micronutrients on yield and quality of Assam lemon. The research work was executed on three years old Assam lemon plants which were planted at the spacing of 3m x 3m during the year 2019 at the Citrus Fruit Block, Department of Fruit Science of the College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh. It is geographically located at 28° 04' 43" N latitude and 95° 19' 26" E longitude with an altitude of 153 m above the mean sea level. Pasighat lies under the humid sub-tropical climate. The average annual rainfall and temperature of this area are 32.8 cm and 25.5 ºC, respectively. The rainy season starts from June and it continues till September with maximum rainfall during July. August is the warmest month of the year while December is the coldest month of the year with the average temperature of $31.6^0$C and $20.5^0$C P C respectively. The design of the experiment followed was Randomized Block Design (RBD) having twelve treatments and each treatment was replicated thrice. In each replication, there were two plants and the total numbers of plants in the whole experiment were seventy-two. The recommended dose of fertilizer (RDF) for Assam lemon 100: 100: 100 g NPK (Source: N- Urea, P- SSP and K- MOP)/plant/ year (www.kiran.nic.in), was applied to all the plants under the investigation. Half of the dose before flowering during the first week of January and the remaining half dose during the second month of June were applied.

The treatments studied were: $T_1$ - Control, $T_2$ - $ZnSO_4$ (0.2%), $T_3$ - $FeSO_4$ (0.2%), $T_4$ - Borax (0.2%), $T_5$ - $CuSO_4$ (0.2%), $T_6$ - $ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%), $T_7$ - $ZnSO_4$ (0.2%) + Borax (0.2%), $T_8$ - $ZnSO_4$ (0.2%) + $CuSO_4$ (0.2%), $T_9$ - $FeSO_4$ (0.2%) + Borax (0.2%), $T_{10}$ - $FeSO_4$ (0.2%) + $CuSO_4$ (0.2%), $T_{11}$ - Borax (0.2%) + $CuSO_4$ (0.2%) and $T_{12}$ - $ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%). For the foliar application of these micronutrients (Sources: $ZnSO_4$- 36.40% Zn, $FeSO_4$- 32.8% Fe, Borax- 10.8% B and $CuSO_4$- 21% Cu), the required amounts of micronutrient sources were dissolved in separate container. Then the pH of the nutrient solutions were checked by using Pen type digital pH meter and it was adjusted by 0.1 N concentrated hydrochloric acid and sodium hydroxide. The application was done on 5th April, 2019 (i.e.) after the complete emergence of spring flush and the onset of fruit setting) on an average of two liters per tree and normal water was used for spraying the plants in control by using Knapsack sprayer. In each spray treatment teepol @ 0.01% was added as sticking gent in prepared solution.

The total numbers of fruits per plant at the time of harvesting (i.e.) during June-July fruits developed attractive green to little yellow colour and they were harvested two times) were recorded. For fruit yield of Assam lemon, fruits from each plant were harvested separately for all treatments and yield per plant was calculated by multiplying total number of fruits per plant with average fruit weight. For recording the readings of physical parameters like fruit weight, fruit length, fruit diameter and juice content, five fruits from each treatment were selected randomly. Then the fruit weight was recorded by using a precision weighing balance and its average weight was expressed in grams

(g). For the fruit length and fruit diameter a digital Vernier caliper was used to record the data and their average fruit length and fruit diameter were expressed in centimeters (cm). Fruit juice content was also measured by using a measuring cylinder and its average juice content per fruit was expressed in milliliters (mL).

The TSS in fruit juice was determined using hand held Refractometer (0 ºB–32 ºB). The titratable acidity of the fruit was determined by titrating the fruit juice against 0.1N NaOH solution using phenolphthalein as an indicator (light pink end point) and expressed as percentage in terms of citric acid (AOAC, 2002).

Total sugar content was estimated by anthrone method as described by Hodge and Hofreiter (1962).

Reducing sugar content was estimated by spectrophotometric method as described by Somogyi (1952). The non-reducing sugar content was obtained by the subtraction of the reducing sugar content from the total sugar content.

Non reducing sugars content = total sugar - reducing sugar

The ascorbic acid content of fruits was determined by the method described by Ranganna (1986) using 2, 6-dichlorophenol Indophenol dye. The samples extracted in meta-phosphoric acid solution were titrated with dye to pink end point. The ascorbic acid content was calculated and expressed as mg per 100 g of fruit weight sample.

The observations recorded during field experiment and data obtained from laboratory analysis were subjected to the statistical analysis of variance for RBD. Significance and non-significance of the variance due to different treatments were determined by calculating the respective 'F' values according to the method described by Gomez and Gomez (2010).

## RESULTS AND DISCUSSION

**Yield and its attributing parameters:** The data depicted in Table 1 exhibited the significant impact of foliar imposition of micronutrients on yield and its attributing parameters. The highest number of fruits per plant (73) at the time of harvesting and fruit yield (11.52 kg/plant) was recorded in $T_{12}$ [$ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%)] while the lowest number of fruits per plant (43) at the time of harvesting and fruit yield (5.60 kg/plant) was obtained in $T_1$ (Control). This might be due to the synergistic effect of different micronutrients as they directly take part in many physiological processes and activity of many enzymes for greater gathering of food materials. Zinc helps in prevention of abscission layer formation and increase the synthesis of tryptophan which is the precursor of auxin synthesis and this facilitates the ovary to remain intact with the shoot, ensuring in minimizing the flower and fruit drop and maximize the retention of fruits in the plants (Gurjar *et al*., 2015). There is also a correlation between fruit drop and internal hormonal level in the plant system. As the level of internal auxin concentration in the plant system is higher, then the fruit retention capacity will be more leading to increase in number of fruits per plant. Iron also plays a major role in cell division and cell enlargement resulting in increasing the fruit size and fruit weight which ultimately leads to the increase in yield of plants. Ganie *et al*. (2013) reported that boron helps in germination of pollen grains and elongation of pollen tube because of which the fruit set, fruit retention and yield of the guava plants were increased. Copper involves in synthesis and stability of chlorophyll responsible to produce food materials required for the growth and development of fruits. Ilyas *et al*. (2015) observed the significant improvement of photosynthetic and fruit yield in *Citrus reticulata* Blanco var. Kinnow on the foliar imposition of Zn, Cu and B. Similarly, Zoremtluangi *et al*., (2019) also proved that the foliar application of Zn, Cu and B obtained the maximum number of fruits per plant and yield per tree. The present report is in conformity with the experimental findings revealed by Bhoyar and Ramdevputra (2016) in guava and Jangid *et al*. (2019) in aonla.

**Physical parameters of fruits:** Most of the physical parameters of the Assam lemon fruit had consequential effect due to the application of different micronutrients and they are presented in Table 1. The highest fruit fresh weight (157.77 g), juice content (152 mL/fruit), fruit length (9.60 cm) and fruit diameter (5.80 cm) were obtained in treatment $T_{12}$[$ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%)] while the lowest fruit fresh weight (130.20 g), juice content (120 mL/fruit), fruit length (7.43 cm) and fruit diameter (4.10 cm) were obtained in treatment $T_1$ (Control).

The overall amelioration in the physical parameters of the Assam lemon fruit might be because of benefaction of different micronutrients in the growth and development of fruits. Zinc facilitates in the

**Table 1. Effect of micronutrients on yield and physical parameters of Assam lemon**

| Treatments | Number of fruits/plant | Fruit yield (kg/plant) | Fruit Fresh weight (g) | Fruit length (cm) | Fruit diameter (cm) | Juice content (mL/fruit) |
|---|---|---|---|---|---|---|
| $T_1$ | 43 | 5.60 | 130.2 | 7.43 | 4.10 | 120 |
| $T_2$ | 58 | 8.40 | 144.60 | 8.57 | 5.00 | 135 |
| $T_3$ | 48 | 6.64 | 138.40 | 7.87 | 4.67 | 129 |
| $T_4$ | 54 | 7.68 | 142.10 | 8.33 | 4.90 | 134 |
| $T_5$ | 45 | 6.00 | 133.30 | 7.60 | 4.27 | 123 |
| $T_6$ | 70 | 10.84 | 155.00 | 9.43 | 5.73 | 149 |
| $T_7$ | 67 | 10.11 | 150.80 | 9.27 | 5.50 | 145 |
| $T_8$ | 63 | 9.34 | 148.13 | 8.90 | 5.33 | 141 |
| $T_9$ | 52 | 7.29 | 140.23 | 8.13 | 4.73 | 131 |
| $T_{10}$ | 47 | 6.38 | 135.70 | 7.77 | 4.43 | 127 |
| $T_{11}$ | 61 | 8.95 | 146.60 | 8.73 | 5.13 | 137 |
| $T_{12}$ | 73 | 11.52 | 157.77 | 9.60 | 5.80 | 152 |
| C.D. (0.05) | 7.22 | 1.17 | 5.02 | 0.85 | 0.54 | 4.01 |
| SEm± | 2.44 | 0.39 | 1.70 | 0.28 | 0.18 | 1.36 |

synthesis of tryptophan, the precursor of auxin synthesis and consequently the auxin level in the fruit increases. This lead to the higher enlargement of cell because of cell vacuolization resulting in increased size of vesicles, dimension of locules and eventually the weight and size of fruit. Therefore, the significant influence of Zn in increasing the fruit fresh weight was revealed by Ghosh and Besra (2000) in sweet orange. Iron and zinc also play a vital role in the enlargement of cell, division of cell and formation of starch. Thus, the additive effect of these micronutrients resulted in increased fruit fresh weight. The rise in fruit fresh weight might be because of the higher translocation of photosynthates to fruits. Similar findings were revealed by Waskela *et al*. (2013) in guava.

Boron also plays a vital role in cell division and cell elongation, thereby increasing the fresh weight of fruit. The increase in fruit fresh weight owing to the combined imposition of Zn and B may be the result of stimulation influence on plant metabolic process. In addition to this, B also activates the sugar and water mobilization in the fruits resulted in increasing the fruit weight as reported by Lakshmipathi *et al*. (2015). Similarly, Ilyas *et al*. (2015) also reported that the foliar imposition of Zn, Cu and B had significant effect on fruit yield with regard to number and fresh weight of fruit.

Trivedi *et al*. (2012) reported that zinc controls the semi-permeability of cell wall through which the movement of water into fruits increases, that results in obtaining the highest juice content in guava. Sajid *et al*. (2012) also revealed that the foliar spray of Zn and B had consequential influence on juice content in fruits of sweet orange. The imposition of micronutrients might have ameliorated the plant health by improving the sugar metabolism and conduction of assimilates as a result of which the fruit juice content increase. Similar outcomes were revealed by the findings of Singh *et al*. (2018) in sweet orange cv. Mosambi. Increase in fruit length might be because

of the direct effect of B and Zn in increasing the cell division and cell elongation process. Similarly, the fruit diameter was notably influenced by the imposition of zinc sulphate and borax. Dutta and Banik (2005) reported that the increase in fruit length and fruit diameter may be due to the improvement in internal physiology of developing fruit with regard to proper supply of water, mineral nutrients and other compounds essential for normal growth and development of fruit and the present result is in conformity with the reports of Yadav *et al*. (2013).

**Quality parameters of fruit:** The quality parameters of fruits were significantly improved by the foliar application of micronutrients and they are presented in Table 2. In this, the treatment $T_{12}$[$ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%)] offered maximum TSS (6.40 ºB), ascorbic acid (49.10 mg/100 g), total sugars (6.30%), reducing sugars (3.90%) and non-reducing sugars (2.40%) content of fruits while the minimum TSS (5.30 ºB), ascorbic acid (34.37 mg/100 g), total sugars (3.73%), reducing sugars (2.53%) and non-reducing sugars (1.20%) were obtained in $T_1$ (Control).

**Table 2. Effect of micronutrients on quality parameters of Assam lemon**

| Treatments | TSS (°Brix) | Ascorbic acid (mg/100g) | Titratable acidity (%) | Total sugar (%) | Reducing sugar (%) | Non reducing sugar (%) |
|---|---|---|---|---|---|---|
| $T_1$ | 5.30 | 34.37 | 4.93 | 3.73 | 2.53 | 1.20 |
| $T_2$ | 5.93 | 40.43 | 4.57 | 4.80 | 2.90 | 1.90 |
| $T_3$ | 5.50 | 35.27 | 4.63 | 5.13 | 3.17 | 1.97 |
| $T_4$ | 5.60 | 39.55 | 4.47 | 4.37 | 2.70 | 1.67 |
| $T_5$ | 5.83 | 37.09 | 4.33 | 4.63 | 2.83 | 1.80 |
| $T_6$ | 6.13 | 42.33 | 3.90 | 6.00 | 3.80 | 1.20 |
| $T_7$ | 6.00 | 46.83 | 3.73 | 5.53 | 3.43 | 2.10 |
| $T_8$ | 6.20 | 48.30 | 3.57 | 5.73 | 3.70 | 2.03 |
| $T_9$ | 5.90 | 45.13 | 3.47 | 5.67 | 3.57 | 2.10 |
| $T_{10}$ | 5.97 | 41.06 | 3.23 | 5.37 | 3.13 | 2.23 |
| $T_{11}$ | 5.80 | 47.70 | 3.67 | 5.47 | 3.27 | 2.20 |
| $T_{12}$ | 6.40 | 49.10 | 3.13 | 6.30 | 3.90 | 2.40 |
| C.D. (0.05) | 0.44 | 3.31 | 0.39 | 0.27 | 0.24 | 0.40 |
| SEm± | 0.15 | 1.12 | 0.13 | 0.09 | 0.08 | 0.13 |

Zinc is required in enzymatic reactions namely hexokinase, carbohydrate and protein synthesis. In addition to this, Boron helps in the transportation of sugar in the form of boron-sugar complex and it also intensifies hydrolysis of carbohydrates into simple sugar. Copper also helps in elevating the photosynthetic efficiency that results in higher rate of photosynthesis. The main product of photosynthesis is sugar, thus increase in the photosynthesis by the additive action of zinc, boron and copper results in more sugar compounds and this led to the accumulation of more total soluble solids in fruit juice. The results of the current investigation are in line with the results obtained by Babu and Yadav (2005) in Khasi mandarin and Singh *et al*. (2018) in Sweet orange cv. Mosambi. Kumari *et al*. (2009) also

reported that the foliar fertilization of Fe, Zn and Cu had significant effect in increasing the TSS of Kinnow mandarin.

Zinc helps in the synthesis of auxin that leads to the increase in ascorbic acid content as reported by Nawaz *et al*. (2008). Similarly, foliar imposition of Zn, B and Cu also helps in increasing the ascorbic acid content in the fruit juice of Sweet orange cv. Mosambi as reported by Singh *et al*. (2018). Increase in sugar content (total sugar, reducing sugar and non-reducing sugar) in the fruit juice of Assam lemon might be because of the active participation of Zn, Cu and B in photosynthesis and faster translocation of sugars from the site of synthesis to the developing fruits. The other reason might be due to the decrease in starch content during deterioration of acid and quick transfer of sugars in the fruit. Thus, the present findings are in line with the outcomes obtained by El-Rahman (2003) in Naval Orange and Bhatt *et al*. (2012) in mango.

Although, the lowest value of titratable acidity (3.13%) of fruit juice was observed in $T_{12}$[$ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%)] while the highest titratable acidity (4.93%) was recorded in $T_1$ (Control). The reduction of titratable acidity might be because of more synthesis of nucleic acids due to more availability of plant metabolites as reported by Ullah *et al*. (2012) in Kinnow mandarin. The other reason might be because of their utilization in respiration and quick transformation of organic acid into sugars as disclosed by Brahmachari *et al*. (1997) in litchi. Similarly, the outcomes are in agreement with the results obtained by Sau *et al*. (2018) in guava.

On the basis of the experimental evidence obtained from the current research work, it is concluded that the imposition of recommended dose of N, P and K fertilizers (100g: 100g: 100g NPK/plant/year) together with foliar spray of $ZnSO_4$ (0.2%) + $FeSO_4$ (0.2%) + Borax (0.2%) + $CuSO_4$ (0.2%) once (two weeks after fruit setting) can be advocated to Assam lemon growers as the most potent measures to enhance the number of fruits per plant, yield per plant and fruit quality that will eventually increase the productivity.

## ACKNOWLEDGEMENT

The authors would like to express the heartfelt and sincere thanks to the Department of Fruit Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, for the guidance and needful help during the entire course of the experiment.

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***(Received on 14.04.2021, Revised on 13.09.2021 and Accepted on 26.10.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 152-163, 2021*

***Original Research Paper***

# Studies on high density planting and nutrient requirement of banana in different states of India

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## ABSTRACT

**An experiment was conducted under the ICAR-All India Coordinated Research Project on Fruits to study the high density planting (HDP) and nutrient requirement of banana at six research centres across the country, including Bhubaneswar (Orissa), Gandevi (Gujarat), Jalgaon (Maharashtra), Jorhat (Assam), Kannara (Kerala) and Mohanpur (West Bengal) to enable higher productivity of banana and profit to farmers. Objective of this study was to explore the possibility of increasing productivity through intervention of only per unit plant population (through planting system) and level of nutrition, but without any interference to the regional choices of variety (e.g. choice variety Nendran for Kerala or Martaman for West Bengal), production system (mono/poly- clone, single/multi-year plantation, and POP of respective states), for which national productivity ranges are much skewed also. Results indicated that intervention of only plant density could increase productivity of banana within the existing system of production and choice of variety of different region or states. The experiment was laid out in RBD with four planting densities ($S_1P_2$, $S_1P_3$, $S_2P_2$and $S_2P_3$, where $S_1$=2m x3m, $S_2$=1.8m x3.6m, $P_2$=2 suckers/hill, $P_3$=3 suckers/hill), three nutrition levels ($F_1$, $F_2$and $F_3$, which is 100%, 75% and 50% of RDF) and one with region-specific conventional planting density and nutrition (100% of RDF) practices as control.The results of this experiment showed that HDP ($S_1P_3$, 5000 plants / ha) in banana, accommodating three suckers per hill at 2m x 3m spacing increased productivity over the conventional system at the Bhubaneswar, Gandevi, Jorhat, Kannara and Mohanpur centres. The increase in productivity due to HDP (5,000/ha) over control was 28.9% (RDF 25%) to 50.6% (RDF 100%) at Bhubaneswar, 15.2% (RDF 25%) to 21.9% (RDF 100%) at Gandevi, 4.0% (RDF 25%) to 7.4% (RDF 100%) at Jorhat, 33.5%(RDF 25%) to 43.5% (RDF 100%) at Kannara and 46.5%(RDF 25%) to 79.0% (RDF 100%) at Mohanpur. The nutrient requirement under HDP was 100% RDF at Kannara, 75% RDF at Bhubaneswar and Mohanpur and 50% RDF at Gandevi and Jorhat centres, which indicates a saving in cost of fertilizer input by 25% -50%. It is therefore, recommended for HDP (5000 plants/ha) in banana, accommodating three suckers per hill at 2m x 3m (6.6 ft x 3.8 ft) spacing with 50% RDF in the agro-climatic regions of Gandevi and Jorhat, with 75% RDF in the agro-climatic regions of Bhubaneswar and Mohanpur and with 100% RDF in the agro-climatic region of Kannara in order to ensure higher productivity and profit to farmers.**

**Keywords**: Banana, productivity, input saving, nutrition strategy and planting

## INTRODUCTION

Sustainable increase in productivity is the key objective of commercial fruit cultivation to meet the per-head demand of fruits for human nutrition. High density planting (HDP), mediated by canopy management, was found to be very useful for increasing the productivity of fruit crops. However, the commonly used canopy management tools for perennial fruit trees (training, pruning and dwarfing rootstocks) were not feasible for canopy management and HDP of herbaceous perennial plants such as banana (Debnath *et al.*, 2015). Productivity in bananas is governed by the 'source' and 'sink' components of the plant system and its usefulness necessitates distinguishing between physiological and agronomic approaches (Turner, 1998). HDP in banana was found to have direct effect on growth and yield parameters, *viz*., pseudostem height, girth, leaf number, leaf area index, absorption of solar light, bunch weight and productivity (Nalina *et al*., 2000; Thippesha *et al*., 2005; Debnath *et al*., 2017). This, therefore, indicated the need forregion-specific fine-tuning of agronomic practices including spacing, plant density, nutrition and so on, for successful HDP in banana. For HDP of cv. Martaman (AAB) in the Gangetic alluvium region of West Bengal, the identified optimum leaf area index (LAI) was 5.50, corresponding to a plant population of 5000/ha, accommodating 3 plants/pit at 2m × 3m spacing (Debnath *et al*., 2015). These technological inputs on HDP in banana through research works are essentially needed for intervention and betterment of the much-skewed distribution of banana productivity across the different states in India. The average national productivity of banana in India is 34.86 t/ha, of which only five states recorded a productivity of more than 45 t/ha - Madhya Pradesh (69.52 t/ha), Gujarat (65.62 t/ha), Andhra Pradesh (56.24 t/ha), Maharashtra (52.04 t/ha) and Uttar Pradesh (45.72 t/ha). In fact, banana is grown in rest of the states with much lower productivity (3.40 to 44.94 t/ha) (Anon, 2018). With this back ground, an experiment was conducted to study the HDPand nutrient requirement of banana across the different states in the country for increasing productivity and profitability of the farmers.

## MATERIALS AND METHODS

The Indian Council of Agricultural Research (ICAR), through its All India Coordinated Research Project (AICRP) on Fruits, conducted an experiment between 2009 to 2015 to study the HDP and nutrient requirement of banana at six research centres across the country, including Bhubaneswar (Orissa), Gandevi (Gujarat), Jalgaon (Maharashtra), Jorhat (Assam), Kannara (Kerala) and Mohanpur (West Bengal) to ensure higher productivity of banana and profit for farmers (Table1). The experiment was laid out in Randomized Block Design (RBD), replicated four times with 15 plants per replication and 13 treatment combinations, including four planting densities ($S_1P_2$, $S_1P_3$, $S_2P_2$ and $S_2P_3$, where $S_1$=2mx3m, $S_2$=1.8m x3.6m, $P_2$=2 suckers/hill, $P_3$=3 suckers/hill), three nutrition levels (F1, F2 and F3=100%, 75% and 50% of RDF) and with one

**Table 1. Soil type, agro-climatic region and location of experimental sites under ICAR-AICRP on Fruits**

| Centre | Soil type, agro-climatic region and location |
|---|---|
| Bhubaneswar<br>OUAT, Odisha | Soil: Saline, lateritic, alluvial, red and mixed red and black; East and South East Coastal Plain; 20$^0$15'N latitude and 85$^0$52' E longitude |
| Gandevi<br>NAU, Gujarat | Soil: Clay loam; Agro-climatic region-I (south Gujarat) and heavy rainfall area; 21$^0$N latitude, 73$^0$E longitude, 7.6 m above mean sea level |
| Jalgaon<br>MPKV, Maharashtra | Soil: Black; Deccan plateau, hot semi-arid eco region; 21$^0$Nlatitude, 74.33$^0$Elongitude |
| Jorhat<br>AAU, Assam | Soil: Sandy loam; upper Brahmaputra valley zone; 26.75$^0$Nlatitude, 94.22$^0$Elongitude |
| Kannara<br>KAU, Kerala | Soil: Clay loam; 10°32'6.5" N latitude, 76°20'9.8" E longitude, 58m above mean sea level |
| Mohanpur<br>BCKV, West Bengal | Soil: Clay-loam; the Gangetic Alluvium region of West Bengal; 23.5$^0$North latitude, 89$^0$ Elongitude, 9.75 m above mean sea level |

region-specific conventional planting density and nutrition (100%RDF) practice as control. For a particular region/state, existing package of practices (POP) was fixed and followed both for conventional density and treatment densities. Compared the impact of density and nutrition level (variable factor) only, while the POP (including irrigation method and amount) was a constant for the same region/state. Details were given above on the variable factors only, viz., plant population ($S_1P_2$, $S_1P_3$, $S_2P_2$& $S_2P_3$) and nutrition levels (F1, F2 & F3). Uniform, healthy sword suckers were disinfected and planted in $1m^3$ pits as per spacing treatments. Region-specific recommended varieties and POP (nutrition, irrigation, protection, and so on) were followed for the respective research centres (Table 2). Initial soil nutrient status was estimated from the soil samples randomly collected

**Table 2. Variety, planting materials, conventional spacing, plant population, recommended fertilizer dose (RDF) and irrigation method followed at different centres**

| Centre | Variety & planting material | Conventional spacing & plant population/ha | Recommended dose of fertilizer (RDF/plant/crop cycle) | Irrigation method followed |
|---|---|---|---|---|
| Bhubaneswar | Grand Nain (AAA), Sucker | 1.8 m x 1.8 m, 3086 | 10 Kg FYM + 200g N + 50g $P_2O_5$ + 200g $K_2O$ | Drip |
| Gandevi | Grand Nain, Sucker | 1.8 m x 1.8 m, 3086 | 10 Kg FYM + 300g N + 90g $P_2O_5$ + 200g $K_2O$ | Drip |
| Jalgaon | Grand Nain, Sucker | 0.9x1.5x2.1m, 4444 (paired row system) | 10 Kg FYM + 200g N + 40g $P_2O_5$ + 200g $K_2O$ | Drip |
| Jorhat | Jahaji (AAA), Sucker | 1.5m x1.5m, 4444 | 12 Kg FYM + 110g N + 33 g $P_2O_5$ + 330 g $K_2O$ | Rainfed |
| Kannara | Nendran (AAB), Sucker | 2 m x 2 m, 2500 | 10 Kg FYM + 190g N + 115 g $P_2O_5$ + 300 g $K_2O$ | Basin |
| Mohanpur | Martaman (AAB), Sucker | 2 m x 2 m, 2500 | 10 Kg FYM + 200g N + 40g $P_2O_5$ + 200g $K_2O$ | Check basin |

**Table 3. Initial soil nutrient status of experimental plots at different centres**

| Centre | Organic carbon (%) | Total nitrogen (%) | Available Soil Nitrogen (N) content (kg/ha) | Available Soil Phosphorus ($P_2O_5$) content (kg/ha) | Available Soil Potassium ($K_2O$) content (kg/ha) |
|---|---|---|---|---|---|
| Bhubaneswar | 0.61 | 0.67 | 200.0 | 67.6 | 134.4 |
| Gandevi | 0.66 | - | 230.0 | 52.8 | 230.0 |
| Jorhat | 0.60 | 0.64 | 192.2 | 40.1 | 119.1 |
| Kannara | 0.70 | 0.70 | 260.0 | 55.0 | 155.0 |
| Mohanpur | 0.78 | 0.70 | 285.0 | 58.0 | 165.0 |

from experimental field during final land preparation (Table 3). Observations on growth characters (*viz*., pseudostem height (m), girth (cm), leaf number, leaf area index, days taken for shooting) and leaf nitrogen, phosphorus and potassium content (N, P & K in %) were recorded at shooting or flowering stage of the plant. The crop duration (days), finger number per bunch, finger weight (g), bunch weight (kg), yield (t/ha), TSS ($^0$B), acidity (%), shelf-life (days) of fruits, yield increase over control (%), B: C ratio and soil nutrient status (available N, $P_2O_5$and $K_2O$ in kg/ha) were recorded after harvest. Quality of fruit was analyzed as per A.O.A.C. (1984) methods. The available nitrogen was determined by using the alkaline potassium permanganate method (Subbiah

and Asija, 1956). The available soil phosphorus was estimated by Olson method (Jackson, 1967). Available soil potassium was determined by using Flame photometric method, whereas Walkley and Black's rapid titration method was used to determine the organic carbon content of the soil (Jackson, 1967). The micro-kjeldahl method as described by Black (1965) was used to estimate the leaf N content. The Leaf P content was estimated by using the Vanado-molybdate yellow colour method and the leaf K content was determined by using Flame photometry (Chapman and Pratt, 1961). The amount of nutrients applied per hectare was estimated on the basis of plant population per hectare under HDP and conventional systems and the recommended fertilizer dose (RDF) at the respective centres, considering that per ton FYM contributed 0.5 kg N, 0.2kg $P_2O_5$ and 0.5 kg $K_2O$. The amount of nutrients removed through fruit harvest from HDP (those that produced higher yield and highest B:C ratio) and conventional systems was calculated based on fruit yield and nutrient removal (6.7 kg N, 1.7 kg $P_2O_5$and 6.7 kg $K_2O$) per ton banana produce (Ganeshamurthy *et al*., 2011). Pooled data for three crop cycles' was analyzed for statistical inference by following the statistical method for RBD, as described by Gomez and Gomez (1983).

## RESULTS AND DISCUSSION

The Major objective of this study was to investigate productivity increase, if any, due to variations in per unit plant population and nutrition level. Yield increase for each region/state was estimated separately, in respect of its variety and POP only, by comparing the yield under HDP & conventional density of that particular variety. It was reflected from the observations that HDP could increase productivity in different region/state with the same variety & POP of respective region, only including intervention of HDP system.

It was observed that the plant growth characters showed significant variations (C.D. at 5%) due to density of planting and a level of nutrition at all centres (Tables 4, 5 and 6). Maximum height of the pseudostem was recorded with a planting density of 5000/ha with 100% RDF (S1P3F1) at all centres

**Table 4. Variations in pseudostem height (H in m) and girth (G in cm) at shooting stage of plant due to different planting densities and nutrition levels**

| Treat-ment | No. of Plants/ha. | Bhubaneswar | | Gandevi | | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H | G | H | G | H | G | H | G | H | G |
| S1P2F1 | 3333 plant/ ha | 2.32 | 55.12 | 1.85 | 61.18 | 1.22 | 61.27 | 3.23 | 42.25 | 2.90 | 64.38 |
| S1P2F2 | | 2.30 | 54.53 | 1.78 | 59.38 | 1.21 | 58.30 | 3.12 | 40.92 | 2.87 | 62.80 |
| S1P2F3 | | 2.28 | 53.82 | 1.73 | 58.05 | 1.18 | 59.40 | 3.06 | 41.35 | 2.83 | 62.53 |
| S1P3F1 | 5000 plant/ ha | 2.41 | 53.91 | 1.98 | 59.88 | 1.81 | 53.30 | 3.38 | 41.15 | 3.02 | 62.42 |
| S1P3F2 | | 2.39 | 51.62 | 1.89 | 59.01 | 1.76 | 55.67 | 3.29 | 40.66 | 2.99 | 61.10 |
| S1P3F3 | | 2.37 | 50.23 | 1.86 | 55.47 | 1.40 | 63.50 | 3.24 | 39.15 | 2.97 | 60.46 |
| S2P2F1 | 3086 plant/ ha | 2.31 | 56.83 | 1.79 | 60.21 | 1.58 | 57.20 | 3.23 | 43.10 | 2.87 | 65.43 |
| S2P2F2 | | 2.27 | 55.74 | 1.75 | 56.77 | 1.12 | 63.10 | 3.14 | 41.70 | 2.86 | 64.83 |
| S2P2F3 | | 2.25 | 54.95 | 1.69 | 56.33 | 1.10 | 56.10 | 3.11 | 42.25 | 2.82 | 63.19 |
| S2P3F1 | 4630 plant/ ha | 2.38 | 54.94 | 2.00 | 58.25 | 1.28 | 57.53 | 3.37 | 41.65 | 2.99 | 62.47 |
| S2P3F2 | | 2.33 | 53.91 | 1.93 | 55.99 | 1.39 | 56.10 | 3.29 | 41.15 | 2.92 | 61.73 |
| S2P3F3 | | 2.31 | 52.87 | 1.86 | 55.98 | 1.36 | 65.10 | 3.25 | 40.00 | 2.90 | 61.33 |
| Control | | 2.20 | 57.01 | 1.87 | 62.78 | 1.13 | 74.10 | 3.05 | 46.85 | 2.78 | 66.18 |
| SEm (±) | | 0.02 | 0.33 | 0.04 | 1.05 | 0.004 | 0.65 | 1.23 | 0.41 | 0.04 | 0.79 |
| C.D. at 5% | | 0.07 | 1.54 | 0.12 | 2.96 | 0.008 | 0.42 | 0.03 | 1.139 | 0.08 | 1.62 |

**Table 5. Variations in leaf number per plant and leaf area index (LAI) at shooting stage of plant due to different planting densities and nutrition levels.**

| Treat-ment | No. of Plants/ ha. | Bhubaneswar | | Gandevi | | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf/ Plant | LAI | Leaf/ Plant | LAI | Leaf/ Plant | LAI | Leaf/ Plant | LAI | Leaf/ Plant | LAI |
| S1P2F1 | 3333 | 10.59 | 3.58 | 20.89 | 1.02 | 24.65 | 2.60 | 11.00 | 5.48 | 12.70 | 3.25 |
| S1P2F2 | plant/ | 10.44 | 3.47 | 20.33 | 0.99 | 26.16 | 2.65 | 10.40 | 5.17 | 12.40 | 3.17 |
| S1P2F3 | ha | 10.32 | 3.36 | 20.04 | 0.97 | 24.33 | 2.44 | 10.20 | 5.29 | 11.90 | 3.05 |
| S1P3F1 | 5000 | 10.21 | 4.35 | 19.79 | 1.00 | 26.64 | 2.83 | 10.80 | 8.16 | 11.60 | 4.45 |
| S1P3F2 | plant/ | 9.82 | 4.22 | 19.47 | 0.98 | 25.62 | 2.58 | 10.40 | 8.11 | 11.30 | 4.35 |
| S1P3F3 | ha | 9.64 | 4.15 | 19.02 | 0.94 | 25.32 | 2.72 | 10.00 | 7.98 | 11.00 | 4.22 |
| S2P2F1 | 3086 | 11.02 | 3.26 | 20.78 | 1.00 | 23.76 | 2.45 | 11.67 | 3.53 | 13.10 | 3.11 |
| S2P2F2 | plant/ | 10.64 | 3.14 | 20.35 | 0.97 | 24.03 | 2.16 | 11.27 | 3.65 | 12.80 | 3.03 |
| S2P2F3 | ha | 10.62 | 3.09 | 19.56 | 0.90 | 23.74 | 2.49 | 10.70 | 3.47 | 12.10 | 2.87 |
| S2P3F1 | 4630 | 10.32 | 4.14 | 19.65 | 0.99 | 26.33 | 2.53 | 10.80 | 5.30 | 11.70 | 4.16 |
| S2P3F2 | plant/ | 10.27 | 4.05 | 19.50 | 0.95 | 26.05 | 2.65 | 10.40 | 5.33 | 11.40 | 4.05 |
| S2P3F3 | ha | 9.84 | 3.92 | 19.30 | 0.93 | 26.15 | 2.71 | 10.50 | 5.14 | 11.00 | 3.91 |
| Control | | 11.24 | 2.70 | 21.53 | 1.04 | 21.45 | 6.48 | 13.50 | 1.72 | 13.60 | 2.61 |
| SEm (±) | | 0.17 | 0.17 | 0.42 | 0.02 | 0.53 | 0.22 | 0.31 | 0.56 | 0.27 | 0.50 |
| C.D. at 5% | | 0.52 | 0.52 | 1.17 | 0.07 | 1.10 | 0.46 | 0.92 | 1.20 | 0.55 | 1.01 |

**Table 6. Variations in days taken for shooting (DS in days) and crop duration (CD in days) due to different planting densities and nutrition levels.**

| Treat-ment | No. of Plants/ha. | Bhubaneswar | | Gandevi | | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DS | CD | DS | CD | DS | CD | DS | CD | DS | CD |
| S1P2F1 | 3333 | 217.4 | 309.3 | 288.5 | 398.1 | 254.7 | 341.7 | 247.5 | 336.1 | 297.1 | 389.1 |
| S1P2F2 | plant/ | 214.3 | 304.2 | 294.8 | 390.3 | 253.3 | 342.9 | 248.2 | 338.7 | 293.3 | 383.3 |
| S1P2F3 | ha | 212.5 | 299.3 | 298.9 | 402.8 | 252.0 | 341.2 | 258.5 | 343.7 | 292.6 | 379.6 |
| S1P3F1 | 5000 | 240.4 | 338.4 | 312.2 | 413.0 | 279.7 | 335.4 | 259.6 | 348.1 | 309.7 | 407.7 |
| S1P3F2 | plant/ | 236.7 | 332.7 | 302.7 | 421.5 | 281.7 | 352.7 | 259.5 | 347.5 | 303.4 | 399.4 |
| S1P3F3 | ha | 219.3 | 312.7 | 311.4 | 420.8 | 284.7 | 350.1 | 265.8 | 354.1 | 298.6 | 391.6 |
| S2P2F1 | 3086 | 218.6 | 308.4 | 291.2 | 393.7 | 245.0 | 332.1 | 239.6 | 329.5 | 295.3 | 385.3 |
| S2P2F2 | plant/ | 216.8 | 303.4 | 285.3 | 383.2 | 242.0 | 326.0 | 240.7 | 330.2 | 293.5 | 380.5 |
| S2P2F3 | ha | 215.5 | 299.3 | 293.8 | 407.8 | 240.7 | 322.9 | 247.6 | 336.1 | 296.1 | 380.1 |
| S2P3F1 | 4630 | 236.6 | 331.5 | 313.7 | 411.5 | 270.7 | 351.0 | 251.5 | 339.5 | 306.4 | 401.4 |
| S2P3F2 | plant/ | 237.4 | 329.8 | 313.3 | 415.7 | 272.3 | 351.6 | 257.3 | 345.3 | 304.2 | 396.2 |
| S2P3F3 | ha | 219.8 | 307.9 | 324.0 | 414.5 | 269.7 | 347.2 | 259.8 | 348.5 | 299.9 | 387.9 |
| Control | | 210.3 | 296.0 | 291.5 | 378.1 | 230.7 | 328.0 | 219.3 | 309.3 | 275.4 | 374.5 |
| SEm (±) | | 3.63 | 5.23 | 6.73 | 6.90 | 0.86 | 0.57 | 1.26 | 1.80 | 8.57 | 8.57 |
| C.D. at 5% | | 10.60 | 15.50 | 19.65 | 19.40 | 1.77 | 1.25 | 4.55 | 5.28 | 17.50 | 17.50 |

except at Gandevi, whereas maximum girth of pseudostem was recorded with conventional planting density and nutrition at all centres. Leaf number per plant at the shooting stage was recorded to be maximum in conventional planting at all centres except Jorhat, however, the leaf area index was recorded to be maximum in highest density of planting with 100% RDF (S1P2F1) at the Bhubaneswar, Kannara and Mohanpur centres. It was seen that more time (days) was required from planting to harvesting under the higher density of planting (5000 plant/ha), when compared to a lower plant population of 3086, 3333 and 4630 /ha. Such variations in plant growth characters *viz*., increase in pseudostem height, leaf area index, durations for shooting and harvesting, but reduction in pseudostem girth and leaf number per plant at shooting, as a result of high-density planting in banana were also established by the findings of Rodriguez *et al.* (2007), Thippesha *et al.* (2007), Pujari *et al*. (2011) and Debnath *et al*. (2015).

Fruit yield and quality parameters were found to vary significantly due to different densities of planting and nutrition levels, across the centres (Tables 7,8 and 9). Finger number per bunch was recorded as being higher under lower density of planting, including control, at all centres except Jalgaon. Similarly, the weight of an individual bunch was also higher under lower density of planting. But the total fruit yield per unit area, that is, the productivity of banana showed steady increase due to increase in the density of planting. Maximum content of total soluble solids and shelf life of fruit were recorded under conventional plant density and nutrition at the Bhubaneswar, Jorhat and Mohanpur centres, whereas non-significant effect was recorded on the total soluble solids content of the fruit at Gandevi and Kannara centres. A plant population of 5000/ha with 100% RDF resulted in maximum fruit acidity at all centres. These results corroborated with the findings of Nalina *et al*. (2000), Thippesha *et al*. (2007), Pujari *et al*. (2011) and Debnath *et al*. (2015).

The per cent increase in productivity over control due to different planting densities and nutrition levels varied from 8.3 to 50.6 at Bhubaneswar, 8.8 to 21.9 at Gandevi, 2.4 to 7.4 at Jorhat, 4.5 to 43.5 at Kannara and 5.7 to 79.0 at Mohanpur centre (Table 10). Maximum productivity and B: C ratio

**Table 7. Variations in finger number (FN) and finger weight (FW in gram) due to different planting densities and nutrition levels**

| Treat-ment | No.of Plants/ha. | Bhubaneswar | | Gandevi | | Jalgaon | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FN | FW | FN | FW | FN | FN | FW | FN | FW | FN | FW |
| S1P2F1 | 3333 plant/ ha | 128.4 | 119.3 | 129.9 | 142.8 | 154.0 | 151.3 | 145.9 | 52.4 | 166.5 | 108.7 | 122.2 |
| S1P2F2 | | 123.6 | 118.4 | 123.0 | 141.6 | 148.0 | 145.1 | 144.1 | 51.0 | 165.2 | 102.4 | 121.1 |
| S1P2F3 | | 112.7 | 116.5 | 116.5 | 133.8 | 125.0 | 150.8 | 140.6 | 50.8 | 157.4 | 96.4 | 119.8 |
| S1P3F1 | 5000 plant/ ha | 124.4 | 114.2 | 113.5 | 128.6 | 131.0 | 173.8 | 123.8 | 52.0 | 148.3 | 104.0 | 119.3 |
| S1P3F2 | | 122.0 | 112.7 | 105.1 | 128.1 | 124.0 | 177.8 | 127.1 | 50.7 | 148.1 | 100.9 | 118.0 |
| S1P3F3 | | 108.9 | 111.3 | 99.7 | 122.3 | 110.0 | 196.6 | 104.3 | 50.3 | 142.5 | 87.5 | 116.6 |
| S2P2F1 | 3086 plant/ ha | 127.3 | 125.2 | 131.0 | 146.7 | 154.0 | 159.4 | 154.5 | 52.0 | 163.4 | 107.5 | 124.8 |
| S2P2F2 | | 124.5 | 123.1 | 126.9 | 140.8 | 142.0 | 175.4 | 150.6 | 51.2 | 159.1 | 103.5 | 123.2 |
| S2P2F3 | | 118.4 | 119.8 | 121.5 | 134.3 | 127.0 | 155.4 | 155.2 | 50.5 | 151.5 | 97.8 | 120.5 |
| S2P3F1 | 4630 plant/ ha | 122.3 | 118.7 | 110.9 | 131.9 | 136.0 | 166.1 | 119.8 | 51.3 | 149.8 | 104.0 | 121.2 |
| S2P3F2 | | 120.7 | 116.4 | 108.0 | 124.7 | 125.0 | 158.9 | 112.3 | 50.7 | 144.3 | 101.0 | 119.9 |
| S2P3F3 | | 108.3 | 115.3 | 105.4 | 118.4 | 110.0 | 188.1 | 102.9 | 50.5 | 139.9 | 88.8 | 117.5 |
| Control | | 130.2 | 129.4 | 133.7 | 147.5 | 147.0 | 269.5 | 161.8 | 56.9 | 168.7 | 110.7 | 125.3 |
| SEm (±) | | 2.97 | 2.91 | 4.01 | 3.55 | 1.95 | 0.31 | 1.68 | 0.19 | 1.95 | 4.31 | 1.05 |
| C.D. at 5% | | 9.01 | 8.92 | 11.3 | 9.98 | 5.69 | 0.67 | NS | 0.53 | 3.66 | 8.81 | 2.15 |

**Table 8. Variations in bunch weight (BW in kg) and yield (Y in t/ha) of fruit due to different planting densities and nutrition levels**

| Treat-ment | No.of Plants/ha. | Bhubaneswar | | Gandevi | | Jalgaon | | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BW | Y | BW | Y | BW | Y | BW | Y | BW | Y | BW | Y |
| S1P2F1 | 3333 | 14.4 | 48.1 | 17.2 | 57.3 | 20.4 | 67.9 | 14.1 | 47.1 | 8.7 | 29.1 | 12.1 | 40.1 |
| S1P2F2 | plant/ | 13.6 | 45.4 | 16.3 | 54.2 | 19.3 | 64.3 | 14.0 | 46.7 | 8.4 | 28.1 | 11.3 | 37.5 |
| S1P2F3 | ha | 13.4 | 44.6 | 15.1 | 50.4 | 14.9 | 49.6 | 13.1 | 43.8 | 8.0 | 26.7 | 10.5 | 34.3 |
| S1P3F1 | 5000 | 12.0 | 59.8 | 15.3 | 76.5 | 18.8 | 93.8 | 15.5 | 77.6 | 7.7 | 38.5 | 11.2 | 55.7 |
| S1P3F2 | plant/ | 11.6 | 57.8 | 14.9 | 74.7 | 17.1 | 85.3 | 15.8 | 78.7 | 7.5 | 37.5 | 10.9 | 54.4 |
| S1P3F3 | ha | 10.2 | 51.2 | 14.5 | 72.3 | 13.9 | 69.3 | 16.1 | 80.2 | 7.2 | 35.8 | 9.2 | 45.6 |
| S2P2F1 | 3086 | 15.1 | 46.8 | 16.7 | 51.5 | 20.8 | 64.2 | 12.6 | 38.9 | 8.5 | 26.2 | 12.3 | 37.8 |
| S2P2F2 | plant/ | 14.3 | 44.2 | 16.1 | 49.6 | 19.6 | 60.4 | 11.4 | 35.1 | 8.1 | 25.2 | 11.6 | 35.5 |
| S2P2F3 | ha | 13.9 | 43.0 | 13.9 | 42.9 | 15.3 | 47.3 | 13.1 | 40.4 | 7.6 | 23.6 | 10.7 | 32.9 |
| S2P3F1 | 4630 | 12.0 | 55.7 | 17.3 | 80.3 | 19.6 | 90.6 | 16.5 | 76.4 | 7.7 | 35.6 | 11.3 | 52.0 |
| S2P3F2 | plant/ | 11.6 | 53.8 | 14.7 | 68.3 | 17.3 | 80.3 | 16.5 | 77.0 | 7.3 | 33.9 | 11.0 | 50.1 |
| S2P3F3 | ha | 10.7 | 49.7 | 14.1 | 65.4 | 14.3 | 66.1 | 16.6 | 77.0 | 7.1 | 32.7 | 9.5 | 43.3 |
| Control | | 15.9 | 49.0 | 20.3 | 62.8 | 21.2 | 94.2 | 17.2 | 74.7 | 10.7 | 26.9 | 12.4 | 31.1 |
| SEm (±) | | 0.52 | 0.60 | 0.71 | 2.45 | 0.90 | 3.31 | 0.08 | 0.06 | 0.05 | 0.56 | 0.55 | 0.64 |
| C.D. at 5% | | 1.54 | 1.80 | 2.09 | 6.90 | 2.65 | 9.65 | 0.18 | 0.12 | 0.15 | 0.98 | 1.13 | 1.30 |

**Table 9. Variations in total soluble solids (TSS in $^0$Brix), acidity (A in %), and shelf-life (SL in days) of fruits due to different planting densities and nutrition levels**

| Treat-ment | No.of Plants/ha. | Bhubaneswar | | | Gandevi | | | Jorhat | | | Kannara | Mohanpur | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TSS | A | SL | TSS | A | SL | TSS | A | SL | TSS | TSS | A | SL |
| S1P2F1 | 3333 | 22.9 | 0.30 | 6.90 | 20.1 | 0.37 | 9.14 | 18.8 | 0.15 | 7.50 | 30.4 | 24.6 | 0.48 | 10.1 |
| S1P2F2 | plant/ | 22.3 | 0.29 | 6.70 | 19.7 | 0.35 | 9.26 | 17.9 | 0.19 | 8.33 | 30.2 | 23.8 | 0.46 | 9.70 |
| S1P2F3 | ha | 22.1 | 0.27 | 6.20 | 19.7 | 0.30 | 8.80 | 19.2 | 0.16 | 8.06 | 30.0 | 23.2 | 0.42 | 9.50 |
| S1P3F1 | 5000 | 22.7 | 0.37 | 6.80 | 19.9 | 0.39 | 8.94 | 20.2 | 0.31 | 8.80 | 30.4 | 24.4 | 0.59 | 9.80 |
| S1P3F2 | plant/ | 21.6 | 0.36 | 6.40 | 19.6 | 0.35 | 9.49 | 19.6 | 0.21 | 9.27 | 30.2 | 23.7 | 0.58 | 9.50 |
| S1P3F3 | ha | 20.9 | 0.34 | 6.00 | 19.8 | 0.32 | 9.85 | 19.5 | 0.26 | 9.08 | 29.9 | 23.1 | 0.56 | 9.10 |
| S2P2F1 | 3086 | 23.1 | 0.33 | 7.00 | 20.0 | 0.35 | 8.93 | 16.7 | 0.16 | 7.73 | 30.0 | 24.8 | 0.55 | 10.2 |
| S2P2F2 | plant/ | 23.0 | 0.33 | 6.80 | 19.6 | 0.33 | 9.03 | 13.7 | 0.21 | 8.60 | 29.8 | 24.5 | 0.55 | 9.80 |
| S2P2F3 | ha | 22.4 | 0.30 | 6.60 | 19.6 | 0.30 | 9.05 | 18.6 | 0.17 | 7.69 | 30.0 | 24.1 | 0.48 | 9.60 |
| S2P3F1 | 4630 | 22.6 | 0.36 | 6.80 | 20.1 | 0.37 | 9.33 | 19.6 | 0.35 | 7.18 | 30.1 | 24.5 | 0.55 | 9.90 |
| S2P3F2 | plant/ | 21.7 | 0.35 | 6.50 | 19.4 | 0.36 | 8.95 | 19.2 | 0.22 | 8.75 | 29.8 | 23.9 | 0.54 | 9.80 |
| S2P3F3 | ha | 21.1 | 0.32 | 6.10 | 19.6 | 0.31 | 9.00 | 21.0 | 0.23 | 8.81 | 29.6 | 23.3 | 0.51 | 9.50 |
| Control | | 23.2 | 0.31 | 7.30 | 20.1 | 0.31 | 8.97 | 24.0 | 0.14 | 11.3 | 29.5 | 25.1 | 0.49 | 10.4 |
| SEm (±) | | 0.21 | 0.01 | 0.18 | 0.23 | 0.01 | 0.20 | 0.25 | 0.01 | 0.19 | 0.2 | 0.33 | 0.05 | 0.28 |
| C.D. at 5% | | 0.64 | 0.04 | 0.54 | NS | 0.02 | 0.56 | 0.53 | 0.01 | 0.39 | NS | 0.67 | 0.10 | 0.58 |

**Table 10. Variations in yield increase over control (YI in %) and B:C ratio due to different planting densities and nutrition levels**

| Treat-ment | No.of Plants/ha. | Bhubaneswar | | Gandevi | | Jalgaon | | Jorhat | | Kannara | | Mohanpur | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | YI | BCR | YI | BCR | YI | BCR | YI | BCR | YI | BCR | YI | BCR |
| S1P2F1 | 3333 | 21.1 | 2.47 | - | 3.90 | -27.9 | 2.32 | - | 2.78 | 8.3 | 2.13 | 29.0 | 2.41 |
| S1P2F2 | plant/ | 14.4 | 2.54 | - | 4.12 | -31.7 | 2.25 | - | 3.06 | 4.5 | 2.02 | 20.7 | 2.56 |
| S1P2F3 | ha | 12.4 | 2.49 | - | 4.31 | -47.3 | 1.77 | - | 3.15 | -0.7 | 2.05 | 10.5 | 2.45 |
| S1P3F1 | 5000 | 50.6 | 2.41 | 21.9 | 4.24 | -0.4 | 2.76 | 4.0 | 3.73 | 43.5 | 2.60 | 79.0 | 2.42 |
| S1P3F2 | plant/ | 45.6 | 2.67 | 19.0 | 4.77 | -9.4 | 2.58 | 5.4 | 3.93 | 39.8 | 2.55 | 75.9 | 2.65 |
| S1P3F3 | ha | 28.9 | 2.32 | 15.2 | 5.40 | -26.4 | 2.16 | 7.4 | 4.94 | 33.5 | 2.47 | 46.5 | 2.34 |
| S2P2F1 | 3086 | 17.8 | 2.44 | - | 3.57 | -31.8 | 2.25 | - | 2.22 | -2.4 | 2.13 | 21.4 | 2.45 |
| S2P2F2 | plant/ | 11.4 | 2.57 | - | 3.84 | -35.9 | 2.16 | - | 2.15 | -6.3 | 2.02 | 14.1 | 2.54 |
| S2P2F3 | ha | 8.3 | 2.43 | - | 3.65 | -49.8 | 1.73 | - | 2.94 | -12.1 | 2.02 | 5.7 | 2.44 |
| S2P3F1 | 4630 | 40.4 | 2.34 | 27.9 | 4.76 | -3.8 | 2.75 | 2.4 | 4.00 | 32.5 | 2.52 | 67.2 | 2.31 |
| S2P3F2 | plant/ | 35.4 | 2.47 | 8.8 | 4.51 | -14.8 | 2.50 | 2.7 | 4.32 | 26.1 | 2.45 | 61.2 | 2.45 |
| S2P3F3 | ha | 25.2 | 2.29 | 4.2 | 5.00 | -29.8 | 2.15 | 3.1 | 4.40 | 21.7 | 2.41 | 39.3 | 2.28 |
| Control | | - | 2.25 | - | 4.57 | - | 2.91 | - | 3.99 | - | 1.74 | - | 2.49 |
| SEm (±) | | - | - | - | - | - | - | - | - | - | - | - | - |
| C.D. at 5% | | - | - | - | - | - | - | - | - | - | - | - | - |

were estimated due to highest planting density of 5000 plants/ha at all centres, except Jalgaon. It varied from 28.9% to 50.6% at Bhubaneswar, 15.2% to 21.9% at Gandevi, 4.0% to 7.4% at Jorhat, 33.5% to 43.5% at Kannara and 46.5% to 79.0% at Mohanpur, over the conventional system (control). However, the estimated B: C ratios varied with the levels of nutrition (50%, 75% and 100% of RDF) within the same planting density of 5000/ ha. For Kannara centre, maximum B: C ratio was 2.65 with 5000 plant/ha and 100% RDF, whereas, for Mohanpur and Bhubaneswar centres, it was 2.65 and 2.67, respectively with 5000 plants/ha and 75% RDF. In case of Jorhat and Gandevi centres, the B:C ratio was 4.94 and 5.40, respectively with 5000 plants/ha and 50% RDF. Hence, there were savings in fertilizer input by 25% at the Mohanpur and Bhubaneswar centres and by 50% at the Jorhat and Gandevi centres. It was noted that although the bunch weight of an individual plant under high density planting decreased, the total number of plants and bunches per unit area was much higher and hence the productivity much higher (Debnath *et al*., 2015). Increase in the photosynthetic canopy surface and light interception under high density planting are reported to be the major contributing factors for higher productivity in banana (Thippesha *et al.*, 2007, Debnath *et al*., 2015).

Significant variations were recorded in the soil nitrogen, phosphorus and potassium content (kg/ha) after harvesting of banana and in the leaf N, P and K content at the shooting stage of the fruit at all centres (Tables 11, 12, 13and 14). As compared with soil nutrient status after harvest inthe conventional system (control), no significant depletion was observed due to the combination of high density planting and nutrition treatment (that resulted in higher yield and maximum B:C ratio) in the available soil nitrogen content (except at the Gandevi, Jalgaon and Jorhat centres), the available soil phosphorus content (except at the Gandevi and Jalgaon centres) and the available soil potassium content (except at the Jalgaon and Jorhat centres). At the Bhubaneswar centre, maximum leaf N and P content was recorded in control, whereas it was lowest in the 5000 plants/ha with 50% RDF treatment. But leaf K content was recorded as being maximum under the 3086 plants/ha with 100% RDF treatment and lowest in the 4630 plants/ha with 50% RDF treatment. However, leaf N, P and

**Table 11. Variations in available soil nitrogen (N) content (kg/ha) after harvest due to different planting densities and nutrition levels**

| Treatment | No.of Pl./ha | Bhubaneswar | Gandevi | Jalgaon | Jorhat | Kannara | Mohanpur |
|---|---|---|---|---|---|---|---|
| S1P2F1 | 3333 | 196.0 | 259.3 | 225.0 | 276.5 | 280.0 | 281.3 |
| S1P2F2 | plant/ | 194.0 | 249.7 | 222.0 | 301.5 | 274.3 | 279.2 |
| S1P2F3 | ha | 187.0 | 244.7 | 208.0 | 234.6 | 289.0 | 272.4 |
| S1P3F1 | 5000 | 191.0 | 265.0 | 211.0 | 194.8 | 312.2 | 278.1 |
| S1P3F2 | plant/ | 185.0 | 256.1 | 209.0 | 205.7 | 356.6 | 274.2 |
| S1P3F3 | ha | 177.0 | 249.7 | 203.0 | 200.2 | 328.8 | 270.5 |
| S2P2F1 | 3086 | 198.0 | 260.2 | 220.0 | 211.6 | 285.6 | 282.3 |
| S2P2F2 | plant/ | 196.0 | 250.5 | 212.0 | 227.0 | 274.2 | 280.3 |
| S2P2F3 | ha | 189.0 | 247.3 | 209.0 | 216.6 | 269.3 | 274.8 |
| S2P3F1 | 4630 | 195.0 | 264.1 | 214.0 | 193.2 | 286.1 | 279.3 |
| S2P3F2 | plant/ | 188.0 | 248.8 | 211.0 | 204.5 | 295.0 | 276.1 |
| S2P3F3 | ha | 182.0 | 244.3 | 205.0 | 183.6 | 268.1 | 271.1 |
| Control | | 198.0 | 263.2 | 214.0 | 206.1 | 266.2 | 282.3 |
| SEm (±) | | 7.62 | - | 0.61 | 2.05 | 3.56 | 5.20 |
| C.D. at 5% | | 15.54 | - | 1.79 | 4.24 | 8.20 | 10.62 |

**Table 12. Variations in available soil phosphorus ($P_2O_5$) content (kg/ha) after harvest due to different planting densities and nutrition levels**

| Treatment | No.of Pl./ha | Bhubaneswar | Gandevi | Jalgaon | Jorhat | Kannara | Mohanpur |
|---|---|---|---|---|---|---|---|
| S1P2F1 | 3333 | 64.0 | 61.0 | 19.8 | 14.2 | 136.4 | 55.5 |
| S1P2F2 | plant/ | 62.0 | 57.8 | 19.3 | 19.2 | 139.9 | 53.4 |
| S1P2F3 | ha | 59.0 | 56.1 | 18.4 | 14.6 | 115.2 | 50.1 |
| S1P3F1 | 5000 | 60.0 | 61.8 | 19.1 | 17.9 | 129.8 | 51.2 |
| S1P3F2 | plant/ | 58.0 | 57.1 | 18.9 | 9.8 | 139.9 | 48.3 |
| S1P3F3 | ha | 55.0 | 54.8 | 17.9 | 17.7 | 128.5 | 43.6 |
| S2P2F1 | 3086 | 67.0 | 61.9 | 19.3 | 10.9 | 115.3 | 56.4 |
| S2P2F2 | plant/ | 64.0 | 57.1 | 18.9 | 10.9 | 126.4 | 54.1 |
| S2P2F3 | ha | 61.0 | 53.9 | 18.3 | 11.5 | 120.8 | 51.3 |
| S2P3F1 | 4630 | 65.0 | 61.4 | 19.2 | 12.2 | 142.6 | 53.5 |
| S2P3F2 | plant/ | 61.0 | 57.1 | 19.2 | 14.6 | 139.6 | 51.4 |
| S2P3F3 | ha | 57.0 | 54.4 | 18.2 | 18.8 | 129.2 | 45.7 |
| Control | | 69.0 | 60.7 | 19.2 | 13.3 | 70.2 | 57.6 |
| SEm (±) | | 5.20 | - | 0.11 | 0.07 | 3.69 | 4.76 |
| C.D. at 5% | | 14.04 | - | 0.31 | 0.15 | 7.25 | 9.97 |

**Table 13. Variations in available soil potassium ($K_2O$) content (kg/ha) after harvest due to different planting densities and nutrition levels**

| Treatment | No.of Pl./ha | Bhubaneswar | Gandevi | Jalgaon | Jorhat | Kannara | Mohanpur |
|---|---|---|---|---|---|---|---|
| S1P2F1 | 3333 | 129.0 | 333.9 | 631.0 | 74.4 | 397.6 | 160.2 |
| S1P2F2 | plant/ | 125.0 | 323.7 | 628.0 | 67.2 | 532.0 | 156.7 |
| S1P2F3 | ha | 120.0 | 316.3 | 614.0 | 77.3 | 425.6 | 150.4 |
| S1P3F1 | 5000 | 124.0 | 327.5 | 622.0 | 69.4 | 436.8 | 154.5 |
| S1P3F2 | plant/ | 118.0 | 319.0 | 619.0 | 50.4 | 565.6 | 151.2 |
| S1P3F3 | ha | 112.0 | 310.8 | 602.0 | 81.8 | 515.2 | 143.2 |
| S2P2F1 | 3086 | 130.0 | 334.7 | 630.0 | 87.3 | 459.2 | 161.4 |
| S2P2F2 | plant/ | 127.0 | 324.8 | 627.0 | 74.9 | 481.6 | 158.1 |
| S2P2F3 | ha | 120.0 | 317.3 | 616.0 | 86.7 | 470.4 | 151.1 |
| S2P3F1 | 4630 | 126.0 | 324.7 | 625.0 | 65.0 | 526.4 | 157.3 |
| S2P3F2 | plant/ | 123.0 | 317.2 | 624.0 | 85.2 | 487.2 | 154.3 |
| S2P3F3 | ha | 118.0 | 310.6 | 607.0 | 79.4 | 414.4 | 149.4 |
| Control | | 130.0 | 330.7 | 622.0 | 71.7 | 330.4 | 162.5 |
| SEm (±) | | 5.84 | - | 2.76 | 3.74 | 2.65 | 6.28 |
| C.D. at 5% | | 14.60 | - | 8.04 | 7.73 | 7.40 | 13.12 |

**Table 14. Variations in leaf N, P and K content (%) at the shooting stage due to different planting densities and nutrition levels**

| Treat-ment | No.of Plants/ha. | Bhubaneswar | | | Jorhat | | | Kannara | | | Mohanpur | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | N | P | K | N | P | K | N | P | K |
| S1P2F1 | 3333 | 2.76 | 0.33 | 3.59 | 2.83 | 0.29 | 4.23 | 2.41 | 0.04 | 0.49 | 2.82 | 0.28 | 3.71 |
| S1P2F2 | plant/ | 2.72 | 0.32 | 3.57 | 3.21 | 0.35 | 5.31 | 1.95 | 0.13 | 0.84 | 2.78 | 0.26 | 3.69 |
| S1P2F3 | ha | 2.62 | 0.27 | 3.50 | 2.93 | 0.19 | 4.84 | 2.31 | 0.14 | 0.83 | 2.68 | 0.23 | 3.60 |
| S1P3F1 | 5000 | 2.74 | 0.33 | 3.60 | 3.04 | 0.20 | 4.88 | 2.28 | 0.11 | 0.85 | 2.80 | 0.26 | 3.70 |
| S1P3F2 | plant/ | 2.67 | 0.29 | 3.58 | 3.04 | 0.14 | 5.44 | 1.67 | 0.13 | 0.78 | 2.73 | 0.24 | 3.62 |
| S1P3F3 | ha | 2.58 | 0.23 | 3.48 | 4.06 | 0.18 | 5.84 | 2.10 | 0.09 | 0.74 | 2.64 | 0.18 | 3.52 |
| S2P2F1 | 3086 | 2.77 | 0.32 | 3.68 | 2.43 | 0.25 | 4.34 | 1.72 | 0.14 | 0.79 | 2.83 | 0.27 | 3.71 |
| S2P2F2 | plant/ | 2.70 | 0.30 | 3.62 | 2.71 | 0.36 | 3.13 | 2.48 | 0.11 | 0.83 | 2.76 | 0.25 | 3.65 |
| S2P2F3 | ha | 2.64 | 0.27 | 3.58 | 2.75 | 0.32 | 4.71 | 2.41 | 0.05 | 0.59 | 2.70 | 0.22 | 3.61 |
| S2P3F1 | 4630 | 2.77 | 0.32 | 3.55 | 2.85 | 0.33 | 4.80 | 2.49 | 0.08 | 0.71 | 2.81 | 0.27 | 3.68 |
| S2P3F2 | plant/ | 2.71 | 0.30 | 3.51 | 2.32 | 0.34 | 4.21 | 2.42 | 0.05 | 0.57 | 2.74 | 0.25 | 3.64 |
| S2P3F3 | ha | 2.63 | 0.24 | 3.41 | 3.41 | 0.23 | 5.34 | 2.58 | 0.04 | 0.66 | 2.66 | 0.19 | 3.54 |
| Control | | 2.79 | 0.34 | 3.65 | 3.45 | 0.30 | 3.11 | 2.81 | 0.04 | 0.50 | 2.85 | 0.20 | 3.72 |
| SEm (±) | | 0.01 | 0.01 | 0.01 | 0.02 | 0.03 | 0.02 | 0.01 | 0.01 | 0.32 | 0.01 | 0.01 | 0.67 |
| C.D. at 5% | | 0.01 | 0.02 | 0.02 | 0.03 | 0.06 | 0.04 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 |

K content showed no specific trend at the Jorhat and Kannara centres. At the Mohanpur centre, minimum leaf N, P and K content was recorded under the highest planting density (5000 plants/ha) with the lowest nutrition level (50% RDF). The nutrients applied to and nutrients removed through fruit harvest from HDP (producing higher yield and highest B:C ratio) and conventional systems were calculated and are presented in Table 15. Under the HDP system, the region-specific, per-plant RDF was increased in proportion to the increase in plant population per unit area, therefore, any remarkable

**Table 15. Nutrient applied to and nutrient removed through fruit harvest from HDP (producing higher yield and highest B:C ratio) and conventional system (control)**

| Centre | HDP producing higher yield and highest B:C ratio | | | | | | Conventional system producing lower yield and lower B:C ratio | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nutrient applied (kg/ha)* | | | Nutrient removed through fruit harvest (kg/ha)** | | | Nutrient applied (kg/ha)* | | | Nutrient removed through fruit harvest (kg/ha)** | | |
| | N | $P_2O_5$ | $K_2O$ | N | $P_2O_5$ | $K_2O$ | N | $P_2O_5$ | $K_2O$ | N | $P_2O_5$ | $K_2O$ |
| Bhubaneswar | 1375 | 550 | 1500 | 387 | 98 | 550 | 848 | 339 | 926 | 266 | 67 | 266 |
| Gandevi | 1875 | 750 | 1500 | 484 | 123 | 750 | 1157 | 463 | 926 | 421 | 107 | 421 |
| Jorhat | 1000 | 525 | 2250 | 537 | 136 | 400 | 889 | 467 | 1999 | 500 | 127 | 500 |
| Kannara | 1325 | 875 | 2000 | 258 | 65 | 530 | 662 | 437 | 1000 | 180 | 46 | 180 |
| Mohanpur | 1375 | 500 | 1500 | 364 | 92 | 550 | 687 | 250 | 750 | 208 | 53 | 208 |

*Estimated on the basis of RDF and planting density in HDP and conventional systems.

**Calculated as per Ganeshamurthy *et al*. (7) and fruit yield from HDP and conventional systems.

depletion in soil and plant nutrient status may not have shown at many centres. It was observed by Debnath *et al*. (6) that the root zone of plants under the high density planting system had more density of effective feeder roots compared to the root zone of plants under the conventional (low density) planting system and it indicated better uptake of applied manures and fertilizers. In present study, the site-specific application of nutrients (RDF) to the high-density feeder root zones of banana plants under HDP might have caused better utilization of applied nutrients, resulting in 25%-50% savings of RDF.

## CONCLUSION

The results of this experiment showed that high density planting (HDP:5000plants/ha) of banana, accommodating three suckers per hill at 2m x3m spacing,increased productivity over the conventional planting system at the Bhubaneswar, Gandevi, Jorhat, Kannara and Mohanpur centres. Under the HDP system, the nutrient requirement was 100% RDF at the Kannara centre, 75% RDF at the Bhubaneswar and Mohanpur centres and 50% RDF at the Gandevi and Jorhat centres. This indicated a savings in cost of fertilizer input by 25% at the Bhubaneswar and Mohanpur centres and by 50% at the Gandevi and Jorhat centres. It was therefore, recommended that HDP (5000 plants/ha) in banana be adopted, accommodating three suckers per hill at 2m x3m (6.6 ft x 3.8 ft) spacing with 50% RDF in the agro-climatic region of Gandevi and Jorhat, with 75% RDF in the agro-climatic region of Bhubaneswar and Mohanpur and with 100% RDF in the agro-climatic region of Kannara for higher productivity and return on investment to farmers.

## ACKNOWLEDGEMENTS

This work was carried out with the financial support of the Indian Council of Agricultural Research (ICAR) and the collaborative organizations (SAUs/ICAR Institutes) under ICAR-All India Coordinated Research Project (AICRP) on Fruits. The authors wish to acknowledge the ICAR and the collaborative organizations for providing the required research facilities and support.

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***(Received on 05.02.2021, Revised on 02.12.2021 and Accepted on 06.12.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 164-176, 2021*

***Original Research Paper***

# Mineral nutrient composition in leaf and root tissues of fifteen polyembryonic mango genotypes grown under varying levels of salinity

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## ABSTRACT

**Mango (*Mangifera indica* L.) is sensitive to salinity and its cultivation in salt affected area is declining day by day. There is a need to find out the rootstocks to sustain under saline conditions which can be used for commercial cultivation of superior cultivars through grafting. To achieve this, the present study was carried out to understand the salt tolerance and sensitive nature of fifteen polyembryonic mango rootstock seedlings (EC-95862, Bappakkai, Vellaikolamban, Nekkare, Turpentine, Muvandan, Kurukkan, Kensington, Olour, Manipur, Deorakhio, Vattam, Mylepelian, Sabre and Kitchener) which were exposed to 0 mM, 25 mM, 50 mM and 100 mM concentration of NaCl+$CaCl_2$ (1:1) salt. The output of this study revealed that there was reduction in $K^+$, $Ca^{++}$, $Fe^{++}$ and $Zn^{++}$ while the content of $Cu^{++}$ and $Mn^{++}$ in both leaf and root tissues were found to increase with gradual increase in salt concentration from 0 to 100 mM. The overall results of this study revealed that the salinity stress caused the alterations in mineral nutrient composition of polyembryonic mango genotypes. Among the fifteen genotypes, the seedlings of Turpentine, Deorakhio, Olour and Bappakkai responded better in maintaining the mineral nutrient status in leaf and root tissues under higher level of salinity.**

**Key words**: $K^+/Na^+$ ratio, mango rootstocks, $Na^+$ and $Cl^-$ accumulation, nutrient composition and salinity

## INTRODUCTION

Mango (*Mangifera indica* L.) is the choicest fruit among Indians. It is commercially grown in tropical and subtropical regions of India. Currently, mango is grown on 2.26 million hectares land with annual production of 21.82 million MT fruits (NHB, 2018). India occupies the first position in mango production, but its low productivity of around 7.3 tonnes $ha^{-1}$ is a matter of great concern. Various abiotic stresses such as drought, salinity, high/low temperature are becoming serious issues for crop production and decline in productivity (Qin *et al.,* 2010). Among these abiotic stresses, salinity is a major problem that affected nearly 20% of the agricultural land (Nellemann, 2009). Mango is considered as a salt sensitive crop (Cooper *et al.,*1952). So, growing mango in salt affected soil and saline irrigation water is becoming a challenging issue to the farmers. Though there are several strategies like leaching, application of high-quality irrigation water, amendments of coarse organic matter, *etc.* available to maintain soil and plant health under saline condition, these are expensive and temporary. Using salt tolerant rootstock would be better option to grow mango in salt affected soils. Several cations ($Na^+$, $Ca^{++}$, $Mg^{++}$) and anions ($Cl^-$, $SO_4^{-}$, $CO_3^{2-}$, etc.) are allied to salinity which are predominantly involved in unbalancing the other essential nutrients required for normal growth and development of plants. These cations and anions at higher concentration may have antagonistic effect on other essential minerals and nutrients. Out of which $Na^+$ and $Cl^-$ are considered as most detrimental to the soil as well as plant system if their accumulation increases in saline conditions (Hasegawa *et al.,* 2000). Thus, the mechanism involved in salt tolerance with response to mineral nutrients depends on the toxic ion exclusion, extrusion and salt dilution capacity of

particular genotypes. So, keeping in mind all these facts the present investigation was carried out to understand the salt tolerance and sensitive nature of fifteen polyembryonic mango genotypes by studying their mineral nutrient composition.

## MATERIALS AND METHODS

### Planting material and growing conditions

The present investigation was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India (12.97°N and 77.56°E) during the years 2015-2017 by growing the seedling plants of fifteen polyembryonic mango genotypes (EC-95862, Bappakkai, Vellaikolamban, Nekkare, Turpentine, Muvandan, Kurukkan, Kensington, Olour, Manipur, Deorakhio, Vattam, Mylepelian, Sabre and Kitchener) under different salinity conditions. The 15 day old germinated seedlings were transplanted in polythene bags (15 x 25 cm) filled with 1.5 kg soil mixture consisting of soil, sand and FYM (1:1:1, w/w) having EC 0.36 $dSm^{-1}$, pH 6.89 and organic carbon 0.53%. These seedlings were grown under polyhouse for four and half months. Then raised seedlings were subjected to salinity stress by irrigating with 0 mM, 25 mM, 50 mM and 100 mM solutions of NaCl+$CaCl_2$ (1:1, w/w) up to saturation level (approximately 150 ml/plant) to maintain optimum soil moisture. The irrigation was given at an interval of four days for 40 days. When visual symptoms of salinity stress as marginal leaf burning started to appear on leaves after 40 days under 100 mM salinity stress, the seedlings were uprooted for destructive sampling. Leaf and root samples were thoroughly washed first with tap water, later with distilled water after uprooting from the experimental pots followed by drying at 67°C in a hot air oven. The dried leaf (with leaf petiole) and root samples of each genotype were ground with a grinder until they became fine powder that was used for nutrient analysis.

### Di-acid digestion protocol

The powdered samples (1.0 g) of leaves and roots were transferred into a conical flask. Di-acid mixture was prepared using nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) in the ratio of 9:4 (v/v). About 10 mL of di-acid mixture was poured into the conical flask containing leaf or root samples and kept overnight for pre-digestion. Next day, that solution was kept on a hot plate at 100°C in the digestion chamber for initial 1 hour followed by an increase in temperature up to 200°C for 2-3 hours till the solution became colorless and white precipitate. The volume of the solution was made up to 100 mL with distilled water and filtered through 'Whatman No. 1' filter paper. These samples were used for mineral and nutrient analysis.

### Mineral nutrient estimation

The $Ca^{++}$content of the di-acid digested samples was estimated using Atomic Absorption Spectrophotometry (AAS) (Sarma *et al.,* 1987). The content of potassium and sodium were estimated using 'Flame Photometer' as procedure mentioned by Jackson (1973). The content of micronutrients ($Fe^{++}$, $Mn^{++}$, $Zn^{++}$, and $Cu^{++}$) of the di-acid digested samples were estimated using Atomic Absorption Spectrophotometry (AAS) (Sarma *et al.,* 1987). The content of chloride in leaf and root samples were determined by the procedure of Skoog *et al.* (1996) where in chloride of leaf and root samples were extracted by adding 50 mL of distilled water to 0.5 g plant tissue and shaking for one hour and filtering through qualitative filter paper (Whatman grade 2, 11cm). Chloride percentage was calculated from the titre value after titrating 10 mL of aliquot of filtered sample against 0.02 N $AgNO_3$ using $K_2CrO_4$ as indicator till the end point attained.

### Statistical analysis

The experiment was laid out in Factorial Completely Randomized Design with six plants in each genotype for individual treatment. For nutrient analysis the randomly collected samples were replicated thrice. The data was analyzed using statistical software SAS 9.3 version and one way analysis of variance (ANOVA) was followed. Significant differences were compared using Fisher's test at P d" 0.05.

## RESULTS AND DISCUSSION

### Sodium and chloride alterations

Content of $Na^+$ in leaves and roots differed among the genotypes. In general the $Na^+$ concentration markedly increased with graded level of salinity and the increase was more in root portion when compared to leaf tissue. In leaf tissue (Table 1) under 25 and 50 mM concentration, the maximum $Na^+$ content was recorded in Kitchener (0.225%) and Vellaikolamban (0.510%), respectively. Whereas, the minimum (0.158%) was recorded in Turpentine and Deorakhio at 25 mM and 0.234% in Turpentine at 50 mM salt stress. The higher

**Table 1. Content of sodium ($Na^+$) and chloride ($Cl^-$) in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**

| Rootstock Seedlings | Sodium (%) | | | | Chloride ((%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 mM | 25 mM | 50 mM | 100 mM | 0 mM | 25 mM | 50 mM | 100 mM |
| EC-95862 | 0.153$^{ab}$ | 0.173$^{b}$ | 0.297$^{cde}$ | 0.448$^{abcde}$ | 1.217 | 1.379$^{abcde}$ | 2.257 | 2.768$^{abc}$ |
| Vattam | 0.138$^{abcd}$ | 0.160$^{b}$ | 0.258$^{def}$ | 0.418$^{cde}$ | 1.115 | 1.179$^{de}$ | 2.198 | 2.487$^{cde}$ |
| Vellaikolamban | 0.154$^{ab}$ | 0.180$^{b}$ | 0.510$^{a}$ | 0.525$^{a}$ | 1.255 | 1.470$^{ab}$ | 2.270 | 2.829$^{ab}$ |
| Nekkare | 0.139$^{abc}$ | 0.161$^{b}$ | 0.265$^{def}$ | 0.413$^{cde}$ | 1.143 | 1.209$^{cde}$ | 2.216 | 2.579$^{bcde}$ |
| Mylepelian | 0.157$^{a}$ | 0.220$^{a}$ | 0.504$^{a}$ | 0.516$^{ab}$ | 1.362 | 1.593$^{a}$ | 2.302 | 2.931$^{a}$ |
| Turpentine | 0.117$^{d}$ | 0.158$^{b}$ | 0.234$^{f}$ | 0.364$^{e}$ | 1.073 | 1.149$^{e}$ | 2.147 | 2.357$^{e}$ |
| Sabre | 0.154$^{ab}$ | 0.182$^{b}$ | 0.310$^{cd}$ | 0.453$^{abcde}$ | 1.287 | 1.439$^{abc}$ | 2.263 | 2.812$^{ab}$ |
| Manipur | 0.153$^{ab}$ | 0.185$^{b}$ | 0.322$^{c}$ | 0.462$^{abcd}$ | 1.240 | 1.406$^{abcd}$ | 2.260 | 2.783$^{abc}$ |
| Kitchener | 0.157$^{a}$ | 0.225$^{a}$ | 0.428$^{b}$ | 0.498$^{abc}$ | 1.312 | 1.548$^{a}$ | 2.272 | 2.905$^{a}$ |
| Kensington | 0.147$^{abc}$ | 0.165$^{b}$ | 0.275$^{cdef}$ | 0.427$^{bcde}$ | 1.190 | 1.295$^{bcde}$ | 2.227 | 2.723$^{abcd}$ |
| Olour | 0.133$^{bcd}$ | 0.159$^{b}$ | 0.252$^{ef}$ | 0.388$^{de}$ | 1.043 | 1.143$^{e}$ | 2.154 | 2.383$^{e}$ |
| Kurukkan | 0.145$^{abc}$ | 0.165$^{b}$ | 0.269$^{def}$ | 0.424$^{bcde}$ | 1.166 | 1.265$^{bcde}$ | 2.231 | 2.653$^{abcde}$ |
| Bappakkai | 0.137$^{abcd}$ | 0.161$^{b}$ | 0.261$^{def}$ | 0.401$^{cde}$ | 1.116 | 1.167$^{e}$ | 2.189 | 2.451$^{de}$ |
| Muvandan | 0.147$^{abc}$ | 0.170$^{b}$ | 0.282$^{cdef}$ | 0.435$^{abcde}$ | 1.205 | 1.373$^{abcde}$ | 2.238 | 2.744$^{abcd}$ |
| Deorakhio | 0.126$^{cd}$ | 0.158$^{b}$ | 0.235$^{f}$ | 0.368$^{de}$ | 1.103 | 1.190$^{de}$ | 2.171 | 2.450$^{de}$ |
| SE (d) | 0.010 | 0.014 | 0.026 | 0.047 | 0.125 | 0.116 | 0.071 | 0.150 |
| LSD ($P \leq 0.05$) | 0.022 | 0.029 | 0.053 | 0.096 | NS | 0.238 | NS | 0.306 |

**Note**: Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes at p= 0.05. Values represented with at least one common letter are not statistically different at $P \leq 0.05$ using Fisher's Least Significant Difference.

salinity stress (100 mM) caused maximum increase in $Na^+$ content in Vellaikolamban (0.525%) followed by Mylepelian (0.516%) and Kitchener (0.498%) whilst, the minimum was found in Turpentine (0.364%), Deorakhio (0.368%) and Olour (0.388%). The $Na^+$ content in root tissues (Table 2) of turpentine increased from 0.103% to 0.247 in 25 mM to 0.304% in 50 mM and to 0.441% at 100 mM salinity stress were the least among the rootstocks. Under 100 mM salinity stress the maximum content of $Na^+$ was found in seedlings of Mylepelian (0.629%).The increase in sodium ion concentration in plant tissues due to salinity stress was a general phenomenon noticed during the course of current study. The results of the study confirmed that genotype Turpentine followed by Deorakhio, Olour and Bappakkai had greater ability to restrict the $Na^+$ uptake from root and their translocation to the leaves. Vellaikolamban rootstock had taken up maximum $Na^+$ from root and transfer to cytoplasm in the leaf tissues while Mylepelian

accumulated more $Na^+$ in the root tissues which might be responsible for more root damage and reduction in dry matter content. This mechanism of $Na^+$ regulation by root and leaf portion in salt tolerant and sensitive mango cultivars was also described by Silva *et al*. (2004).

Marginal variation among the genotypes with respect to $Cl^-$ content in leaf and root tissues was observed and salinity stress caused significant increase in $Cl^-$ accumulation in leaf and root tissues (Table 1 and 2). The $Cl^-$ content in leaf tissues among the genotypes varied from 1.043% to 1.362% and it increased upto 1.143% in Olour and 1.593% in Mylepelian when plants exposed to 25 mM salinity stress. At 50 and 100 mM salinity stress the maximum content of $Cl^-$ was noticed in Mylepelian (2.302% and 2.931%, respectively), whilst minimum content was observed in Turpentine 2.147% at 50 mM and 2.357% at 100

**Table 2. Content of sodium ($Na^+$) and chloride ($Cl^-$) in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**

| Rootstock Seedlings | Sodium (%) | | | | Chloride ((%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 mM | 25 mM | 50 mM | 100 mM | 0 mM | 25 mM | 50 mM | 100 mM |
| EC-95862 | 0.191$^{e}$ | 0.279$^{bc}$ | 0.386$^{abc}$ | 0.549$^{abcd}$ | 0.362$^{abc}$ | 1.203$^{abcd}$ | 1.314$^{abcdef}$ | 2.336$^{bcd}$ |
| Vattam | 0.141$^{h}$ | 0.258$^{defg}$ | 0.325$^{c}$ | 0.505$^{cdef}$ | 0.322$^{bcd}$ | 1.062$^{defgh}$ | 1.176$^{efgh}$ | 2.100$^{cdefg}$ |
| Vellaikolamban | 0.240$^{bc}$ | 0.292$^{ab}$ | 0.389$^{abc}$ | 0.581$^{abc}$ | 0.397$^{a}$ | 1.288$^{abc}$ | 1.477$^{abc}$ | 2.577$^{abc}$ |
| Nekkare | 0.166$^{g}$ | 0.263$^{def}$ | 0.366$^{bc}$ | 0.522$^{bcdef}$ | 0.350$^{abcd}$ | 1.097$^{cdefg}$ | 1.196$^{efgh}$ | 2.173$^{bcdefg}$ |
| Mylepelian | 0.245$^{a}$ | 0.303$^{a}$ | 0.476$^{a}$ | 0.629$^{a}$ | 0.418$^{a}$ | 1.393$^{a}$ | 1.539$^{a}$ | 2.873$^{a}$ |
| Turpentine | 0.103$^{k}$ | 0.247$^{g}$ | 0.304$^{c}$ | 0.441$^{f}$ | 0.285$^{d}$ | 0.876$^{h}$ | 1.003$^{h}$ | 1.669$^{g}$ |
| Sabre | 0.236$^{c}$ | 0.286$^{b}$ | 0.389$^{abc}$ | 0.575$^{abc}$ | 0.385$^{ab}$ | 1.263$^{abc}$ | 1.453$^{abcd}$ | 2.455$^{abcd}$ |
| Manipur | 0.201$^{d}$ | 0.280$^{bc}$ | 0.388$^{abc}$ | 0.552$^{abcd}$ | 0.373$^{ab}$ | 1.239$^{abcd}$ | 1.389$^{abcde}$ | 2.383$^{abcd}$ |
| Kitchener | 0.241$^{ab}$ | 0.290$^{ab}$ | 0.439$^{ab}$ | 0.602$^{ab}$ | 0.420$^{a}$ | 1.344$^{ab}$ | 1.517$^{ab}$ | 2.630$^{ab}$ |
| Kensington | 0.185$^{f}$ | 0.266$^{cde}$ | 0.379$^{bc}$ | 0.529$^{bcde}$ | 0.355$^{abcd}$ | 1.148$^{bcde}$ | 1.246$^{cdefgh}$ | 2.251$^{bcde}$ |
| Olour | 0.109$^{j}$ | 0.255$^{efg}$ | 0.328$^{c}$ | 0.476$^{def}$ | 0.292$^{cd}$ | 0.901$^{gh}$ | 1.050$^{gh}$ | 1.735$^{fg}$ |
| Kurukkan | 0.117$^{i}$ | 0.261$^{defg}$ | 0.337$^{c}$ | 0.514$^{cdef}$ | 0.355$^{abcd}$ | 1.123$^{cdef}$ | 1.218$^{defgh}$ | 2.205$^{bcdef}$ |
| Bappakkai | 0.138$^{h}$ | 0.258$^{defg}$ | 0.331$^{c}$ | 0.494$^{def}$ | 0.315$^{bcd}$ | 0.980$^{efgh}$ | 1.139$^{fgh}$ | 2.030$^{defg}$ |
| Muvandan | 0.186$^{f}$ | 0.271$^{cd}$ | 0.380$^{bc}$ | 0.542$^{bcde}$ | 0.357$^{abcd}$ | 1.185$^{bcd}$ | 1.280$^{bcdefg}$ | 2.288$^{bcde}$ |
| Deorakhio | 0.109$^{j}$ | 0.252$^{fg}$ | 0.321$^{c}$ | 0.465$^{ef}$ | 0.299$^{cd}$ | 0.931$^{fgh}$ | 1.125$^{fgh}$ | 1.789$^{efg}$ |
| SE (d) | 0.0023 | 0.0069 | 0.045 | 0.039 | 0.0357 | 0.098 | 0.122 | 0.247 |
| LSD ($P \leq 0.05$) | 0.0046 | 0.0140 | 0.0918 | 0.0810 | 0.073 | 0.200 | 0.249 | 0.504 |

**Note**: Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes at P = 0.05. Values represented with at least one common letter are not statistically different at $P \leq 0.05$ using Fisher's Least Significant Difference.

mM salinity stress. With regard to root $Cl^-$ content, in 25 mM salt treated plants it increased from 0.876% to 1.39%. The $Cl^-$ content in 50 and 100 mM salinity stress increased upto 1.539% and 2.873% in Mylepelian seedlings and 1.003% and 1.669% in Turpentine genotype, respectively. The probability of toxicity through increasing concentration of chloride ions was the maximum compared to other ions which caused the specific toxicity in the plant system under saline conditions. Generally in mango, the potassium deficiency could cause the leaf scorching, but the toxicity of chloride was more pronounced to cause the leaf scorching and marginal chlorosis (Naqvi, 2007). The results of this study justified the chloride exclusion capacity of Turpentine, Olour and Deorakhio root tissues which also slowed down the translocation of $Cl^-$ to the leaf tissue that might helped in maintenance of proper growth by mitigating the toxic effect. Study of Nigam and Misra (2004) was also in support of the results of the present study that salt tolerance and sensitive capacity of rootstocks related to their $Cl^-$ exclusion ability.

**$K^+/Na^+$ ratio influenced by salinity stress**

The $K^+/Na^+$ ratio in leaves and roots is shown in Tables 3 and 4. The decreasing trend in the $K^+/Na^+$ ratio with gradual increase in salinity stress was observed. The minimum value of $K^+/Na^+$ ratio [7.574 (under 25 mM), 1.377 (under 50 mM) and 0.717 (under 100 mM)] was noticed in Mylepelian leaf tissues. The maximum was recorded in Turpentine (16.828; 4.776; and 2.603 under 25mM, 50mM and 100mM, respectively). In root tissues, the $K^+/Na^+$ ratio was reduced from 8.215 (25mM) to 0.704 (100mM) in Mylepelian and 12.126 (25mM) to 2.730 (100mM) in Turpentine. Potassium, an essential nutrient, could have contributed for lowering down the osmotic potential in plant system exclusively in roots and for maintaining water balance and turgor pressure of xylem tissues under saline conditions (Marschner, 1995). The down regulation of $K^+$ under saline condition led accumulation of $Na^+$ ions in the tissues (Bandeh-Hagh *et al.,* 2008). So, in this regard the greater capacity of Turpentine, Deorakhio and Olour to withstand higher salinity might be the result of efficiency of these rootstock seedlings for discriminatory uptake of $K^+$ over $Na^+$. The genotypes which maintained higher potassium content and lower sodium content in their tissues had the ability to tolerate the salinity stress. The rootstocks Turpentine, Deorakhio and Olour maintained higher $K^+/Na^+$ ratio in leaf and root tissues as they excluded $Na^+$ by absorbing less amounts in root and in leaf. As per Samra (1985), mango could generally accumulate about 2.5 to 3 times more sodium than other species. Hence many mango varieties are sensitive to salt. Whereas, Schmutz (2000) reported higher $K^+$ and $K^+/Na^+$ ratios in roots of *M. zeylanica* which showed tolerance to salinity.

**Nutrient composition under salinity stress**

Calcium content in leaf tissues (Table 3) at 25 mM salinity stress decreased from 2.050% to 1.670%. The genotype Turpentine showed higher amount of $Ca^{++}$content (1.665%) while the lower amount was recorded in Mylepelian (1.469%) at 50 mM salinity level. Under 100 mM salinity, the decrease in $Ca^{++}$content was noticed in Turpentine (1.467%) and Mylepelian (1.224%). The marginal decrease in $Ca^{++}$ content of root tissues (Table 4) from 0 to 25 mM was in the range of 3.628% to 2.336% in Turpentine and 2.368% to 2.038% in Mylepelian seedlings. At 100 mM salinity stress, the maximum amount of $Ca^{++}$content was recorded in Turpentine (1.626%) and minimum was in Mylepelian (1.279%). Calcium, an essential element, could not only play a greater role to contain salinity but might also have contributed significantly for normal growth and development of plant. The increasing level of salinity caused the reduction in calcium content of leaf and root tissues in all mango genotypes. The higher amount of calcium was recorded in leaf and root tissues of Turpentine followed by Deorakhio and Olour which showed the ability of these rootstocks to minimize the ion specific toxicity particularly $Na^+$ and $Cl^-$ through the maintenance of the membrane integrity (Rengel, 1992). Similar findings were also documented by Khayyat *et al.* (2016) in pomegranate.

The decreasing trend of $Fe^{++}$ content was noticed in both leaf and root tissues with gradual increase in salinity levels from 25 to 100 mM salt concentration. Root tissues depicted the higher amount of $Fe^{++}$ (Fig. 2) when compared to leaf tissues (Fig. 1). The $Fe^{++}$ content of leaf tissues decreased with increasing salinity levels as in 25 mM [328.90 ppm (Turpentine) to 234.00 ppm (Mylepelian)], in 50 mM [232.99 ppm (Turpentine) to 157.06 ppm (Mylepelian)] and 189.42 ppm (Turpentine) to 128.69 ppm (Mylepelian) in 100 mM. The amount of $Fe^{++}$ in root tissues reduced from 264.35 to 206.12 ppm in Mylepelian and 463.41 to

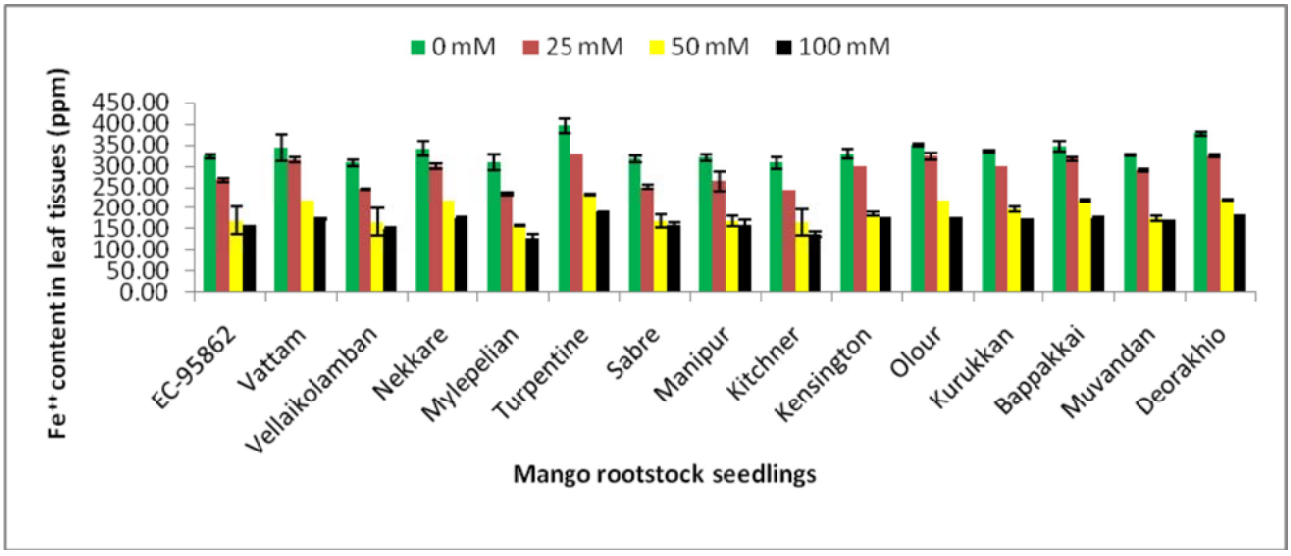
**Table 3. Content of $Ca^{++}$ and $K^+/Na^+$ in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**

| Rootstock Seedlings | $Ca^{++}$(%) | | | | $K^+/Na^+$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 mM | 25 mM | 50 mM | 100 mM | 0 mM | 25 mM | 50 mM | 100 mM |
| EC-95862 | 2.253$^{ef}$ | 1.726$^{fg}$ | 1.485$^{c}$ | 1.310$^{ef}$ | 19.198$^{def}$ | 11.861$^{cde}$ | 3.357$^{f}$ | 1.356$^{efgh}$ |
| Vattam | 2.798$^{abcd}$ | 1.923$^{bcd}$ | 1.617$^{ab}$ | 1.399$^{bc}$ | 22.364$^{bcde}$ | 13.730$^{abcd}$ | 4.248$^{abc}$ | 1.828$^{bcde}$ |
| Vellaikolamban | 2.154$^{f}$ | 1.704$^{fg}$ | 1.478$^{c}$ | 1.298$^{f}$ | 16.386$^{ef}$ | 9.789$^{ef}$ | 1.543$^{h}$ | 0.856$^{hi}$ |
| Nekkare | 2.628$^{bcde}$ | 1.924$^{bcd}$ | 1.597$^{ab}$ | 1.364$^{cd}$ | 21.864$^{cdef}$ | 13.385$^{bcd}$ | 4.100$^{bcde}$ | 1.734$^{bcde}$ |
| Mylepelian | 2.134$^{f}$ | 1.670$^{g}$ | 1.469$^{c}$ | 1.224$^{g}$ | 14.848$^{f}$ | 7.574$^{f}$ | 1.377$^{h}$ | 0.717$^{i}$ |
| Turpentine | 3.133$^{a}$ | 2.050$^{a}$ | 1.665$^{a}$ | 1.467$^{a}$ | 31.009$^{a}$ | 16.828$^{a}$ | 4.776$^{a}$ | 2.603$^{a}$ |
| Sabre | 2.157$^{f}$ | 1.723$^{fg}$ | 1.480$^{c}$ | 1.308$^{f}$ | 16.518$^{ef}$ | 11.252$^{de}$ | 2.696$^{g}$ | 1.060$^{ghi}$ |
| Manipur | 2.192$^{ef}$ | 1.725$^{fg}$ | 1.481$^{c}$ | 1.308$^{f}$ | 19.120$^{defg}$ | 10.979$^{de}$ | 2.702$^{g}$ | 1.145$^{fghi}$ |
| Kitchener | 2.140$^{f}$ | 1.694$^{g}$ | 1.472$^{c}$ | 1.291$^{f}$ | 16.129$^{ef}$ | 7.556$^{f}$ | 1.651$^{h}$ | 0.846$^{hi}$ |
| Kensington | 2.546$^{cdef}$ | 1.847$^{de}$ | 1.492$^{c}$ | 1.343$^{def}$ | 19.845$^{cdef}$ | 12.481$^{cde}$ | 3.684$^{def}$ | 1.483$^{defg}$ |
| Olour | 2.972$^{abc}$ | 1.950$^{bc}$ | 1.627$^{ab}$ | 1.429$^{ab}$ | 26.202$^{abc}$ | 16.020$^{ab}$ | 4.382$^{ab}$ | 2.110$^{abc}$ |
| Kurukkan | 2.590$^{bcdef}$ | 1.860$^{cde}$ | 1.560$^{bc}$ | 1.363$^{cde}$ | 20.417$^{defg}$ | 12.531$^{cde}$ | 3.783$^{cdef}$ | 1.646$^{cdef}$ |
| Bappakkai | 2.888$^{abc}$ | 1.927$^{bcd}$ | 1.625$^{ab}$ | 1.428$^{ab}$ | 23.947$^{bcd}$ | 14.640$^{abc}$ | 4.236$^{abcd}$ | 1.948$^{bcd}$ |
| Muvandan | 2.336$^{def}$ | 1.796$^{ef}$ | 1.487$^{c}$ | 1.312$^{def}$ | 19.986$^{cdef}$ | 12.084$^{cde}$ | 3.576$^{ef}$ | 1.410$^{defg}$ |
| Deorakhio | 3.041$^{ab}$ | 1.966$^{ab}$ | 1.660$^{a}$ | 1.439$^{ab}$ | 28.969$^{ab}$ | 16.153$^{ab}$ | 4.688$^{a}$ | 2.205$^{ab}$ |
| SE (d) | 0.228 | 0.048 | 0.047 | 0.026 | 3.397 | 1.541 | 0.275 | 0.265 |
| LSD ($P \leq 0.05$) | 0.467 | 0.099 | 0.097 | 0.053 | 6.936 | 3.147 | 0.561 | 0.541 |

**Note**: Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes at P= 0.05. Values represented with at least one common letter are not statistically different at $P \leq 0.05$ using Fisher's Least Significant Difference.

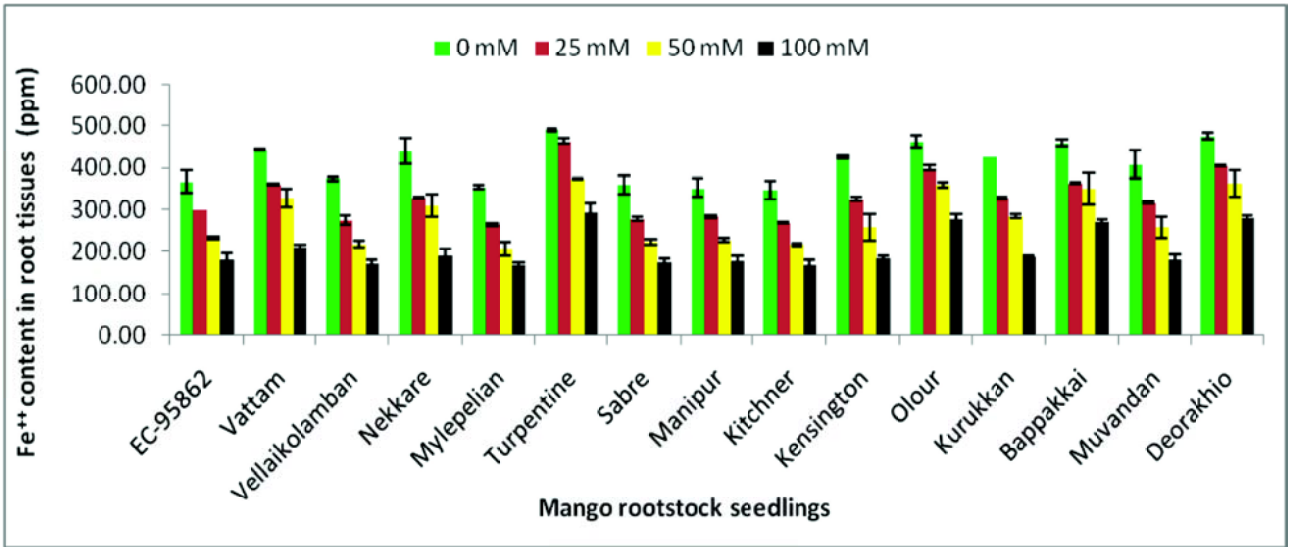
374.29 ppm in Turpentine with increase in salt stress from 25 to 50 mM. While at 100 mM salinity stress, the $Fe^{++}$content varied from 168.98 to 294.42 ppm among the genotypes. The results of the current study depicted the reduction in iron content of both leaf and root tissues with graded level of salinity stress, it might be due to the down regulation of $Fe^{++}$ due to toxicity of $Na^+$ and $Cl^-$. Iron had contributed for combating the adverse effect of salinity by reducing the level of $Na^+$ (Mozafari and Ghaderi, 2018) in grape cuttings. In leaf tissues the $Zn^{++}$ content (Fig. 3), ranged from 88.66 ppm to 125.76 ppm under 25 mM treatments while at 50 and 100 mM salinity stress it decreased from 55.17 to 47.44 ppm in Mylepelian and 97.21 to 84.07 ppm in Turpentine genotype, respectively. The content of $Zn^{++}$ in root portion (Fig. 4) reduced from 56.12 ppm (25 mM) to 53.32 ppm (50 mM) in Vellaikolamban and 80.07 ppm (25 mM) to 74.80 ppm

**Fig. 1. Iron ($Fe^{++}$) content in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Fe^{++}$ content in leaf tissues was significant at $P \leq 0.05$ (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-38.314, 25 mM-22.063, 50 mM-45.796, 100 mM-14.819)

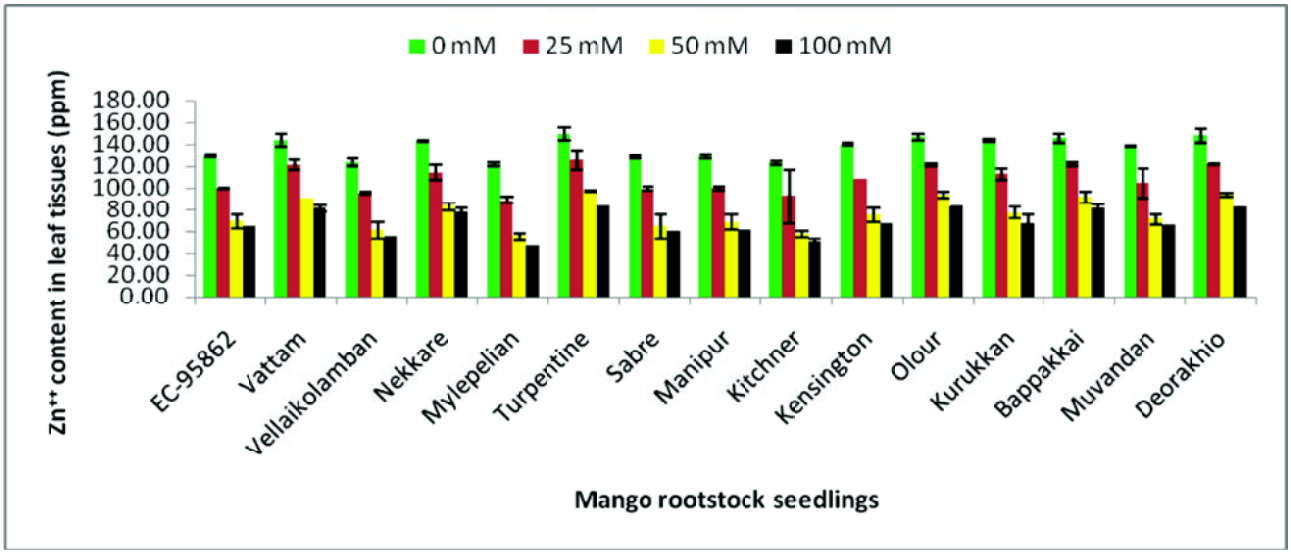
**Fig. 2. Iron ($Fe^{++}$) content in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Fe^{++}$ content in root tissues was significant at $P \leq 0.05$ (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-51.473, 25 mM-12.702, 50 mM-57.098, 100 mM-31.826)

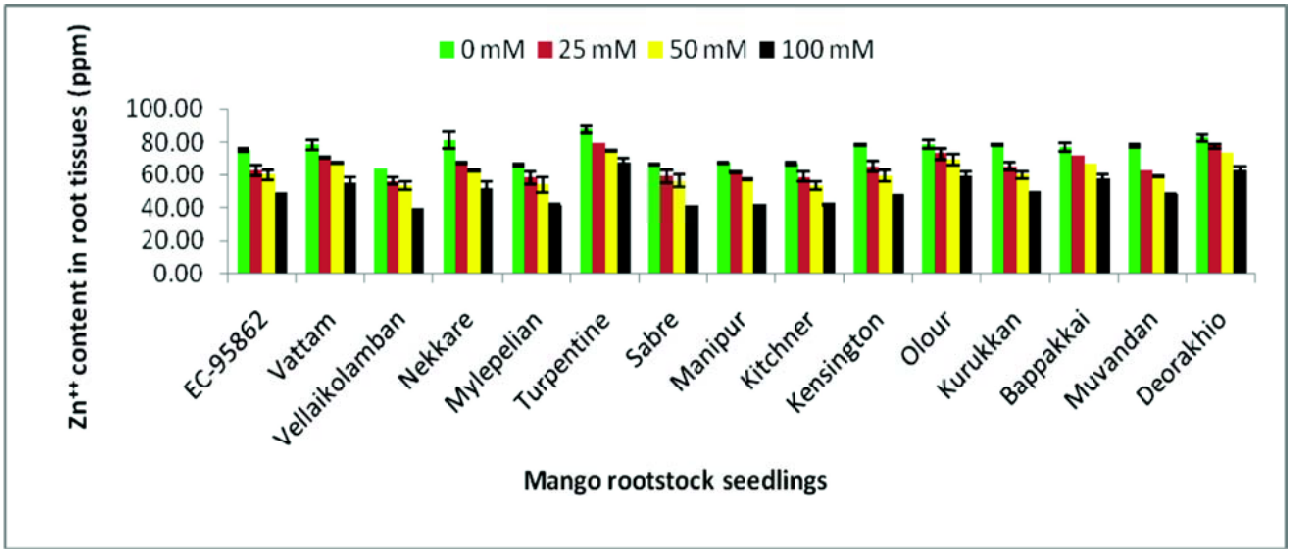
(50 mM) in Turpentine. At 100 mM salinity stress, maximum amount of $Zn^{++}$ was found in Turpentine (68.01 ppm) followed by Deorakhio (62.83 ppm) and Olour (58.93 ppm), whilst the least amount was recorded in Vellaikolamban (38.42 ppm) followed by Mylepelian (41.29 ppm) and Sabre (41.42 ppm). Zinc, an essential element, could not only involve in different metabolic activities like carbohydrate, protein, and nucleic acid synthesis but might also have contributed for activity of antioxidant enzymes during stress condition. The results indicated the reduction in $Zn^{++}$ content with increase in salinity stress. The reduction in $Zn^{++}$ under salinity might be a cause of damage to the $Zn^{++}$ carriers and pumps under higher salinity

**Fig. 3. Zinc ($Zn^{++}$) content in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Zn^{++}$ content in leaf tissues was significant at $P \leq 0.05$ (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-9.428, 25 mM-23.46, 50 mM-15.755, 100 mM-8.425)

**Fig. 4. Zinc ($Zn^{++}$) content in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Zn^{++}$ content in root tissues was significant at $P \leq$" 0.05 (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-5.878, 25 mM-6.865, 50 mM-6.987, 100 mM-5.867)

(Kholova *et al.,* 2009). The higher $Zn^{++}$ content was found in Turpentine followed by Deorakhio and Olour which showed their ability to combat the stress condition.

The $Cu^{++}$ content found to markedly increased with increasing level of salinity in both leaf and root tissues. The data about $Cu^{++}$ content in leaf tissue is presented in Fig. 5. The $Cu^{++}$ content increased under 25 mM salinity with 73.00 ppm in Turpentine, whereas 53.46 ppm in Kitchener. Under 50 and 100 mM salinity, maximum amount was noted in Turpentine (100.21 ppm and 143.31 ppm,) and the minimum in Mylepelian (68.81ppm and 104.94 ppm), respectively. With regards to root tissues (Fig. 6), under 25 mM

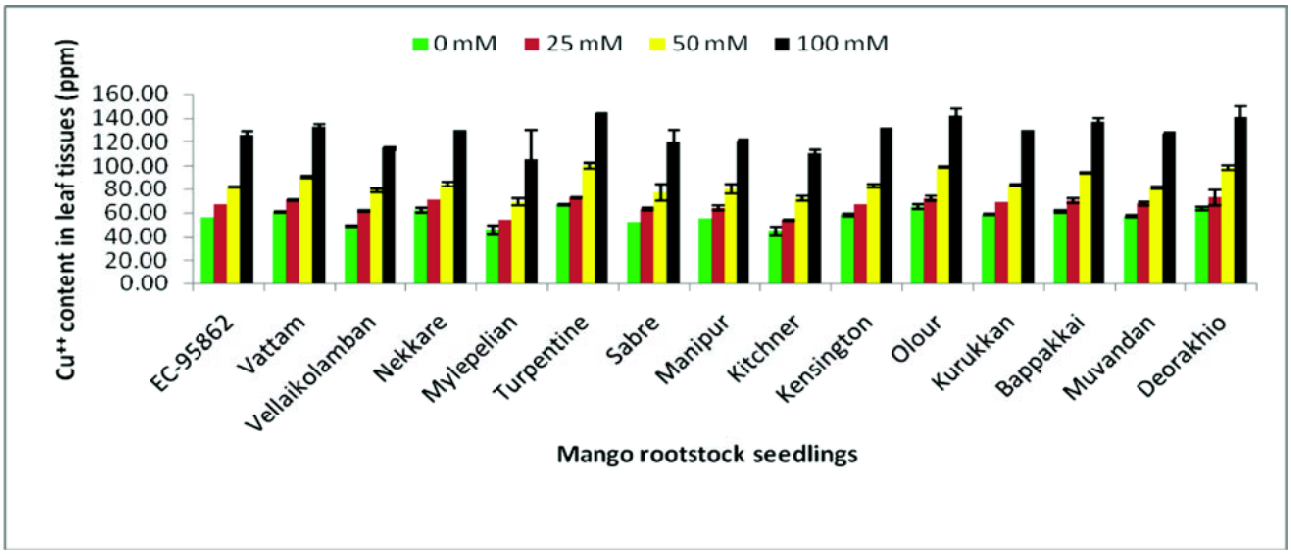
**Table 4. Content of $Ca^{++}$ and $K^+/Na^+$ ratio in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**

| Rootstock Seedlings | $Ca^{++}$(%) | | | | $K^+/Na^+$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 mM | 25 mM | 50 mM | 100 mM | 0 mM | 25 mM | 50 mM | 100 mM |
| EC-95862 | 2.507$^{efg}$ | 2.153 | 1.712 | 1.482$^{cde}$ | 14.540$^{efg}$ | 9.143$^{efgh}$ | 5.422$^{cd}$ | 1.307$^{efgh}$ |
| Vattam | 2.870$^{cd}$ | 2.253 | 1.821 | 1.524$^{bc}$ | 20.471$^{cd}$ | 10.393$^{bcde}$ | 7.222$^{ab}$ | 1.824$^{bcde}$ |
| Vellaikolamban | 2.416$^{g}$ | 2.131 | 1.695 | 1.382$^{fg}$ | 11.353$^{fg}$ | 8.611$^{gh}$ | 4.637$^{de}$ | 1.035$^{ghi}$ |
| Nekkare | 2.846$^{cde}$ | 2.179 | 1.784 | 1.522$^{bc}$ | 17.203$^{de}$ | 10.167$^{cdef}$ | 6.365$^{bc}$ | 1.666$^{cdef}$ |
| Mylepelian | 2.368$^{g}$ | 2.038 | 1.626 | 1.279$^{h}$ | 11.025$^{g}$ | 8.215$^{h}$ | 3.670$^{e}$ | 0.703$^{i}$ |
| Turpentine | 3.628$^{a}$ | 2.336 | 2.032 | 1.626$^{a}$ | 29.460$^{a}$ | 12.126$^{a}$ | 8.053$^{a}$ | 2.730$^{a}$ |
| Sabre | 2.461f$^{g}$ | 2.133 | 1.703 | 1.418$^{ef}$ | 11.720$^{fg}$ | 8.787$^{gh}$ | 4.871$^{cde}$ | 1.103$^{fghi}$ |
| Manipur | 2.492ef$^{g}$ | 2.148 | 1.706 | 1.439$^{def}$ | 13.827$^{efg}$ | 9.014$^{fgh}$ | 5.109$^{cde}$ | 1.195$^{fghi}$ |
| Kitchener | 2.402$^{g}$ | 2.098 | 1.646 | 1.314$^{gh}$ | 11.304$^{fg}$ | 8.648$^{gh}$ | 4.656$^{de}$ | 0.965$^{hi}$ |
| Kensington | 2.801$^{cdef}$ | 2.155 | 1.755 | 1.511$^{bc}$ | 15.319$^{ef}$ | 9.854$^{cdefg}$ | 5.552$^{cd}$ | 1.474$^{defgh}$ |
| Olour | 3.410$^{ab}$ | 2.311 | 1.928 | 1.543$^{bc}$ | 27.170$^{ab}$ | 10.990$^{abc}$ | 7.228$^{ab}$ | 2.169$^{abc}$ |
| Kurukkan | 2.833$^{cde}$ | 2.177 | 1.760 | 1.517$^{bc}$ | 24.257$^{bc}$ | 10.160$^{cdef}$ | 6.337$^{bc}$ | 1.610$^{cdefg}$ |
| Bappakkai | 3.131$^{bc}$ | 2.265 | 1.875 | 1.489$^{cde}$ | 21.162$^{cd}$ | 10.497$^{bcd}$ | 7.110$^{ab}$ | 1.898$^{bcd}$ |
| Muvandan | 2.586$^{defg}$ | 2.155 | 1.718 | 1.491$^{cd}$ | 15.198$^{efg}$ | 9.643$^{defg}$ | 5.531$^{cd}$ | 1.387$^{defgh}$ |
| Deorakhio | 3.248$^{b}$ | 2.317 | 1.978 | 1.577$^{ab}$ | 28.571$^{a}$ | 11.65$^{ab}$ | 7.575$^{ab}$ | 2.277$^{ab}$ |
| SE (d) | 0.175 | 0.154 | 0.158 | 0.035 | 2.102 | 0.628 | 0.758 | 0.289 |
| LSD ($P \leq 0.05$) | 0.358 | NS | NS | 0.072 | 4.2919 | 1.283 | 1.547 | 0.590 |

**Note**: Each value represents the mean value of three samples. NS indicates non significant differences among the genotypes at P= 0.05. Values represented with at least one common letter are not statistically different at $P \leq 0.05$ using Fisher's Least Significant Difference.

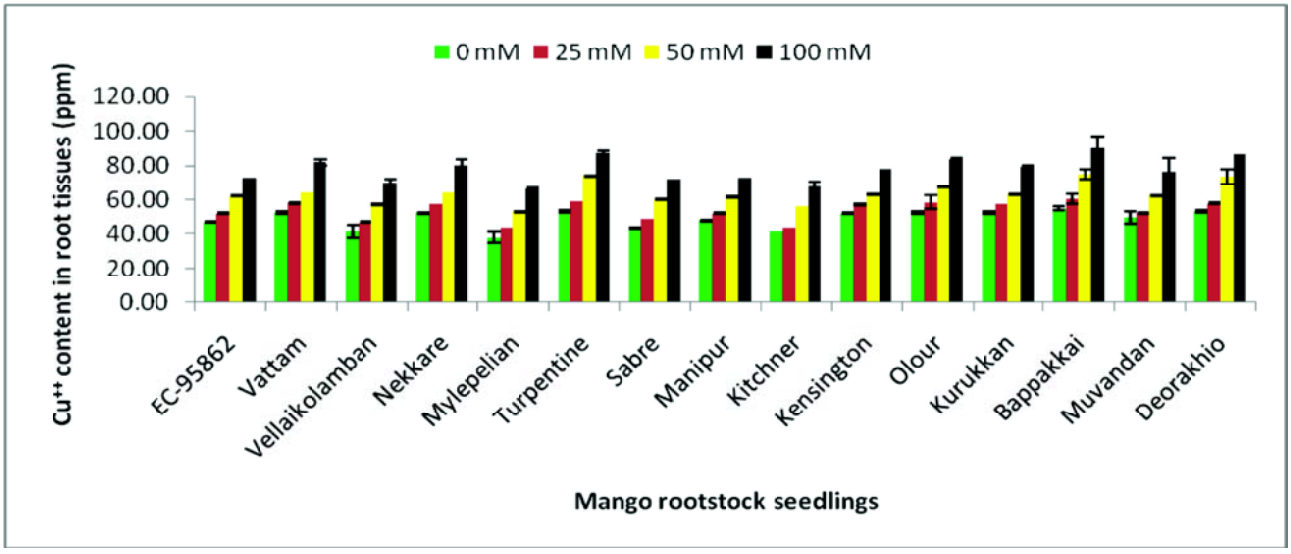
salt stress content of $Cu^{++}$ varied from 43.39 ppm (Mylepelian) to 60.23 ppm (Bappakkai). When plants were exposed to 50 and 100 mM salinity stress, lower amount of $Cu^{++}$ was noticed (53.01 ppm and 66.10 ppm) in Mylepelian. While, higher amount was recorded in Bappakkai 75.23 ppm in 50 mM and 90.34 ppm in 100 mM treatments. The $Mn^{++}$ content was also found to increase with increase in salinity levels (25-100 mM). The higher amount of $Mn^{++}$ was recorded in the leaf tissues (Fig. 7) than in root tissues (Fig. 8). The $Mn^{++}$ concentration in leaf tissue increased from 57.69 ppm to 64.120 ppm in Turpentine and 63.83 ppm to 79.60 ppm in Mylepelian when salt levels increased from 0 to 25 mM concentration. At 50 mM salinity stress, maximum amount of $Mn^{++}$ was recorded (108.88 ppm) in Mylepelian and least was (81.97 ppm) in Turepentine. Under 100 mM salinity stress the increasing trend of $Mn^{++}$was recorded 116.52 (Turpentine) to163.01 ppm (Mylepelian). In the root tissues, content of $Mn^{++}$

**Fig. 5. Copper ($Cu^{++}$) content in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Cu^{++}$ content in leaf tissues was significant at $P \leq 0.05$ (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-4.714, 25 mM-5.913, 50 mM-7.502, 100 mM-22.632)

**Fig. 6. Copper ($Cu^{++}$) content in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**

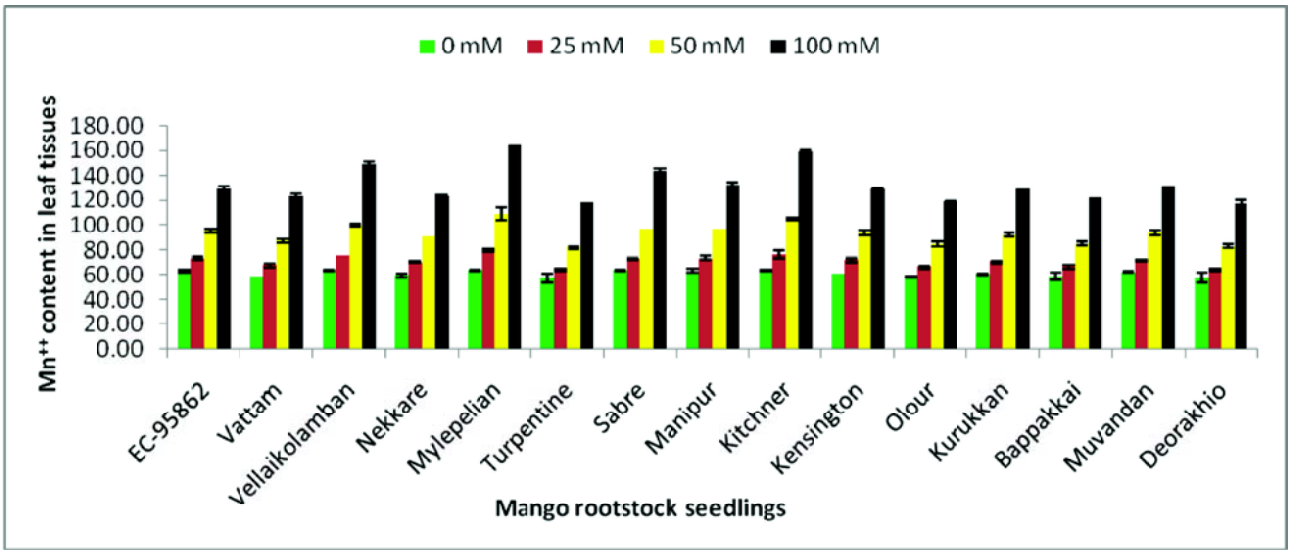



Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Cu^{++}$content in root tissues was significant at $P \leq 0.05$ (at 0 mM, 25 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5%= 0 mM-4.683, 25 mM-3.828, 50 mM-3.841, 100 mM-9.353)

increased from lower (39.33 ppm) to higher 48.70 ppm under 25 mM salinity level. The drastic increase in $Mn^{++}$ content from 58.75 to 106.22 ppm was recorded in Mylepelian when the salinity stress increased from 50 mM to 100 mM whereas the least increase was observed in Turpentine (49.43 to 65.82 ppm) at same level of salinity.

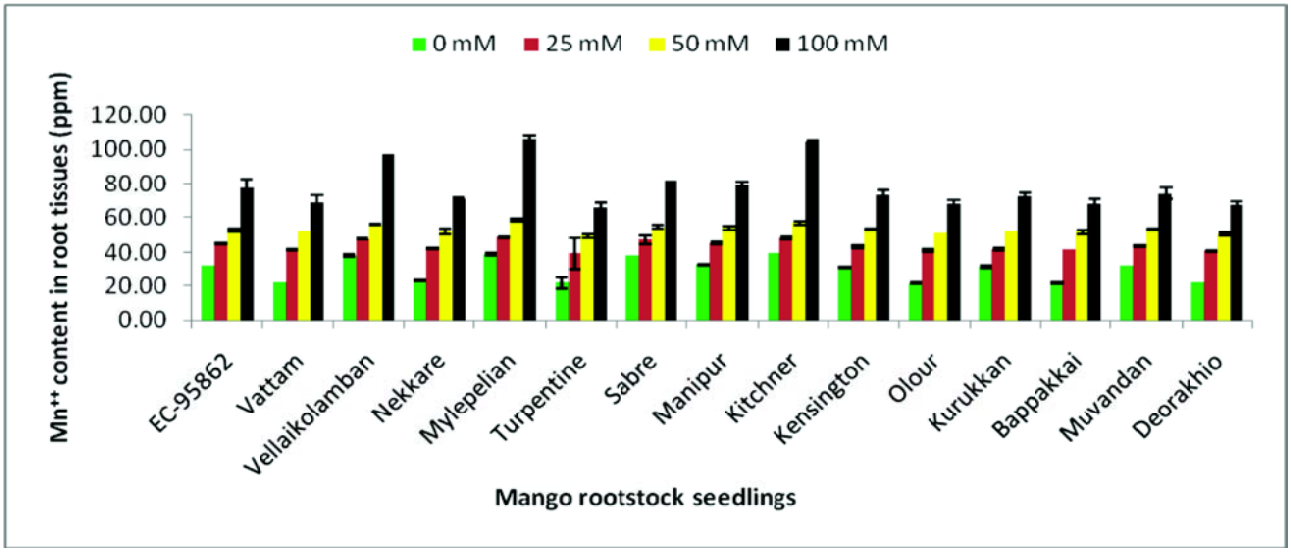
The $Cu^{++}$ in plants could play a vital role in increasing activity of several antioxidant enzymes (Lombardi and Sebastiani, 2005) through which it might have contributed for ameliorating the adverse effect of salinity by scavenging the reactive oxygen species (ROS) generation. The micronutrient $Cu^{++}$could also act as an essential element for phenolic compound

**Fig. 7. Manganese ($Mn^{++}$) content in leaf tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Mn^{++}$ content in leaf tissues was significant at $P \leq 0.05$ (at 25 mM and 50 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-NS, 25 mM-3.609, 50 mM-4.757, 100 mM-NS)

**Fig. 8. Manganese ($Mn^{++}$)content in root tissues of polyembryonic mango rootstock seedlings under different salinity levels**




Each value represents the mean value of three samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity on $Mn^{++}$ content in root tissues was significant at $P \leq 0.05$ (at 0 mM, 50 mM and 100 mM salt concentration) using Fisher's Least Significant Difference. (LSD at 5% = 0 mM-2.634, 25 mM-NS, 50 mM-2.523, 100 mM-7.765)

synthesis and its deficiency reduced the phenol content in plant tissues (Dicko *et al.,* 2006) which might have imparted defense mechanism against ROS generation (Ksouri *et al.,* 2007). Despite the fact, application of $Mn^{++}$ to plants under saline stress had improved their tolerance nature (Cramer, 1992). The toxicity of this element resulted in metabolic alterations and imbalance in ion homeostasis at cellular level (Ducic and Polle, 2005). The current findings showed the more membrane damage in the tissues of Mylepelian, Kitchener and Vellaikolamban which indicated their sensitive nature to salinity compared to tolerant genotypes like Turpentine, Deorakhio and Olour in

which the amount of $Mn^{++}$ was in sufficient amount and not in higher or toxic level.

## CONCLUSION

Results of the present study confirmed that the salinity stress (0 mM, 25 mM, 50 mM and 100 mM) has imbalanced the mineral nutrient composition in all the fifteen polyembryonic mango rootstock seedlings. Though there were variations noticed in macro and micro nutrient contents in response to salinity, yet at higher level of salinity the seedlings of Turpentine, Olour and Deorakhio have maintained balance of nutrients by avoiding the toxic effect of $Na^+$ and $Cl^-$ ions. These rootstocks are found to restrict more prominently the up-regulation of $Cl^-$ ions from root to leaf tissues and maintained the $K^+/Na^+$ ratio under salinity stress. On the basis of overall results with respect to mineral nutrient content of the fifteen rootstock seedlings have showed their tolerance nature to salinity stress in the order of Mylepelian, Kitchener, Vellaikolamban, Sabre, Manipur, EC95862, Muvandan, Kensington, Kurukkan, Nekkare, Vattam, Bappakkai, Olour, Deorakhio and Turpentine.

## ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR - Indian Institute of Horticultural Research, Bengaluru, India for providing the requisite facility during the course of research work.

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***(Received on 27.05.2021, Revised on 26.07.2021 and Accepted on 22.11.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 177-184, 2021*

***Original Research Paper***

# Optimization of $GA_3$ concentration for improved bunch and berry quality in grape cv. Crimson Seedless (*Vitis vinifera* L)

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## ABSTRACT

**Crimson Seedless is a coloured grape, gaining popularity in India for its attractive colour, bunch and berry quality with better shelf life. In cultivation of any seedless grape variety, application of $GA_3$ at different stages is very much essential to produce good quality berries and bunches. However, this variety is highly sensitive to excess application which adversely affects bunch quality. Thus, there is a need to standardize mild dose of $GA_3$ for rachis elongation which will help to reduce bunch compactness to a greater extent. Hence, an experiment was initiated to standardize concentration of $GA_3$ for rachis elongation of Crimson Seedless grapes. Three different concentrations of $GA_3$ (viz., 5 ppm, 7.5 ppm, and 10 ppm) were sprayed during pre bloom stage and compared with unsprayed control. Among different treatments, pre-bloom spray of $GA_3$@5 ppm could produce less compact bunches with highest average bunch weight, berry weight, berry length and TSS. However, bunches sprayed with 7.5 ppm and 10 ppm $GA_3$ could also produce good quality bunches, average berry weight with TSS. Because of severe coiling of rachis at 7.5 ppm and 10 ppm $GA_3$ spraying, bunches were too straggly compared to spraying of 5 ppm $GA_3$. The control bunches without $GA_3$ produced very compact clusters with less average bunch weight, berry weight, berry diameter and berry length.**

**Keywords**: Crimson Seedless, cluster compactness, fruit quality, $GA_3$, grapes and rachis elongation

## INTRODUCTION

Grape cultivation in India is highly remunerative owing to its high foreign exchange with maximum net returns to grape growers. Thompson Seedless is the preferred variety by growers and more than 70% of the area under grape cultivation is occupied by Thompson Seedless and its clonal selections like Tas-A-Ganesh, Sonaka, Manik Chaman *etc*. Though Thompson Seedless is the internationally accepted table grape across the globe, in recent years many new green and colored varieties are dominating in the export market. The important varieties are Crimson Seedless, Fantasy Seedless, Red Globe, Autumn Royal *etc*. Due to change in the international export market scenario, the area under coloured grape varieties is steadily increasing in mild tropical climatic regions of India especially in southern India. The important cultivars grown there are Flame Seedless, Sharad Seedless (Syn: Kishmish Cheyrni) and its clonal selections, Red Globe, Crimson Seedless etc. Though most of the cultural practices are similar to that of Thompson Seedless, their response is different for growth regulator application and canopy management practices. Coloured grape variety Crimson Seedless is gaining importance in recent years due to their superior quality with respect to bunch and berry parameters and extended shelf life.

Gibberellic acid (GA) is commonly used in grape cultivation to improve size of berries and length of clusters. Though grapevine cultivars shows large variation in response to applied GA, the reasons for such variations are unclear. This variation in response of different varieties to $GA_3$ might be possible due to variation in GA signalling components and/or availability of bioactive GA (Acheampong *et al.,* 2017). Unlike seeded varieties of grapes, berries of the small stenospermic grape varieties like Thompson Seedless, Flame Seedless, and Crimson Seedless etc. will have lower concentration of GA as they carry

rudimentary seed traces due to abortion of endosperm following fertilization (Cheng *et al.,* 2013). Hence, external application of $GA_3$ is routinely followed to stimulate development of berries in stenospermic varieties for commercial acceptance of berry size in addition to flower thinning and rachis elongation (Weaver, 1965; Harrell and Williams, 1987). Thompson Seedless grapes require quite higher concentration of $GA_3$ which is to be applied at different stages of cluster development to attain desirable bunch and berry qualities (Chadha and Shikhamany, 1999). Without the knowledge on concentration of $GA_3$ to be applied to Crimson Seedless, some of growers used similar concentrations as used for Thompson Seedless which resulted in adverse effects on bunch and berry quality parameters. However, application of higher concentration of $GA_3$ at different stages of berry development in Crimson Seedless grapes is found to be toxic and not advisable. Higher concentration of $GA_3$ results in excessive berry thinning (straggly clusters) and shot berry formation, as well as an unacceptable reduction in fruitfulness in the following year (Dokoozlian *et al.,* 2000). Higher concentration of $GA_3$ sometimes causes lignifications and contortion of the rachis (Aguero *et al.,* 2000). Iqbal *et al.* (2011) suggested that GA rates @ 20 g/ ac effective for berry sizing are detrimental to the productivity and fruit quality of Crimson Seedless. Hence, there was a need to optimize the concentration of $GA_3$to elongate rachis which can improve the overall bunch and berry quality parameters. Higher concentration of $GA_3$ used arbitrarily was found to have adverse effect wherein it caused severe coiling of rachis. Under tropical climatic conditions of India, no information is available on concentration of $GA_3$ to be used to improve rachis elongation in Crimson Seedless grapes. Hence, the present investigation was taken up to standardize the concentration of $GA_3$ to be sprayed at pre-bloom stage to improve bunch and berry characters.

## MATERIALS AND METHODS

This study was undertaken at the experimental vineyard of ICAR - Indian Institute of Horticultural Research (ICAR - IIHR) located at Hessaraghatta, Bengaluru during three consecutive years 2016-17 to 2018-19. It is situated at an elevation of 890 meters above sea level, $12^0$ 68' North latitude and $77^0$38' East latitude. Four year old vines of cv. Crimson Seedless grafted on Dogridge rootstock and trained on to 'Y' trellis were utilized for imposition of treatments. The spacing followed was 3.3m × 2.0m. Throughout the experiment regular soil management and plant protection practices were followed in compliance with the schedule developed for successful grape cultivation in the region. Similar to the practices followed in most of the tropical grape growing countries, the vines were pruned twice in a year once after harvest of previous crop which is popularly known as foundation pruning. This pruning usually coincides with summer season and is done to encourage canes with fruitful buds. Again on these developed canes, one more pruning was done retaining 5-6 buds per cane, encouraging cluster development which is usually called as fruit pruning. Different concentrations of $GA_3$ *viz*., 5 ppm (5 mg/L), 7.5 ppm (7.5 mg/L) and 10 ppm (10 mg/L) were sprayed at panicle emergence stage (23-28 days after pruning, EL stage 15) along with one treatment as control (water spray). The stock solution of $GA_3$ was prepared just before spraying, by dissolving 1g of $GA_3$ in 5 ml absolute alcohol and make up the volume to 1 litre using distilled water. From this stock solution desired concentrations were made with suitable dilutions. The experiment was laid out as randomized block design with 4 treatments and seven replications. Each treatment consisted of six vines. In each replication 20 clusters were tagged to record all the bunch and berry quality parameters. Berry physiochemical analysis was performed immediately after harvest. Average berry weight, berry diameter and berry length were measured as per the standard procedures using electronic balance and measuring scale. Cluster compactness was calculated using number of berries per bunch and total length of rachis and first five rachillae. Berry total soluble solids (TSS) was measured using temperature compensated refractometer calibrated at Room Temperature of 25°C. Titratable acidity was measured using titration method where in 10 ml of grape juice was titrated against 0.1 N sodium hydroxide using phenolphthalein as indicator. Peel anthocyanin concentration was estimated as per the procedure reported by Fuleki, (1969) using spectrophotometer and quantity of anthocyanin in the sample was calculated using cyanidin hydrochloride as standard and expressed as mg/100g fresh weight. Total phenol content in grape juice was estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) as per the method developed by Singleton and Rossi, (1965). Total sugar was estimated by the method developed by Somyogi, (1952) and expressed in g/100gFW.The

average of three years observations were used for statistical analysis. SPSS for Windows version 9.0 and Microsoft Excel 2003 were used to carry out statistical analysis and graphical data presentation.

## RESULTS AND DISCUSSION

Significant differences among the treatments were recorded for rachis length in response to different concentrations of $GA_3$ applied. The clusters treated with $GA_3$ @ 5 ppm recorded highest total rachis length of 124.90 cm followed by those treated with $GA_3$ @ 7.5 ppm which recorded rachis length of 89.52cm (Table 1). The least length of the rachis (55.68cm) was recorded in untreated control. Though higher rachis length of more than 124.90 cm was recorded when $GA_3$ was applied at 10 ppm, there was severe coiling of rachis which affected the bunch quality at later stages of berry development with respect to shape, appearance, lignified rachis etc. Statistically significant differences among the treatments were recorded for bunch compactness. $GA_3$ at 5 ppm recorded the less bunch compactness (0.94 berries / cm of rachis length) among all the treatments resulting in development of loose cluster, while in treatment where no $GA_3$ application was applied, it recorded maximum bunch compactness (2.59 berries/cm of rachis length)

**Table 1. Influence of different concentration of $GA_3$ on bunch characters of grape cv. Crimson Seedless (mean of three years)**

| Treatments | Total length of Rachis (cm) | Total number of berries per bunch | Bunch compactness (no. of berries/cm of rachis) | Bunch weight (g) |
|---|---|---|---|---|
| $GA_3$ at 5ppm | 124.90 | 102.40 | 00.94 | 507.42 |
| $GA_3$ at 7.5ppm | 089.50 | 110.42 | 01.26 | 482.04 |
| $GA_3$ at 10ppm | 132.90 | 119.11 | 01.11 | 499.55 |
| Control | 055.60 | 140.75 | 02.59 | 442.56 |
| SEM ± | 009.80 | 010.52 | 00.18 | 037.20 |
| CD(P=0.05) | 029.50 | NS | 00.54 | NS |

*NS: Non Significant

resulting in very tight clusters. Though $GA_3$ @ 7.5 and 10 ppm could produce loose clusters, their bunch shape was not desirable due to coiling of rachis. Application of $GA_3$ at different concentrations has brought significant changes in cluster morphological parameters like rachis length, length of internodes, rachis weight etc. Rachis elongation is the most essential phenomenon to produce loose grape bunches. Application of $GA_3$ has brought significant changes in rachis length compared to control clusters and which might be due to lot of biochemical events which takes place at cellular level. There was negative correlation (-0.743) between the total rachis length and cluster compactness (Fig 1) which means, more the rachis length the number of berries per unit length is less indicating loose clusters. The bunch morphological

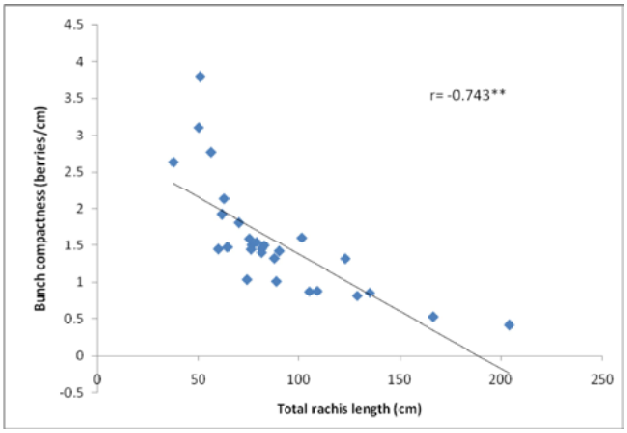


**Fig. 1. Correlation between total rachis length and cluster compactness in grape cv. Crimson Seedless**

**Correlation is significant at the 0.01 level (P<0.01)

parameters of the present experiment are in accordance with established reports on the application of $GA_3$ for improved berry and bunch characters (Looney and Wood, 1977; Molitor *et al.,* 2012; Weaver, 1958; Weaver, 1975). The rachis elongation is a complex process which requires enhanced carbon metabolism of sugar accumulation by phloem area expansion. The increased rachis elongation in our studies might be due to over expression of some proteins involves in these processes which belong to biological processes like generation of precursor metabolites, cellular protein metabolic process, responses to abiotic stimulus and protein processes (Ghule *et al.,* 2019a). The process of rachis elongation in response to applied $GA_3$ has been studied extensively at different levels *viz.,* phenotypic, physiological and transcriptomes (Domingos *et al.,* 2016; Upadhyay *et al.,* 2018). Most of these studies have indicated cell wall loosening and cell enlargement as the key physiological processes which are essential for rachis elongation to make grape clusters less compact. Schopfer (2001) and Liszkay *et al,* (2004) in their studies reported that hydroxyl radicals generated via Fenton reaction with $H_2O_2$ as the substrate which helps in cell wall loosening and cell enlargement. Similarly some of the proteins associated with cell biogenesis like IRX15-LIKE like proteins which are involved in secondary wall participate in xylan biosynthesis as they are major hemicelluloses in secondary cell walls of most of dicotyledonous plants (Brown *et al.,* 2011). Similarly, the process of cell wall elongation and wall loosening involves significant alterations in the properties of cell wall polysaccharides. Nunan *et al.* (2001) predicted the activation of some of the enzymes that participate in cell wall modification. In our study also, the protein EOCPF 1 (β galactosidase BG1) belonging to carbohydrate, monosaccharide, and galactose metabolism might have played a key role in elongating the cell wall which usually exists with other proteins, viz. pectin methylesterase, polygalacturonase, and xyloglucan endotransglycosylase.

Though no difference was recorded for total bunch weight in response to application of different concentrations of $GA_3$ which is a factor of number of berries per cluster, $GA_3$ at 5 ppm recorded maximum bunch weight (507.48g) among the all treatments while treatment without $GA_3$ application recorded the least bunch weight (442.54g). But, application of $GA_3$ brought a significant difference in individual berry weight wherein $GA_3$ @ 5 ppm registered maximum berry weight (4.93g) followed by $GA_3$@ 7.5ppm (4.85g). The least average berry weight was recorded in untreated control $T_4$ (3.98g). Some of the mechanisms proposed for $GA_3$ action are increased activity of soluble invertase (Pérez and Gómez, 2000) and subsequent change in water potential of berries and modulation of aquaporin genes by $GA_3$ (Espinoza *et al.* 2009) to increase the water content of berries during berry growth. Recent proteome and transcriptome-based analyses *(*Cheng *et al.,*2015*;* Wang *et al.,* 2012) have also shown $GA_3$-mediated modulation of several genes involved in cell expansion and cell wall modification which might be responsible for the increase in berry size and volume. In a study to see the effect of $GA_3$ on berry sizing in Thompson Seedless grapes, Ghule *et al,* (2019 b) reported the increased size of berries in $GA_3$ applied bunches and was attributed to increase level of peroxidase as early response and suppressed level of catalase and glutaredoxin as late response and concluded that berry enlargement might have influenced by expression of antioxidant enzymes such as catalase and peroxidise which was also suggested by Wang *et al.* (2017).

No significant difference was recorded for berry quality parameters like berry diameter, Total soluble solids etc (Table 2). However, titratable acidity was found to be highest in control vines (0.52%) while the least acidity (0.41%) was recorded in clusters treated with 5 ppm $GA_3$. Observations on anthocyanin concentration are presented in Table 3. Significant differences among the treatments were recorded. Among all treatments bunches treated with $GA_3$ @ 7.5ppm (247.914mg/100g) registered maximum anthocyanin concentration (Table 3) followed by $GA_3$@ 5ppm $T_1$ (177.327 mg/100g). The least anthocyanin concentration was recorded in bunches with no $GA_3$ application i.e., $T_4$ (167.143 mg/100g). The highest anthocyanin concentration in treatment with 7.5 ppm $GA_3$ might be due to its lower total sugar concentration which has exhibited negative correlation (r= -0.413, Fig 2) and vice versa in treatments with $GA_3$ @ 5 ppm and 10 ppm**.** The sugar conversion into anthocyanin biosynthesis is reported by few workers in different flowers and fruit crops as reported by Ozer *et al.* (2012). Our findings are in accordance with that of Peppi *et al.* (2006), where the application of gibberellic acid ($GA_3$) was effective at increasing the

**Table 2. Influence of different concentration of $GA_3$ on berry quality parameters of grape cv. Crimson Seedless (mean of three years)**

| Treatments | 50 berry weight (g) | Average berry weight (g) | Berry diameter (mm) | Berry length (mm) | TSS ($^0$B) | Acidity (%) |
|---|---|---|---|---|---|---|
| $GA_3$at 5 ppm | 246.41 | 4.92 | 17.31 | 25.82 | 18.52 | 0.24 |
| $GA_3$at 7.5 ppm | 242.51 | 4.81 | 17.30 | 25.43 | 17.57 | 0.32 |
| $GA_3$at 10 ppm | 233.44 | 4.63 | 17.43 | 24.64 | 17.66 | 0.41 |
| Control | 199.35 | 3.92 | 16.82 | 22.77 | 18.21 | 0.51 |
| SEM ± | 8.843 | 0.17 | 0.27 | 0.47 | 0.40 | 0.054 |
| CD (P = 0.05) | 26.27 | 0.53 | NS | 1.40 | NS | 0.16 |

*NS: Non Significant

**Table 3. Influence of different concentration of $GA_3$ on berry quality parameters of grape cv. Crimson Seedless (mean of three years)**

| Treatments | Anthocyanin concentration (mg/100g) | Total phenols (mg/100g) | Total sugars (g/100g) |
|---|---|---|---|
| $GA_3$ at 5ppm | 177.30 | 112.70 | 18.20 |
| $GA_3$ at 7.5ppm | 247.90 | 172.60 | 15.90 |
| $GA_3$ at 10ppm | 173.70 | 217.60 | 16.00 |
| Control | 167.10 | 155.10 | 17.40 |
| SEM ± | 016.50 | 023.73 | 00.38 |
| CD(P=0.05) | 049.40 | 071.06 | 01.13 |

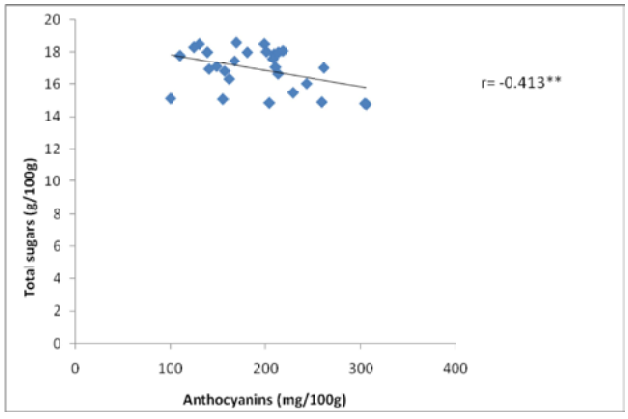


**Fig. 2. Correlation between anthocyanins and total sugars in grape cv. Crimson Seedless**

**Correlation is significant at the 0.01 level (P<0.01)

anthocyanins content of grape variety Flame Seedless. The use of higher concentrations of $GA_3$ (over 50 ppm) leads to a reduction in the content of anthocyanins in berries (Rusjan, 2010) and this in turn has an adverse effect on the organoleptic properties of varieties with red and blue color of the skin intended for consumption in fresh condition.

Significant differences among the treatments were recorded with respect to total phenol content wherein, bunches treated with $GA_3$ @ 10ppm (217.605 mg/100g) registered maximum total phenols followed by $GA_3$ @ 7.5ppm $T_2$ (172.664mg/100g). The least total phenol was recorded in clusters treated with $GA_3$ @ 5ppm $T_1$ (112.752mg/100g). $GA_3$ (highest 3 concentrations) and CPPU treatments (highest 2 concentrations) significantly increased the total phenol content of the grapes after cold storage Avenant *et al* (2017). Increased phenol content of 'Regal Seedless' was correlated with an increased astringent taste (Fraser, 2007), with serious negative implications regarding consumer preferences and market access. Application of higher concentration of GA3 might not only reduce the physical appearance of cluster with respect to lignifications of rachis but also reduce the chemical properties with respect to reduced sugar

content and more phenolic compounds as evidenced in present study which is in accordance with the findings of Avenant *et al.* (2017).

Significant differences among the treatments were recorded for total sugars. Among all treatments bunches treated with $GA_3$ at 5 ppm (18.211g/100g) registered maximum total sugars followed by bunches without $GA_3$ application (17.444g/100g). The least total sugars was recorded in bunches treated with $GA_3$ at 7.5 ppm (15.914g/100g). The increase in reducing, non-reducing and total sugars might be ascribed to the conversion of starch and acids into sugars in addition to continuous mobilization of sugars from leaves to berries (Singh *et al.,* 1993). Singh and Khanduja, (1977) further reported that the application of $GA_3$ in Pusa Seedless showed increased sugars and decreased acidity content. Application of $GA_3$ at rachis elongation stage might have stimulated internal synthesis of $GA_3$ in young berries which might have increased the sink drawing ability leading to more accumulation of sugars in treated berries than in control. The phloem loading capacity is increased or stimulated by application of $GA_3$ in many crops which helps in better translocation of photosynthates synthesized in leaves to young berries via phloem vessels. Application of $GA_3$ modifies phloem loading, phloem area and increased expression of sugar transporters to enhance carbon metabolism (Murcia *et al.,* 2016). A ten-fold increase in some of the genes involved in sugar transport and metabolism was observed in Malbec grapes compared to control. A positive correlation was observed between photosynthesis and stomatal conductance in $GA_3$ treated vines (Murcia *et al.,* 2016). Berry growth is stimulated due to increase in rate of cell division as well as cell elongation (Dokoozlian and Peacock, 2001). Plant hormones have strong effects on berry growth and development (Guerios *et al.,* 2016) among them, GAs take part in a critical function in berry sizing and enlargement (Weaver and McCune, 1960). In the last few years, the effect of exogenous $GA_3$ application on grape berry growth and cell enlargement has been studied by several researchers; however, the basic mode of action of $GA_3$ to produce maximum berry size is not very clear.

$GA_3$ applications may also have negative effects on grapevine, including excessive reduction of the number of berries per cluster, the production of grassy or herbaceous flavors in the fruit, a reduction in tissue winter hardiness and a reduction in node fruitfulness. These phytotoxic effects of GA tend to become more pronounced in the seeded varieties. Considering the above findings from the present study and other supported results from different workers, it might be summarized that $GA_3$ at 5 ppm might be optimum for bringing about desirable changes in bunch morphology in Crimson Seedless. Super or suboptimal level of $GA_3$might result in adverse effect on bunch characters.

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***(Received on 21.08.2021, Revised on 11.10.2021 and Accepted on 17.11.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 185-192, 2021*

***Original Research Paper***

# RGAP molecular marker for resistance against yellow mosaic disease in ridge gourd [*Luffa acutangula* (L.) Roxb.]

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## ABSTRACT

**Yellow mosaic disease caused by *Tomato leaf curl New Delhi virus* (ToLCNDV) causes 100 per cent losses in ridge gourd under epidemic conditions, particularly in the tropics and sub-tropics of India. Plant breeding approaches led by virus resistance marker-assisted selection have gained increased momentum in breeding for hasten the development of resistant varieties. In the present study, an effort had been made to identify molecular markers linked to yellow mosaic disease resistance loci in an $F_2$ population derived from a cross between susceptible 'Arka Prasan' and resistant 'IIHR-Sel-1' of ridge gourd. All the molecular markers were amplified in parents, and one polymorphic marker clearly distinguished the contrasting parents. The primers LaRGAP 63 produced a polymorphic DNA fragment that co-segregated with yellow mosaic disease reaction phenotypically in the $F_2$ population. The identified marker will be helpful to the breeders for introgression of resistance loci into the elite background.**

**Keywords:** molecular breeding, resistance, Ridge gourd, ToLCNDV and yellow mosaic

## INTRODUCTION

Yellow mosaic disease caused by the tomato leaf curl New Delhi virus (ToLCNDV) is a major production constraint in ridge gourd [*Luffa acutangula* (L.) Roxb.] cultivation. It results in heavy crop losses, especially in the tropical and sub-tropical regions as high disease dissemination through whitefly (*Bemisia tabaci*). It was first observed in India, causing viral disease symptoms on tomato (*Solanum lycopersicum* L.) (Padidam *et al.,* 1995), and recently it has invaded many new countries with extended host range (Zaidi *et al.,* 2016). Considering the potential of pathogen and health and environmental risk associated with pesticide use, identification, and introduction of genetic resistance in ridge gourd cultivars is a priority for breeders. Source of genetic resistance have been confirmed in gourds and other cucurbits (Islam *et al.,* 2010; Lopez *et al.,* 2015; Prasanna *et al.,* 2015a; Prasanna *et al.,* 2015b; and Saez *et al.,* 2016). Romay *et al.* (2019) reported a single recessive gene (*bgm-1*) with two independent minor genes (*Bgm-2* and *ToLCDV*) inheritance in melon accession IC-274014. Rai *et al.* (2014) reported monogenic recessive control for leaf curl virus in an inter specific cross (PBC 535 × Bhut Jolokia) of chili. In tomato, one major recessive gene (*ty-5*) with one additional gene was reported in resistant source 'Fla 8753' (Hutton *et al.,* 2012). Islam *et al.* (2010) observed single dominant gene action for yellow mosaic virus resistance in sponge gourd, DSG-6 and DSG-7 (*L. acutangula* L).

Incorporation of resistance alleles in the cultivated varieties require some biotechnological intervention as it recover resistant material fast with high precision. Molecular marker linked to various traits has been identified in gourd (Islam *et al.,* 2011; Karthik *et al.,* 2016). Genetic markers have been identified and leaf curl resistance genes were mapped on genetic map of tomato (Zamir *et al.,* 1994; Chague *et al.,* 1997; Ji *et al.,* 2007b; and Ji *et al.,* 2009). De Castro *et al.* (2007b) identified co-dominant cleaved amplified polymorphic sequence (CAPS) marker linked to tomato leaf curl virus resistance genes, *Ty-1* in tomato. The resistant genes were introgessed from *S. habrochaites*, *S. lycopersicon*, *S. pimpinellifoium*. Garcia *et al*. (2007) reported two sequence characterized amplified region (SCAR) markers (TG0302 and TY-2R1) linked to Tomato leaf curl virus resistance gene (*Ty-2*) in tomato. Ji *et al. (2007a)* detected co-dominant SCAR primer FLUW25 linked to leaf curl virus resistance genes (*Ty-3/Ty-3a*) in the LA2779 lines derived from tomato wild species *S.*

*chilense*. Identification and mapping of the mungbean yellow mosaic virus resistance gene have been reported by several researchers (Souframanien and Gopalakrishna, 2006; Gupta *et al.,* 2013; Alam*et al.*, 2014; Singh *et al.*, 2020). With this background, here we report a RGAP marker linked to yellow mosaic disease resistance in *L. acutangula* (L.) Roxb.

## MATERIALS AND METHODS

### Plant materials

This experiment was conducted in the $F_2$ population developed from a cross between Arka Prasan and IIHR-Sel-1. Arka Prasan is a commercial high yielding ridge gourd variety with long and tender fruits, however susceptible to yellow mosaic disease. *L. acutangula* genotype IIHR-Sel-1 is a yellow mosaic disease resistant advanced breeding line maintained as inbred after at least six generations of self-pollination at the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru (Kaur *et al.,* 2021b). Both the genotypes were true-to-type with highly uniform plant types due to continuous self-pollination.

Controlled crosses were made between the susceptible 'Arka Prasan' (used as a female parent; $P_1$) and the resistant 'IIHR-Sel-1' (used as a male parent; $P_2$) ridge gourd genotypes to develop $F_1$ generations. The $F_1$ population was self-pollinated to produce the subsequent $F_2$ population in the spring-summer season. In the field, 30 plants of each $P_1$, $P_2$, and $F_1$ (Arka Prasan × IIHR-Sel-1), whereas 252 plants of $F_2$ population were evaluated under high disease pressure conditions with high epidemics of whitefly, the insect vector for disease transmission during the spring-summer season under field conditions (Kaur *et al.,* 2020). Randomized complete block design with three replications was followed for current experiment. Plants were evaluated on a standardized 6-point interaction phenotype scale (Sohrab, 2005) where 0= no symptoms, 1= mild mosaic pattern in young leaves covering >10% area, 2= mosaic pattern in young leaves covering >25% area, 3 = mosaic pattern in young leaves covering>50% area, blistering and puckering of leaves, 4= widespread mosaic pattern in young leaves covering >75% area, distortion of leaves and 5= widespread mosaic pattern in young leaves covering >75% area, distortion of leaves and stunting of the plants. Eight scorings were taken at the weekly interval after the first disease symptoms (18 days after transplanting) on susceptible lines and last week data were used to link with the marker.

### DNA extraction and primer selection

DNA of parents (Arka Prasan and IIHR-Sel-1) $F_1$'s and segregating population ($F_2$) was extracted from young and healthy leaves using the procedure of cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). The two-sequence related amplified polymorphism (SRAP) primers in four combinations previously reported (Islam *et al.* 2010) were used for molecular marker validation. Fourteen resistance gene analog polymorphism (RGAP) primers were designed using Primer 3.0 from nucleotide-binding sites leucin rich repeat (NBS-LRR) sequence analogs (Karthik, 2016) and screened in the current study. PCR reaction was performed in 15.0 µl aliquots containing 10X assay buffer, 15.0 mM MgCl2, 1.0 mM dNTPs, 3U Taq DNA polymerase, five µm primers, 30 ng template DNA, and sterile double distilled water in an Eppendorf thermocycler. The PCR profile starts with an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing as per the primers for 30 sec, extension at 72°C for 1 min followed by a final extension at 72°C for 5 min and storage at 4°C. The amplified products separated on 4 % agarose gel were visualized with ethidium bromide staining under the gel documentation system (UVITECH limited, Cleaver Scientific, United Kingdom).

### Scoring of molecular profiles

The molecular markers with polymorphism between resistant and susceptible parents were selected. Primers showing polymorphism between the resistant and susceptible parents were used to screen the segregating $F_2$ population. The goodness of fit of the observation from the $F_2$ plants' genotyping with the expected Mendelian ratio was tested using chi-square analysis (Panse and Sukhatme, 1985).

### Cloning and sequencing of PCR amplicons

Target amplified products of predicted size were gel excised and purified using MinElute Gel extraction kit (Cat# 28704, QIAGEN, GmbH and Hilden, Germany). The purified PCR products were cloned into the plasmid vector pTZ57R/T using InsT/A clone PCR product cloning kit (Cat# K 1214, MBI, Fermentas) following the manufacturer's instruction and transformed into competent cells of *E. coli* strain

DH5a using the standard heat-shock method. Recombinant clones were selected through blue/ white colony screening on LB plates supplemented with antibiotic ampicillin (1000 mg/ml), IPTG (0.1 M) and X-gal (20 mg/ml). The positive clones were confirmed for the presence of inserts by EcoRI digestion of the plasmids and electrophoresis on ethidium bromide-stained 2.0% (w/v) agarose gel. The clones showing expected inserts were sequenced from the Medaxin DNA Sequencing facility, Bangalore, Karnataka India.

The sequences obtained were subjected to NCBI (www.ncbi.nlm.nih.gov) BLAST N search, and the sequence showing the highest scores were retrieved. The sequence data were assembled and analyzed using the software BioEdit version 7.0.9.

## RESULTS AND DISCUSSION

Three SRAP and RGAP markers previously found to be co-segregating with yellow mosaic disease resistant loci were monomorphic between the contrasting genotypes. Since linked markers failed to differentiate resistant and susceptible genotypes, attempts were made to identify molecular markers linked to yellow mosaic resistance loci. All the markers amplified in the parental lines, of which most of the primers were found monomorphic. One primer, LaRGAP 63, showed polymorphism and distinguished the contrasting parents (Fig 1). The marker LaRGAP 63 showed amplified product size (460 bp) in susceptible plants, while a smaller product size (455 bp) was observed in the resistant plants. Similar to susceptible parent, 460bp sizes were detected in $F_1$ plants (Fig 1). Marker was of the dominant nature, hence failed to detect any heterozygous band in the hybrid. The marker LaRGAP 63 was used for individual plant genotyping of 252 plants in the $F_2$ population. PCR amplification of 252 DNA samples with LaRGAP 63 showed the segregation pattern of 3:1 (susceptible: resistant) with ($\chi 2 = 0.09$; $p = 0.77$) indicating co-segregation of markers with phenotypic data where $F_2$ was fitted in 3:1 (susceptible: resistant) and single recessive gene action was observed for trait inheritance (Table 1). LaRGAP 63 marker amplified at 455bp in resistant genotype (IIHR-Sel-1) and 61 $F_2$ individuals whereas 460 bp band size was detected in susceptible genotypes, $F_1$ hybrid and 191 $F_2$individuals (Fig. 1). Marker LaRGAP 63 was found to be co-segregated with phenotypic observations (80.95 %) for yellow mosaic resistance in the genetic background of IIHR-Sel-1 (Table 1). Upon cloning and sequencing of amplified products, LaRGAP 63 in resistant and susceptible parents, the five nucleotides difference was confirmed. In the resistant parent, the deletion of 5 nucleotides was observed compared to the susceptible parent (Fig 2). Further sequence analysis with BLASTN tool (www.ncbi.com) and BioEdit 7.0, LaRGAP 63 showed 85.4% homology with mosaic virus resistance gene in cucurbits (Fig 3).

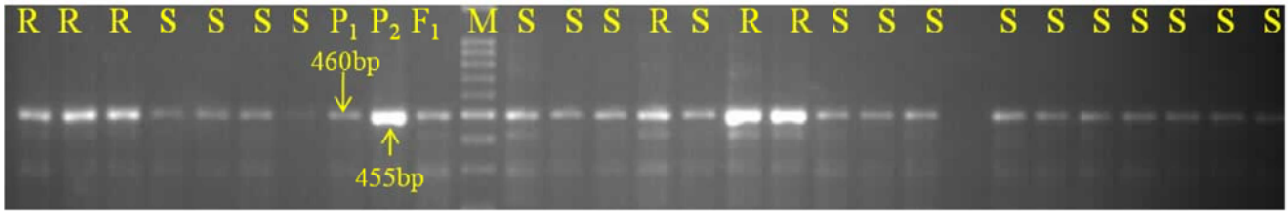


**Figure 1. Genotyping of $F_2$ population with LaRGAP 63**
($P_1$- Arka Prasan, $P_2$- IIHR-Sel-1, $F_1$-Hybrid, S-Susceptible, R-Resistant and M-100 bp ladder)

**Table 1. Segregation pattern of LaRGAP 63 marker locus using 252 $F_2$ plants derived from the cross of Arka Prasan × IIHR-Sel-1**

| Marker | Observed $F_2$ plants (Genotypic) | | Expected ratio | $\chi 2$ value | P value at 5% | Observed $F_2$ plants (Phenotypic) | | Co-segregation with trait (%) |
|---|---|---|---|---|---|---|---|---|
| | Resistant (R) | Susceptible (S) | R:S | | | R | S | |
| LaRGAP 63 | 61 | 191 | 1:3 | 0.09 | 0.77 | 68 | 184 | 80.95 |

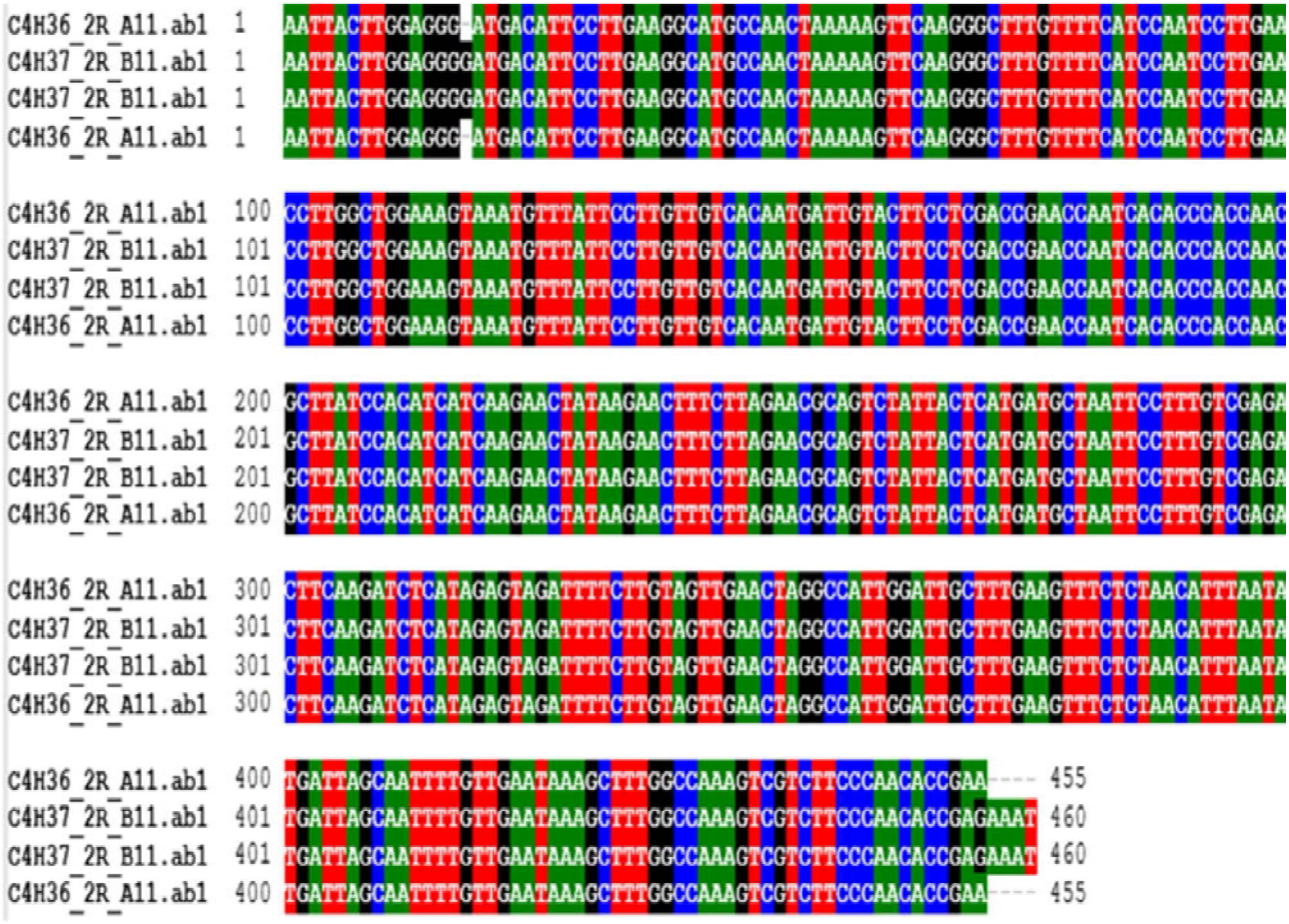


**Figure 2. Sequence analysis of LaRGAP 63 displaying nucleotide difference between the resistant and susceptible parents**

(C4H36_2R_All.ab1 and C4H36_2R_All.ab1 corresponds to genomic sequence of the amplified segment of the resistant genotype, IIHR-SEL-1 and susceptible genotype, Arka Prasan respectively)

Phenotypic evaluation combined with marker aided selection is important in molecular breeding. Molecular mapping of the ToLCNDV resistance gene was carried out using $F_2$ segregating population. Based on phenotypic evaluation for disease reaction in the $F_2$ population, a segregation pattern was found the best fit for 3:1 (susceptible: resistant). Thus, indicating that inheritance of resistance was under the control of a recessive gene (Kaur *et al*., 2021a). Islam *et al.* (2011) reported two SRAP markers linked to ToLCNDV resistance gene in sponge gourd. Due to genome synteny between ridge gourd and sponge gourd (Wu *et al*., 2016), both the previously identified markers were employed for validation in resistant *Luffa* genotype IIHR-Sel-1. Both the markers were amplified in susceptible and resistant genotypes but did not show any polymorphism. One RGAP marker (LaRGAP 63) has shown polymorphism between resistant and susceptible genotypes. PCR amplification of derived $F_2$ population with LaRGAP 63 showed the segregation pattern of 3:1 (susceptible: resistant) indicating the involvement of a recessive gene for marker inheritance (Table 1). Sequence analysis of LaRGAP63 showed 85.40 and 84.29 per cent homology with mosaic virus resistance protein and disease-resistant protein RPP-4, respectively. A number of RGAs were found to be linked with virus, bacteria and nematodes resistance in many crop species (Kanazin *et al*., 1996; Leister *et al*., 1996; Yu *et al*., 1996; Speulman *et al*., 1998; Spielmeyer *et al*., 2000). Saha *et al*. (2013) also identified one molecular marker (sgRGC 18) linked with ToLCNDV resistance in sponge gourd. Cultivated *Luffa* species are cross-compatible; however, fruit-related traits need to be studied. Pandey *et al*. (2018) found 56 polymorphic microsatellite markers for diversity analysis among *Luffa* species. Cucumber derived SSR markers were highly transferable to *L. hermaphrodita* (60.2%), *L.*

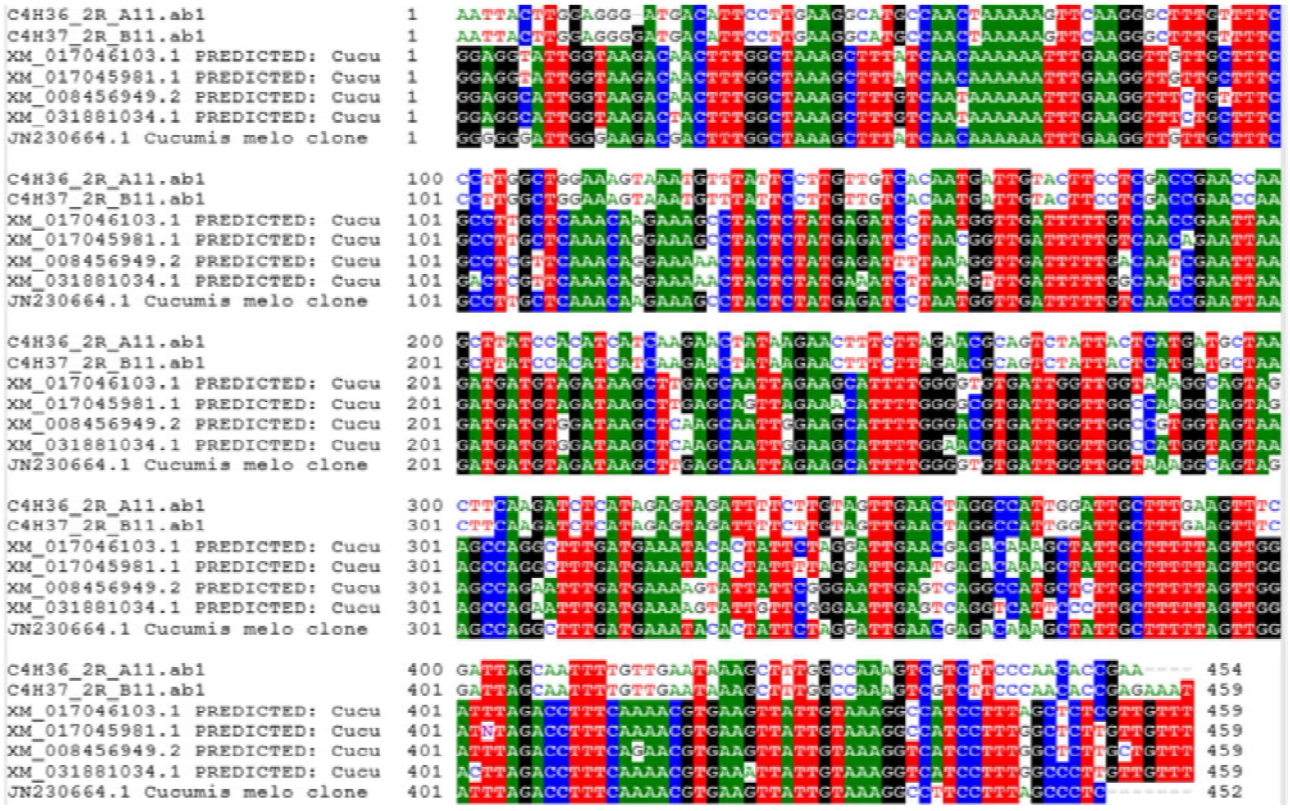


**Figure 3. LaRGAP 63 nucleotides sequence similarity with known mosaic virus resistance protein available in public domain**

(C4H36_2R_All.ab1 and C4H36_2R_All.ab1 corresponds to genomic sequence of the amplified segment of the resistant genotype, IIHR-SEL-1 and susceptible genotype, Arka Prasan respectively. Amplified genomic sequence was aligned with mosaic virus resistance genes available in public domain www.ncbi.com)

*acutangula* (68%) and *L. aegyptica* (61.16%) and therefore can be used for marker assisted selection in *Luffa* species.

Wu *et al.* (2016) constructed a genetic linkage map using an $F_2$ population derived from an interspecific cross between S1174 (ridge gourd) and 93075 (sponge gourd) spans over 1436.12 cM and the average distance between the markers is 8.11cM. Total 177 EST-SSR markers were employed which were distributed over 14 linkage group with an average of 102.58 cM per linkage group. These studies are more relevant for interspecies horticultural traits incorporation.

Saez *et al*. (2020) reported that major QTLs linked to ToLCNDV resistance was located at chromosome 8. Souframanien and Gopalakrishna (2006) identified ISSR marker (ISSR8111357) linked to mungbean yellow mosaic virus resistance gene in black gram. ISSR8111357 was validated using diverse germplasm differing in the disease reactions. ISSR8111357 marker was converted to co-dominant SCAR marker. Upon screening, the SCAR marker clearly differentiated the resistant and susceptible plants. Hoque and Rabbani (2009) found four polymorphic RAPD markers distinguishing 28 accessions of ridge gourd. Wu *et al*. (2014) designed 641 expressed sequence tags- simple sequence repeats (EST-SSR) primer pairs with sponge gourd transcriptome analysis out of which 494 were amplified and two hundred and one (40.69%) revealed polymorphisms between the sponge gourd [*L. cylindrica* (L.) Roem.] and ridge gourd [*L. acutangula* (L.) Roxb.]. These polymorphic markers were found highly transferable in six other cucurbits *viz*. pumpkin, cucumber, wax gourd, bitter gourd and bottle gourd. Therefore, these can be employed inmarker-assisted selection for the improvement of *Luffa* species.

The identified yellow mosaic disease linked marker LaRGAP 63 sharing homology with the mosaic virus resistance protein in cucurbits is vital to facilitate early plant selection and further pyramiding for resistance

loci for different virus isolates in common ridge gourd background. As the identified marker is dominant, it needs to be converted into a co-dominant to increase its efficiency for the ridge gourd's molecular resistance breeding program.

## ACKNOWLEDGEMENT

Authors gratefully acknowledge Indian Institute of Horticultural Research, Bengaluru for resource facilitations.

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***(Received on 14.09.2021, Revised on 24.12.2021 and Accepted on 31.12.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 193-198, 2021*

***Original Research Paper***

# Genetic divergence study in bitter gourd (*Momordica charantia* L.)

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## ABSTRACT

**The genetic divergence of forty bitter gourd genotypes was studied for sixteen different parameters by adopting Mahalanobis $D^2$ statistics using Tocher'smethod. The genotypes were grouped into six clusters irrespective of geographic divergence, indicating no parallelism between geographic and genetic diversity. A maximum of 32 genotypes entered in cluster I, followed by 4 genotypes in cluster II. The cluster III, IV, V and VI had single genotypes each. Maximum inter cluster distance observed between cluster II and cluster IV followed by cluster IV and cluster V and cluster II and V. This indicates, the genotypes belonging to cluster II (GYB-3-1-2, Bit-3-1-2-1, Bit-3-1-1-1, ArkaHarit), cluster IV (IC-68238) and cluster V (Bit-18-1-1) are more diverse and hence, hybridization between genotypes of respective cluster may improve the yield and quality of bitter gourd.**

**Keywords:** Bitter gourd, clusters, $D^2$ analysis and genetic divergence

## INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is considered as a valuable vegetable crop for its nutritional and medicinal properties, but it is neglected in terms of genetic and molecular breeding. Even though bitter gourd has a relatively broad phenotypic species variation due to diverse morphological traits, the studies on multi variate analysis is limited (Singh *et al*., 2013). Genetic divergence has been considered as an important factor in discriminating the genetically diverse parents for efficient and successful hybridization programme in order to get potential transgressive segregants and also provide new recombination of genes in the gene pool.

$D^2$ statistics (Mahalanobis, 1936) is highly acceptable as it provides a measure of magnitude for divergence between two genotypes under comparison. Grouping of genotypes based on $D^2$ analysis will be useful in choosing suitable parental lines for hybridization. Therefore, the present study was conducted to identify suitable parents out of 40 bitter gourd genotypes to initiate a breeding programme by identifying the clusters that are diverse and contain genotypes with good performance.

## MATERIALS AND METHODS

The present investigation was carried out at the Department of Vegetable Science, College of Horticulture, Yelachenahalli, Mysuru district, Karnataka during 2017-18. The experimental materials comprised of 40 indigenous genotypes of bitter gourd including some of the commercially released varieties from different institutes of India as listed in Table 1. The experiment was laid out in a randomized complete block design (RCBD) with two replications. The spacing used in this experiment was 120×90 cm. The recommended NPK fertilizer doses and cultural practices along with plant protection measures were followed to raise a commercial crop (Choudhary *et al*., 2003). Five randomly chosen plants in each replication of each entry were labelled and used for recording the observations. The mean of five plants was taken for analysis. Observations were recorded for 16 parameters like Vine length (m), Number of branches per vine, Duration of crop (days), Node at which first female flower appears, Days to first female flower opening, Days to 50 per cent flowering, Days to first fruit picking, Fruit length (cm), Fruit diameter (cm), Average fruit weight (g), Number of fruits per vine, Fruit yield per vine (kg), Fruit yield per hectare (t), Number of seeds per fruit, Flesh thickness (mm)

and Ascorbic acid (mg/100g). The data were subjected to multivariate analysis of genetic divergence using Mahalanobis $D^2$ statistic. Grouping of entries was done by Tocher's method (Rao, 1952).

**Table 1. List of genotypes and their sources of collection**

| Sl. No. | Genotypes | Source | Sl. No. | Genotypes | Source |
|---|---|---|---|---|---|
| 1 | Preethi | KAU, Vellanikkara | 21 | Bit-10-1-1 | COH, Kolar, Karnataka |
| 2 | Yellapur Local-2 | Yellapur, Karnataka | 22 | West Bengal Local-2 | West Bengal |
| 3 | Bit-25-2-1 | COH, Kolar, Karnataka | 23 | Kotla Local-1 | Rajastan |
| 4 | Meghnaa-2 | Masood seeds, Bangladesh | 24 | Bit-10-1-2 | COH, Kolar, Karnataka |
| 5 | Jhalawar Local-3 | Jalawar, Rajastan | 25 | Bit-5-1-4-1 | COH, Kolar, Karnataka |
| 6 | CO-1 | TNAU, Coimbatore | 26 | Contai Bolder | Barasat Agri Hybrid seeds, West Bengal |
| 7 | Bit-22-1-1-3 | COH, Kolar, Karnataka | 27 | Bit-1-2-2-4 | COH, Kolar, Karnataka |
| 8 | Bit-9-2-4-1 | Maharashtra | 28 | Bit-18-1-1 | Varanasi, Uttar pradesh |
| 9 | GYB-3-1-2 | Tamil Nadu | 29 | Jhalawar Local-1 | Jalawar, Rajastan |
| 10 | Bit-1-2-3 | COH, Kolar, Karnataka | 30 | Bit-3-1-1-1 | Tamil Nadu |
| 11 | Yellapur Local-1 | Yellapur, Karnataka | 31 | GYB-5-1-5-2 | COH, Kolar, Karnataka |
| 12 | Bit-37-2-1 | COH, Kolar, Karnataka | 32 | Bit-22-1-1-1 | COH, Kolar, Karnataka |
| 13 | DEB-505 | Debgiri Pvt Ltd. Kolkatta | 33 | Bit-9-2-1-2 | Maharashtra |
| 14 | Bit-3-1-2-1 | Tamil Nadu | 34 | GYL-2 | COH, Kolar, Karnataka |
| 15 | Bit-9-3-2-3 | Maharashtra | 35 | GYB-2-2 | COH, Kolar, Karnataka |
| 16 | Bit-5-1-2-1 | COH, Kolar, Karnataka | 36 | Katahi | Hyderabad |
| 17 | West Bengal Local-1 | West Bengal | 37 | Bit-35-1-1 | Odisha |
| 18 | Jhalawar Local-2 | Jalawar, Rajastan | 38 | Bit-31-2-2 | COH, Kolar, Karnataka |
| 19 | Super Green | Super Seeds, Odissa | 39 | ArkaHarit | IIHR, Bengaluru |
| 20 | IC-68238 | NBPGR, New Delhi | 40 | Bit-9-1-4-1 | Maharashtra |

## RESULTS AND DISCUSSION

The results from the analysis of variance for 16 characters indicated significantly high differences among 40 genotypes of bitter gourd under study. These 40 genotypes were grouped into six clusters. The distribution of genotypes into 6 clusters were presented in Table 2. Cluster I is the largest cluster having 32 genotypes followed by cluster II with four genotypes (GYB-3-1-2, Bit-3-1-2-1, Bit-3-1-1-1and ArkaHarit). Cluster III (Yellapur Local-2), cluster IV (IC-68238), cluster V (Bit-18-1-1) and cluster VI (Jhalawar Local-2) had one genotype each. The genotypes collected from different geographical regions were present in same clusters indicating that there was no association between geographical distribution and genetic diversity as reported earlier by Bhagwat *et al.* (2013) in bitter gourd.

The intra and inter-cluster $D^2$ and D values among 6 clusters are furnished in the Table 3. and illustrated in Figure 1. Intra-cluster average $D^2$ values ranged from 0 to 104.02. Among the clusters, cluster II had the maximum intra-cluster distance (104.02) followed by cluster I (96.08). The clusters like III, IV, V and cluster VI had no inter cluster distance (zero) as they were represented by single genotypes. The maximum inter cluster $D^2$ value was found between cluster II and VI (1620.05) followed by cluster IV and VI (1262.95), cluster II and V (1098.44), cluster II and cluster III

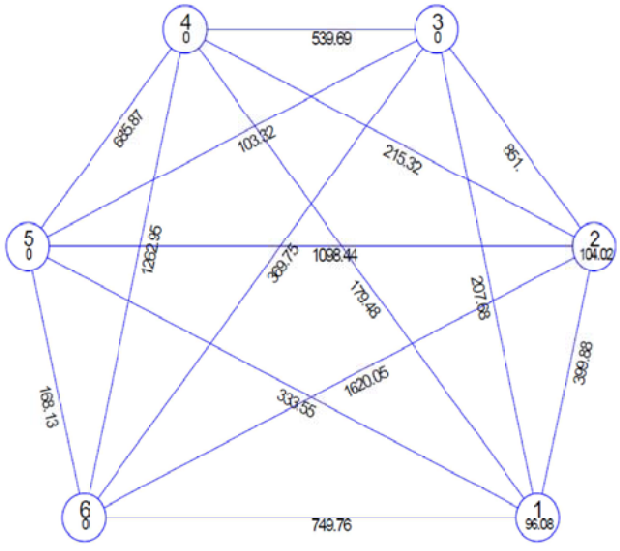
**Table 2. Cluster composition based on $D^2$ statistics in bitter gourd**

| Cluster | Number of genotypes | Genotypes included in the cluster |
|---|---|---|
| I | 32 | Preethi, Bit-25-2-1, Meghnaa-2, Jhalawar Local-3, CO1, Bit-22-1-1-3, Bit-9-2-4-1, Bit-1-2-3, YellapurLocal-1, Bit-37-2-1, DEB-505, Bit-9-3-2-3, Bit-5-1-2-1, West Bengal Local-1, Super Green, Bit-10-1-1, West Bengal Local-2, Kotla Local-1, Bit-10-1-2, Bit-5-1-4-1, Contai Bolder, Bit-1-2-2-4, Jhalawar Local-1, GYB-5-1-5-2, Bit-22-1-1-1, Bit-9-2-1-2, GYL-2, GYB-2-2, Katahi, Bit-35-1-1, Bit-31-2-2, Bit-9-1-4-1 |
| II | 4 | GYB-3-1-2, Bit-3-1-2-1, Bit-3-1-1-1, ArkaHarit |
| III | 1 | YellapurLocal-2 |
| IV | 1 | IC-68238 |
| V | 1 | Bit-18-1-1 |
| VI | 1 | Jhalawar Local-2 |

**Table 3. Intra-cluster (diagonal) and inter-cluster $D^2$ and D values in bitter gourd genotypes**

| | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| I | 96.08 (9.80) | 399.88 (19.20) | 207.68 (14.41) | 179.48 (13.34) | 333.55 (18.26) | 749.76 (27.38) |
| II | | 104.02 (10.12) | 851.00 (29.17) | 215.32 (14.67) | 1098.44 (33.14) | 1620.05 (40.25) |
| III | | | 0.00(0.00) | 539.69 (23.23) | 103.32 (10.16) | 369.75 (19.23) |
| IV | | | | 0.00 (0.00) | 685.87 (26.19) | 1262.95 (35.54) |
| V | | | | | 0.00 (0.00) | 168.13 (12.96) |
| VI | | | | | | 0.00 (0.00) |

Figures in parenthesis denotes corresponding D values




**Mahalanobis Euclidean Distance (Not to the scale)**

**Fig1. Intra-cluster and inter-cluster distance of bitter gourd genotypes (Trocher's method)**

Highest inter cluster distance was found in cluster II and VI, suggesting that hybridisation between the genotypes from these clusters may lead to high heterotic effects and better segregants (Rabbani *et al*., 2012). Similarly, lowest inter cluster distance was observed in cluster III and V indicating that, genotypes exhibited higher genetic similarity (Tyagi *et al*., 2017).

The Per cent contribution of sixteen characters towards total divergence in bitter gourd genotypes is shown in Table 4. Among all the characters, ascorbic acid contributed the maximum (37.31%) to the diversity by taking first rank in 291 times out of 780 combinations, followed by fruit length (15.64% with 122 times ranked first), fruit diameter (14.36% with 112 times ranked first), flesh thickness (11.92% with 93 times ranked first), number of seeds per fruit (9.49% with 74 times ranked first), days to first female flower opening (6.92% with 54 times ranked first), average fruit weight (1.28% with 10 times ranked first). While, there was little and negligible

**Table 4. Per cent contribution of sixteen characters towards total divergence in bitter gourd genotypes**

| Sl. No. | Characters | No. of times ranked first | Per cent germplasm contribution |
|---|---|---|---|
| 1 | Vine length (m) | 4 | 0.51 |
| 2 | Number of branches per vine | 7 | 0.90 |
| 3 | Duration of crop (days) | 0 | 0.00 |
| 4 | Node at which first female flower appears | 1 | 0.13 |
| 5 | Days to first female flower opening | 54 | 6.92 |
| 6 | Days to 50 per cent flowering | 0 | 0.00 |
| 7 | Days to first fruit picking | 0 | 0.00 |
| 8 | Fruit length (cm) | 122 | 15.64 |
| 9 | Fruit diameter (cm) | 112 | 14.36 |
| 10 | Average fruit weight (g) | 10 | 1.28 |
| 11 | Number of fruits per vine | 6 | 0.77 |
| 12 | Fruit yield per vine (kg) | 6 | 0.77 |
| 13 | Fruit yield per hectare (t) | 0 | 0.00 |
| 14 | Number of seeds per fruit | 74 | 9.49 |
| 15 | Flesh thickness (mm) | 93 | 11.92 |
| 16 | Ascorbic acid (mg/100g) | 291 | 37.31 |
| | Total | 780 | 100.00 |

contribution from number of branches per vine (0.90%), number of fruits per vine (0.77%), fruit yield per vine (0.77%), vine length (0.51%) and node at which first female flower appears (0.13%). Similar results were reported by Sidhu and Pathak, 2016 in bitter gourd. However, the duration of crop, days to 50 per cent flowering, days to first fruit picking and fruit yield per hectare had no contribution towards genetic divergence. Similar findings were also observed by Sundaram (2008) and Bhagwat *et al.* (2013). Apart from the divergence, the performance of genotypes and the character with maximum contribution towards divergence should also be given due consideration which appear as desirable for improvement of bitter gourd (Deepa and Mariyappan, 2013).

Cluster means of forty genotypes showed that mean values of cluster varied for all the sixteen characters studied. Cluster II, V an VI performed better for the biometric parameters studied. Among the clusters, cluster VI was generally poor and cluster I as well as cluster III were intermediate in number of fruits per vine and fruit yield (Table 5.). Cluster II with four genotypes showed early flowering, flowering at lower node and early fruit picking. Cluster II had smaller fruits but the number of fruits per vine was highest. Cluster VI with one genotype had longer fruits (30 cm), lower fruit diameter with high average fruit weight and higher ascorbic acid content (112.43). Higher number of branches, longer duration of crop and higher fruit yield was noticed in cluster V with one genotype (Bit-18-1-1). Highest vine length was observed in the cluster III (3.67 m). Cluster I with maximum number of genotypes showed intermediate performance for almost all the characters observed. The best cluster with yield and yield components studied was cluster V followed by cluster III and cluster I. By using these elite germplasms, there is a scope for varietal improvement in bitter gourd.

Inter-crossing of genotypes based on the mean performance for their characters would be effective for further crop improvement. To develop early varieties with small fruits and higher number of fruits per vine, cluster II would be effective as it showed early flowering. Selection from cluster I would be useful in breeding moderately early flowering, intermediate yield with longer crop duration. Cluster VI can be used in breeding for longer fruits with greater average fruit

**Table 5. The cluster mean of sixteen characters for six clusters in bitter gourd genotypes**

| Sl. No. | Characters | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI |
|---|---|---|---|---|---|---|---|
| 1 | Vine length (m) | 2.71 | 1.91 | 3.67 | 1.84 | 2.58 | 2.33 |
| 2 | Number of branches per vine | 10.54 | 8.15 | 10.60 | 8.50 | 11.00 | 9.40 |
| 3 | Duration of crop (days) | 95.79 | 85.56 | 92.13 | 86.50 | 98.50 | 94.00 |
| 4 | Node at which first female flower appears | 15.39 | 11.33 | 15.50 | 14.10 | 14.30 | 16.50 |
| 5 | Days to first female flower opening | 41.46 | 35.69 | 37.60 | 42.55 | 41.35 | 44.50 |
| 6 | Days to 50 per cent flowering | 44.10 | 37.88 | 41.50 | 44.75 | 47.50 | 44.77 |
| 7 | Days to first fruit picking | 58.74 | 50.59 | 56.00 | 58.00 | 61.50 | 59.50 |
| 8 | Fruit length (cm) | 16.94 | 7.05 | 22.43 | 13.49 | 29.05 | 30.00 |
| 9 | Fruit diameter (cm) | 4.82 | 4.46 | 4.48 | 5.48 | 5.04 | 2.85 |
| 10 | Average fruit weight (g) | 84.68 | 36.91 | 85.60 | 79.00 | 88.70 | 91.85 |
| 11 | Number of fruits per vine | 12.87 | 14.76 | 13.10 | 12.10 | 14.60 | 8.85 |
| 12 | Fruit yield per vine (kg) | 1.11 | 0.53 | 1.14 | 0.96 | 1.28 | 0.69 |
| 13 | Fruit yield per hectare (t) | 10.24 | 4.92 | 10.55 | 8.87 | 11.82 | 6.38 |
| 14 | Number of seeds per fruit | 18.06 | 8.75 | 23.50 | 10.50 | 20.50 | 18.25 |
| 15 | Flesh thickness (mm) | 6.09 | 4.63 | 4.94 | 9.41 | 6.69 | 4.11 |
| 16 | Ascorbic acid (mg/100g) | 94.56 | 101.45 | 84.10 | 100.50 | 102.42 | 112.43 |

weight and higher ascorbic acid content, as the demand is increasing in our country. To breed varieties with higher yield and late flowering, selection from cluster V would be useful.

## CONCLUSION

Genetic divergence has been considered as an important factor in discriminating the genetically diverse parents for efficient and successful hybridization programme in order to get potential transgressive segregants and also provide new recombination of genes in the gene pool.Maximum inter cluster distance observed between cluster II and cluster IV followed by cluster IV and cluster V and cluster II and V. This indicates, the genotypes belonging tocluster II (GYB-3-1-2, Bit-3-1-2-1, Bit-3-1-1-1, ArkaHarit), cluster IV (IC-68238) and cluster V (Bit-18-1-1) are more diverse and hence, hybridization between genotypes of respective cluster may improve the yield and quality of bitter gourd.

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***(Received on 07.07.2021, Revised on 27.11.2021 and Accepted on 06.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 199-205, 2021*

***Original Research Paper***

# Combining ability studies to develop superior hybrids in bell pepper (*Capsicum annuum* var. *grossum* L.)

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## ABSTRACT

**General combining ability (GCA) among parents and specific combining ability (SCA) of crosses were studied to identify horticulturally superior crosses for growth, yield and component traits in bell pepper. Experimental material included 21 $F_1$ hybrids developed by crossing seven parents in half diallel mating design. Parents and crosses were planted in randomized complete block design (RCBD) during *Rabi* 2019 to estimate the effects of combining ability. Among parents, Arka Mohini showed good GCA effects for most of the traits (number of secondary branches, early flowering and harvesting, fruit weight and yield) whereas among crosses, Arka Mohini × CW308, Arka Mohini× California Wonder and Yolo Wonder × California Wonder were identified as potential hybrids for yield and attributing traits based on SCA effects.**

**Key words** : Bell pepper, half-diallel mating, general combining ability, hybrids, specific combining ability and yield

## INTRODUCTION

Bell pepper (*Capsicum annuum* var. *grossum*), also known as capsicum, sweet pepper or Shimla mirch is a popular vegetable worldwide for its pleasant flavour and delicate taste. Further, it is an abundant source of ascorbic acid, vitamin A and other minerals (Sharma *et al*. 2013). It belongs to the family Solanaceae and have a diploid chromosome number 2n=24. Both green as well as coloured (red and yellow) fruits of bell pepper have gained a status of high value crop in India. The demand for bell pepper in recent years has increased with the emergence of continental food industry (Sood *et al*. 2010). It always fetches premium price in the market because of its regular demand and inadequate supply owing to average productivity. The basic reason for this is lack of superior quality indigenous varieties and hybrids with high yield and tolerance to biotic as well as abiotic stresses. Indian bell pepper seed market is dominated by imported private sector hybrids and varieties, which increases the input cost for the farmers. Hence, there is an urgent need to strengthen the crop improvement programme for developing new varieties or hybrids in this crop capable of satisfying the needs of farmers as well as consumers. For development of $F_1$ hybrids, selection of parents is of utmost importance. Parents are generally selected based on their combining ability. Here, combining ability refers to the ability of lines or parents to combine well during hybridization process so that desirable genes or characters get transmitted to their progenies (Fasahat *et al.* 2016). General combining ability and specific combining ability are the two main types of combining ability. The study on general combining ability of parents and specific combining ability of the crosses helps in identification of best parents and crosses respectively. Further, the combining ability of the parents also depends upon the nature of genetic system operating in them which predicts the efficiency of selection. Keeping this in view, the objective of this investigation was to work out general combining ability (GCA) among parents and specific combining ability of crosses (SCA) to identify the promising hybrids.

## MATERIALS AND METHODS

The study was conducted at ICAR- Indian Institute of Horticultural Research, Hessaraghatta lake post, Bengaluru-89 during the year 2019-2020 for two seasons. During *Kharif,* 2019 hybrids were developed using seven diverse and elite capsicum genotypes *viz*., Arka Mohini, Arka Gaurav, Arka Basant, Yolo Wonder, California Wonder, UHFBP-4 and CW-308. They were crossed in half diallel fashion to obtain twenty-one cross combinations/hybrids. In *Rabi*, 2020, seedlings of 7 parents and 21 crosses were transplanted in open field in randomized block design with three replications at a spacing of 60x30 cm. The standard cultural practices were followed as per the package of practices of bell pepper by Indian Institute of Horticulture Research, 2011.Observations were recorded on number of primary branches (NPB), number of secondary branches (NSB), plant height (cm) (PH), days to 50% flowering (DF), days to first harvest (DFH), fruit length in cm (FL), fruit width in cm (FW), number of lobes per fruit (NLF), pericarp thickness in cm (PT), average fruit weight in gm (AFW) , number of fruits per plant (NFP), total yield per plant in gm (YP). Indostat software was used for statistical analysis of the data.

## RESULTS AND DISCUSSION

Analysis of variance for GCA was found significant for all the traits except NPB and analysis of variance for SCA was found significant for all the traits (Table 1).With respect to GCA and SCA variance, there was predominance of SCA for all the studied traits indicating the presence of non-additive gene action which could be attributed to dominance and epistatic components like dominance x dominance and additive 'x' dominance type of interactions indicating sufficient scope for heterosis breeding. The parents and crosses were scored based on their GCA and SCA status. Significantly negative GCA and SCA was scored as "-1" and non-significant GCA and SCA was scored as "0" whereas "+1" score was given to significantly positive GCA and SCA effects. By taking these scores into consideration, parents and hybrids were classified as poor, average and good combiners (Table 2 & 3). Arka Mohini was identified as good general combiner for NSB, DF, DFH, AFW and YP. Arka Basant for PH, DFH, FL and NFP whereas, Yolo wonder for DFH and PT (Table 2).

In SCA studies, crosses based on Arka Mohini, Arka Basant and Yolo Wonder as one of the parents exhibited good combining effects. Arka Mohini x Yolo Wonder, Arka Mohini x CW308, Arka Mohini x UHFBP4, Arka Basant x California Wonder and Arka Basant x CW308, Yolo Wonder x California Wonder exhibited good SCA effects for most of the traits (Table 3). Arka Mohini based crosses showed higher yield attributed to more number of big and heavy fruits per plant. Arka Mohini x Yolo Wonder showed good SCA for traits like NPB, NSB, PT, AFW and YP; Arka Mohini x CW308 for PH, FW, NFP, AFW and YP; Arka Mohini x UHFBP4 for NSB, PT, NFP, AFW and YP whereas, Arka Basant based hybrids showed earliness along with higher yield. Arka Basant x California Wonder exhibited good SCA for PH, DF, DFH, NFP, AFW and YP; and Arka Basant x CW308 for FL, PT, NFP, AFW and YP. Yolo Wonder x California Wonder exhibited good SCA for NPB, NSB, NLF, PT, NFP, AFW and YP. The results Obtained indicates that traits like NPB, NPS, PH, DF, DFH, FW, PT, NFP, AFW and YP are governed by non-additive genes hence, highly amenable for exploitation through heterosis. Similar results were reported by Hegde (2019), Praveen *et al*. (2017) and Aditika (2018) for NPB, NSB and PH in capsicum. Kaur *et al.* (2018), Praveen *et al*. (2017) and Devi *et al.* (2018) reported non additive gene action for earliness traits in capsicum. Kamble *et al*. (2009), Hegde (2016), Praveen *et al*. (2017) and Devi *et al*. (2018) have also reported good SCA for fruit length and fruit width. Kaur *et al.* (2018), Aditika (2018) and Devi *et al*. (2018) have reported high SCA effects for pericarp thickness and average number of fruits per plant. Good SCA for average fruit weight and yield has been reported by Galal *et al*. (2018) and Aditika (2018) supporting the present investigation. Based on the general combining ability of parents and specific combining ability of crosses, only three crosses showing good SCA coupled with good or, average GCA of the parents involved in it *viz*., Arka Mohini x CW308, Arka Mohini x Yolo Wonder and Yolo Wonder x California Wonder with GG and GA interactions (table 3) are identified for future considerations. Further studies on the heterosis of the traits in the developed crosses will be useful in identifying the best heterotic combinations among them.

**Table 1. ANOVA for combining ability**

| | df | NPB | NSB | PH | DF | DFH | FL | FW | NLF | PT | NFP | AFW | YP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GCA | 6 | 0.05 | 0.24* | 50.19* | 47.62* | 71.10* | 1.12* | 0.15* | 0.08* | 0.003* | 1.12* | 159.13* | 9763.45 * |
| SCA | 21 | 0.05* | 0.24 * | 18.01 * | 27.90 * | 32.65 * | 1.81* | 0.07* | 0.05* | 0.007* | 1.39* | 133.86* | 34156.48* |
| Error | 54 | 0.03 | 0.06 | 5.03 | 8.16 | 6.13 | 0.29 | 0.04 | 0.03 | 0.001 | 0.06 | 2.92 | 43.88 |

*: Significance at p= 0.05; GCA: General combining ability, SCA: Specific combining ability, NPB: No. of primary branches, NSB: No. of secondary branches, PH: Plant height, DF: Days to 50% flowering, DFH: Days to first harvest, FL: Fruit length, FW: Fruit width, NLF: No. of lobes per fruit, PT: pericarp thickness, AFP: Average fruit per plant, AFW: Average fruit weight, YP: Yield per plant

**Table 2. Overall general combining ability (GCA) of parents for different traits**

| Sl. No. | Parents | NPB | NSB | PH | DF | DFH | FL | FW | NLF | PT | NFP | AFW | YP | Total | | GCA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | +ve | -ve | |
| 1. | Arka Mohini | 0 | +1 | -1 | +1 | +1 | 0 | 0 | 0 | 0 | 0 | +1 | +1 | 5 | 1 | Good |
| 2. | Arka Gaurav | 0 | 0 | 0 | -1 | 0 | 0 | 0 | +1 | 0 | -1 | -1 | -1 | 1 | 4 | Poor |
| 3. | Arka Basant | 0 | 0 | +1 | 0 | +1 | +1 | -1 | -1 | 0 | +1 | -1 | -1 | 4 | 4 | Average |
| 4. | Yolo Wonder | 0 | 0 | 0 | 0 | +1 | 0 | 0 | 0 | +1 | 0 | +1 | 0 | 3 | 0 | Good |
| 5. | California Wonder | 0 | 0 | +1 | 0 | 0 | -1 | 0 | 0 | -1 | 0 | 0 | +1 | 2 | 2 | Average |
| 6. | UHFBP-4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | -1 | -1 | 0 | 3 | Poor |
| 7. | CW308 | 0 | 0 | 0 | -1 | 0 | 0 | +1 | 0 | +1 | 0 | 0 | 0 | 2 | 1 | Good |

NPB: No. of primary branches, NSB: No. of secondary branches, PH: Plant height, DF: Days to 50% flowering, DFH: Days to first harvest, FL: Fruit length, FW: Fruit width, NLF: No. of lobes per fruit, PT: pericarp thickness, NFP: Number of fruits per plant, AFW: Average fruit weight, YP: Yield per plant

**Table 3. Overall specific combining ability (SCA) of crosses for different traits**

| S.No. | Crosses | NPB | NSB | PH | DF | DFH | FL | FW | NLF | PT | NFP | AFW | YP | Total | | SCA effects | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | +ve | -ve | Crosses | Parents |
| 1. | AM x AG | 0 | 0 | 0 | -1 | -1 | 0 | 0 | 0 | +1 | 0 | 0 | -1 | 1 | 3 | P | G x P |
| 2. | AM x AB | 0 | 0 | -1 | +1 | 0 | 0 | 0 | 0 | -1 | +1 | +1 | +1 | 4 | 2 | A | G x P |
| 3. | AM x YW | +1 | +1 | 0 | -1 | -1 | 0 | 0 | 0 | +1 | 0 | +1 | +1 | 5 | 2 | G | G x G |
| 4. | AM x CW | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | -1 | -1 | 0 | 4 | P | G x A |
| 5. | AM x UHF BP4 | 0 | +1 | -1 | 0 | -1 | 0 | 0 | 0 | +1 | +1 | +1 | +1 | 5 | 2 | G | G x P |
| 6. | AM x CW308 | 0 | 0 | +1 | -1 | -1 | 0 | +1 | 0 | -1 | +1 | +1 | +1 | 5 | 3 | G | G x G |
| 7. | AG x AB | 0 | 0 | +1 | 0 | 0 | 0 | +1 | 0 | +1 | 0 | -1 | -1 | 3 | 2 | A | P x P |
| 8. | AG x YW | 0 | 0 | 0 | 0 | -1 | 0 | 0 | +1 | -1 | 0 | +1 | -1 | 2 | 3 | P | P x G |
| 9. | AG x CW | 0 | +1 | 0 | 0 | 0 | 0 | 0 | 0 | +1 | 0 | +1 | +1 | 4 | 0 | A | P x A |
| 10. | AG x UHFBP4 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | +1 | -1 | 0 | +1 | +1 | 3 | 2 | A | P x P |
| 11. | AG x CW308 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | +1 | 0 | -1 | +1 | 2 | 1 | A | P x G |
| 12. | AB x YW | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | +1 | -1 | -1 | -1 | 1 | 3 | P | P x G |
| 13. | AB x CW | 0 | 0 | +1 | +1 | +1 | +1 | 0 | 0 | -1 | +1 | +1 | +1 | 7 | 1 | G | P x A |
| 14. | AB x UHFBP4 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | +1 | -1 | 0 | +1 | -1 | 2 | 3 | P | P x P |
| 15. | AB x CW308 | 0 | 0 | 0 | -1 | -1 | +1 | 0 | 0 | +1 | +1 | +1 | +1 | 5 | 2 | G | P x G |
| 16. | YW x CW | +1 | +1 | 0 | 0 | 0 | 0 | 0 | +1 | +1 | +1 | +1 | +1 | 7 | 0 | G | G x A |
| 17. | YW x UHFBP4 | 0 | 0 | 0 | 0 | 0 | +1 | 0 | 0 | +1 | 0 | -1 | +1 | 3 | 1 | A | G x P |
| 18. | YW x UHFCW308 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | +1 | -1 | 1 | 2 | P | G x G |
| 19. | CW x UHFBP4 | 0 | +1 | +1 | -1 | -1 | -1 | 0 | 0 | 0 | 0 | +1 | +1 | 4 | 3 | A | A x P |
| 20. | CW x CW308 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | +1 | +1 | 2 | 1 | A | A x G |
| 21. | UHFBP4 x CW308 | 0 | 0 | 0 | 0 | 0 | +1 | 0 | 0 | +1 | 0 | -1 | -1 | 2 | 2 | A | P x G |

NPB: No. of primary branches, NSB: No. of secondary branches, PH: Plant height, DF: Days to 50% flowering, DFH: Days to first harvest, FL: Fruit length, FW: Fruit width, NLF: No. of lobes per fruit, PT: pericarp thickness, NFP: Number of fruits per plant, AFW: Average fruit weight, YP: Yield per plant, AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder

**Supplementary Table 1 : General combining ability effects (GCA) of parents growth and yield parameters**

| Sl.No. | Parents | NPB | NSB | PH | DF | DFH | FL | FW | NLF | PT | NFP | AFW | YP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Arka Mohini | 0.09 | 0.28* | -4.72* | 3.55* | 2.89* | -0.25 | 0.05 | -0.003 | -0.01 | 0.08 | 8.19 * | 47.37* |
| 2. | Arka Gaurav | 0.02 | -0.12 | 0.08 | -2.49* | -3.88 | -0.08 | 0.10 | 0.14 * | 0.01 | -0.58 * | -1.86 * | -35.07* |
| 3. | Arka Basant | -0.02 | -0.007 | 1.87* | 2.14 | 3.45* | 0.50 * | -0.18 * | -0.14* | 0.01 | 0.60 * | -3.78 * | -39.91* |
| 4. | Yolo Wonder | -0.04 | 0.07 | -1.28 | -1.12 | 2.11* | 0.27 | -0.02 | -0.06 | 0.02 * | 0.002 | 2.38 * | 3.33 |
| 5. | California Wonder | -0.09 | -0.15 | 1.93* | 0.21 | -1.95 | -0.57 * | -0.03 | 0.06 | -0.03 ** | 0.05 | -0.97 | 35.80* |
| 6. | UHFBP-4 | 0.10 | 0.08 | 1.08 | 0.36 | -0.92 | -0.05 | -0.13 | 0.04 | -0.01 | -0.19 * | -3.81 * | -12.19* |
| 7. | CW308 | -0.06 | -0.17 | 1.03 | -2.64* | -1.69 | 0.17 | 0.20 * | -0.03 | 0.02* | 0.04 | -0.15 | 0.67 |
| SEm± | | 0.05 | 0.07 | 0.69 | 0.88 | 0.76 | 0.05 | 0.06 | 0.05 | 0.001 | 0.073 | 0.53 | 2.04 |
| CD at 5% | | 0.12 | 0.19 | 1.69 | 2.16 | 1.87 | 0.41 | 0.14 | 0.12 | 0.01 | 0.18 | 1.29 | 5.00 |

*: Significance at p= 0.05; NPB: No. of primary branches, NSB: No. of secondary branches, PH: Plant height, DF: Days to 50% flowering, DFH: Days to first harvest, FL: Fruit length, FW: Fruit width, NLF: No. of lobes per fruit, PT: pericarp thickness, AFP: Average fruit per plant, AFW: Average fruit weight, YP: Yield per plant

**Supplementary Table2 : Specific combining ability effects (SCA) for crosses**

| Sl.No. | Crosses | NPB | NSB | PH | DF | DFH | FL | FW | NLF | PT | NFP | AFW | YP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | AM×AG | -0.12 | 0.28 | -2.48 | -6.23* | -4.71* | 0.41 | 0.11 | 0.03 | 0.10* | -0.13 | 0.43 | -47.83* |
| 2. | AM×AB | 0.19 | 0.34 | -5.23* | 7.14* | 0.95 | -0.36 | 0.08 | 0.05 | -0.03* | 1.06* | 9.75* | 223.50* |
| 3. | AM×YW | 0.38* | 0.49* | -1.08 | -7.27 * | -5.05* | -0.10 | 0.07 | 0.004 | 0.06* | 0.53 | 5.89* | 115.16* |
| 4. | AM×CW | -0.23 | -0.55* | -2.29 | 3.07 | 2.36 | 0.28 | -0.02 | -0.08 | -0.02 | -1.18* | -4.46* | -202.04* |
| 5. | AM×UHFBP4 | 0.24 | 0.76* | -4.20* | -3.08 | -4.01* | 0.23 | 0.13 | 0.17 | 0.06* | 1.33* | 7.79* | 150.59* |
| 6. | AM×CW308 | 0.13 | -0.22 | 9.61* | -5.08* | -6.57* | 0.18 | 0.41* | 0.004 | -0.05* | 1.16* | 8.72* | 202.99* |
| 7. | AG×AB | 0.07 | -0.09 | 5.86* | -3.15 | -3.27 | -0.14 | 0.41* | 0.007 | 0.07* | -0.11 | -11.32* | -159.49* |
| 8. | AG×YW | 0.18 | -0.38 | -3.16 | -1.57 | -5.27* | 0.44 | -0.049 | 0.36* | -0.06* | 0.12 | 5.72* | -30.76* |
| 9. | AG×CW | -0.03 | 0.72* | 3.56 | 3.10 | -1.86 | -0.16 | -0.02 | 0.007 | 0.13* | 0.44 | 0.94* | 117.23* |
| 10. | AG×UHFBP4 | 0.04 | -0.51* | 1.51 | -3.04 | -1.89 | 0.08 | 0.08 | 0.32* | -0.03* | 0.52 | 16.84* | 182.83* |
| 11. | AG×CW308 | 0.16 | 0.30 | 0.83 | 3.29 | 3.55 | -0.23 | 0.18 | -0.11 | 0.07* | 0.76 | -8.12* | 85.39* |
| 12. | AB×YW | 0.23 | 0.39 | 3.29 | -2.53 | -3.93 | -0.96 | 0.03 | -0.22 | 0.07* | -0.96* | -8.23* | -107.83* |
| 13. | AB×CW | 0.19 | -0.12 | 3.64* | 7.81* | 8.14* | 1.91* | 0.10 | -0.07 | -0.10* | 1.29* | 2.02* | 135.08* |
| 14. | AB×UHFBP4 | -0.05 | -0.15 | -1.31 | 8.99* | 3.77 | -0.73 | -0.11 | 0.31* | -0.14* | -0.46 | 3.52* | -59.20* |
| 15. | AB×CW308 | -0.19 | -0.32 | -1.06 | -4.68* | -8.45* | 3.08* | 0.09 | -0.06 | 0.09* | 2.31* | 21.28* | 286.86* |
| 16. | YW×CW | 0.36* | 0.50* | 0.062 | -3.60 | -0.86 | -0.30 | 0.33 | 0.28* | 0.06* | 1.93* | 14.99* | 211.16* |
| 17. | YW×UHFBP4 | -0.16 | 0.07 | 0.78 | 2.92 | 2.77 | 3.36* | 0.24 | 0.13 | 0.09* | -0.56 | -13.91* | 133.23* |
| 18. | YW×CW308 | -0.24 | -0.24 | 1.69 | 1.92 | -3.79 | -1.14* | 0.12 | -0.07 | 0.002 | -0.22 | 7.60* | -42.61* |
| 19. | CW×UHFBP4 | -0.006 | 0.73* | 7.90* | -4.75* | -4.82* | -0.74* | 0.02 | -0.16 | 0.01 | 0.63 | 12.22* | 137.06* |
| 20. | CW×CW308 | 0.02 | 0.32 | -4.98* | -1.08 | -1.38 | 0.13 | 0.02 | 0.18 | -0.02 | -0.63 | 3.93* | 34.75* |
| 21. | UHFBP4×CW308 | 0.087 | -0.38 | -1.26 | -1.23 | -2.75 | 1.14* | 0.10 | 0.13 | 0.08* | -0.53 | -9.21* | -78.02* |
| **SEm±** | | **0.12** | **0.19** | **1.71** | **2.18** | **1.89** | **0.41** | **0.14** | **0.12** | **0.01** | **0.41** | **0.14** | **0.12** |
| **CD at 5%** | | **0.26** | **0.40** | **3.57** | **4.55** | **3.94** | **0.86** | **0.29** | **0.25** | **0.03** | **0.86** | **0.29** | **0.25** |

*: Significance at p= 0.05; NPB: No. of primary branches, NSB: No. of secondary branches, PH: Plant height, DF: Days to 50% flowering, DFH: Days to first harvest, FL: Fruit length, FW: Fruit width, NLF: No. of lobes per fruit, PT: pericarp thickness, AFP: Average fruit per plant, AFW: Average fruit weight, YP: Yield per plant; AM: Arka Mohini, AG: Arka Gourav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder

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***(Received on 26.08.2021, Revised on 26.11.2021 and Accepted on 30.11.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 206-214, 2021*

***Original Research Paper***

# SSR marker development in *Abelmoschus esculentus* (L.) Moench using transcriptome sequencing and genetic diversity studies

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## ABSTRACT

**Okra [*Abelmoschus esculentus* (L.) Moench] also known as bhindi or lady's finger is an important vegetable crop in India, West Africa, South Africa, Brazil, USA and Turkey. It belongs to the family Malvaceae. Okra is mainly grown in tropics and subtropics of the world. The studies regarding the molecular marker development are very limited; still there is no SSR marker development from comprehensive transcriptome data in this crop. This study presents the first comprehensive transcriptome data, using RNA from different parts of okra such as root, stem, leaf, bud, flower, different stages of developing pod and from twenty days old plantlets of heat, drought and salt stressed. A total of 10,492 SSRs were identified in this study. Among these tri repeats (2112) were found to be predominant followed by di (1285), tetra (149), penta (24) and hexa repeats. Thirty-four SSRs were standardized for PCR and screened in 36 okra genotypes and accessions. Among these, 18 SSR primers were found to be highly polymorphic with the PIC values more than 0.5. And the overall results of analysis showed that expected heterozygosity ranged from 0.125 to 0.971 with a mean of 0.593; the values for observed heterozygosity ranged from 0.000 to 0.839 with the mean of 0.203; the number of allele per locus ranged from 1 to 30 and the polymorphic information content (PIC) ranged from 0.119 to 0.955 with the mean value of 0.554. The genic SSR markers developed will help in germplasm characterization mapping, genetic diversity studies, molecular assisted breeding and also in gene discovery.**

**Key words:** *Abelmoschus esculentus,* microsatellite markers, next generation sequencing, RNA sequencing and transcriptome

## INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] also known as bhendi or lady's finger is an important vegetable crop in India, West Africa, South Africa, Brazil, USA and Turkey. It belongs to the family Malvaceae and is mainly grown in tropics and subtropics of the world (Priyavathi *et al*. 2018). The total okra production in the world was found to be 9.8 Million-ton pods with an area of around 2.0 million ha and in India it is 6.1 Million ton with an area of around 5.14 lakhs ha followed by Nigeria (FAOSTAT, 2018).

The chromosome number is reported variously for this species as 2n=130 and also 2n=72, invariably the chromosome number was found to be 2n=130 with the genome size of 1.6 Gb (Joshi and Hardas 1956). It was reported that there are two kinds of *A. esculentus* L. as diploid 2n=60-70 and as a tetraploids 2n=120-130, this could be due to irregularities in the chromosome movement during the cell division of mitotic phase (Nwangburuka *et al*. 2011). Further this polyploidy level was assessed through the chloroplast DNA (cpDNA) intronic spacer and revealed that *A. esculentus* are the closest relatives of two wild species that is *A. ficulneus* and *A. moschatus* (Ramya and Bhat 2012).

Molecular markers have paved way for the assessment of genetic variations and genetic relationships among and within the species (Chakravarthi and Naravaneni 2006; Yuan *et al*. 2014, 2015). Molecular marker techniques like RFLP, RAPD, AFLP and SSR are

widely used for genetic characterization and crop improvement (Sawadogo *et al*. 2009). Especially in the less researched species, transcriptome analysis plays a vital role for the development of molecular markers (Strickler *et al*. 2012). Recently, the first report on genomic SSR marker in okra were developed using Next-Generation Sequencing technology (NGS) which was used for the assessment of genetic relatedness and cross species transferability (Ravishankar *et al*. 2018). SSR markers play a key role in many applications of plant genetics and breeding due to its codominant inheritance, multi-allelic nature, high reproducibility and good genome coverage (Bertini *et al*. 2006). There are some studies reported on SSR developed using transcriptome through NGS in okra (Schafleitner *et al*. 2013; Zhang *et al*. 2017) and transcriptome data on *M. balbisiana* and *M. acuminate* ssp. using illumina GA II X technology (Ravishankar *et al*. 2015). With the advent of sequencing technology, RNA sequencing has become an efficient and convenient technique for the SSR detection (Ronoh *et al*. 2018; Xu *et al*. 2017). However, these studies used transcriptome from one or very few tissues, which may not completely cover genic SSRs in the okra genome. Keeping this in view in this study, we present the first comprehensive characterization of combined okra transcriptome from root, stem, leaf, bud and flower, different stages of developing pods and from the abiotic stressed plantlets (drought, heat and salt). Here we also report SSR markers which would greatly help in mapping genes and linkage map development.

**Table 1. Genotypes and the accessions used in the study**

| Genotypes /Accessions | |
|---|---|
| 1. Pule Vimukha | 19. IC-0282266 |
| 2. Azad bendi | 20. IC-0128903 |
| 3. Punjab 7 | 21. IC-0128885 |
| 4. Kashi Kranthi | 22. IC-0085595 |
| 5. Varsha Upahar | 23. IC-0397980 |
| 6. Parbhani Kranthi | 24. IC-0282296 |
| 7. Kashi Leela | 25. IC-0282232 |
| 8. Pusa Sawani | 26. IC-0128891 |
| 9. Shakthi | 27. IC-0069242 |
| 10. Punjab Padmini | 28. IC-0433743 |
| 11. Azad Bendi 3 | 29. IC-0069302 |
| 12. Kashi Vibhuthi | 30. IC-0433628 |
| 13. IC-0600808 | 31. IC-0043750 |
| 14. IC-0602363 | 32. IC-0600832 |
| 15. IC-0128888 | 33. IC-0397271 |
| 16. IC-0282274 | 34. IC-0560493 |
| 17. IC-0469655 | 35. IC-0282233 |
| 18. IC-0043752 | 36. IC-0600256 |

## MATERIALS AND METHODS

### Plant material and DNA isolation

Thirty-six okra genotypes including, a few varieties from germplasm collection were used in this study (Table 1). Young leaves were collected from the okra plants which were maintained at Indian Council Of Agricultural Research- Indian Institute of Horticultural Research Bengaluru India (ICAR-IIHR), and the total genomic DNA was isolated by using the modified CTAB method (Ravishankar *et al*. 2000) with the repetition of chloroform: isoamylalcohol (24:1) for three to five times till the mucilage was removed. Finally. sDNA concentration was determined using Nano drop (NABI micro digital) by taking the absorption at 260 and 280nm.

### RNA isolation and Sequencing

For the transcriptome sequencing we isolated RNA from tissues of root, stem, leaf, bud, flower, different stages of developing pod and from twenty days old seedlings were stressed for heat ($40^0$C for 4h), salt (200mM NaCl) for two days and drought (five days of dehydration) of accession IIHR-299 using by Trizol method where 30 mg of the sample were ground into fine powder using liquid nitrogen and 1ml of Trizol (TAKARA BIO INC. Japan) was added to it and centrifuged at 12,500 rpm for 20 min, to the supernatant equal amount of chloroform was added and centrifuged at 12,500 rpm for 15 min and equal amount of isopropanol was added to the supernatant and precipitated at $-80^0$C for 1hr followed by centrifugation at 12,500 rpm for 15 min and the pellet was washed using 75% ethanol and dried pellet was dissolved using DEPC water and the RNA integrity was examined by gel electrophoresis. RNA purity was examined using Nano drop (NABI micro digital) and the equal amount of RNA from each samples were pooled and sent for RNA sequencing.

### Sequencing, quality control and *de novo* assembly

RNAseq was done at Sandoor Speciality Diagnostics Pvt. Ltd. Hyderabad facility using Illumina Hiseq

platform following manufactures instructions. Paired end cDNA library are from the pooled sample (root, stem, leaf, bud, flower, different parts of developing pods, drought stress, heat stress and salt stress plantlets) to get comprehensive okra transcriptome. Then Quality control were carried out to filter out the adaptors low quality reads >20% of bases and the unknown nucleotides with >5% reads. The clean reads was used for calculating the proportion of nucleotides with quality value larger than 20 (Q20). *De-novo* assembly was done using Trinity software assembly with the default parameters for generating contigs and transcripts (Grabherr *et al*. 2011). The NGS data was submitted to NCBI (SRR 13451946).

**Mining of SSR primer and designing**

The assembled unigenes were further examined for the presence of microsatellites using MISA software (Suping *et al.* 2013). A total of 10492 SSR Primers were identified and 2532 SSR primers were designed using Primer 3.0 software (Untergasser *et al*. 2012). A total of 51 SSR primers were randomly selected and these were used for PCR standardization and amplification of 36 okra genotypes.

**PCR conditions and genotyping**

For the amplification of mined SSRs, fluorescent based M13 tailed PCR assay was performed (Oetting *et al.* 1995). And all the primers at 5' end were labelled with standard M13 tail (Schuelke 2000). A total of 51 SSR markers were initially synthesised and screened with pooled okra DNA. Further the primers which amplified, clear bands were screened over 36 okra genotypes. The PCR conditions employed are as follows initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation, annealing and polymerization steps (94°C for 30s, 50-60°C and 72°C for 1 min) and a final extension of 72°C for 8 min. PCR amplification was carried out in 20 $\mu$L volume containing 75-100 ng of okra DNA, 2 $\mu$L of 10x Taq Buffer (Tris pH with 15mM $MgCl_2$), 1.5 $\mu$L of $MgCl_2$ (25mM of $MgCl_2$), 0.5$\mu$L of dNTPs (10mM), 0.5 $\mu$L of forward primer M13 tail (5 pM), 1 $\mu$L of reverse primer M13 tail (5 pM), 0.5 $\mu$L of probes FAM,VIC, NED and PET (5 pM), 0.2 $\mu$L of *Taq* polymerase (5 units per $\mu$L) (Genei. Pvt. Ltd Bengaluru) and 9.8 $\mu$L of nuclease free water. All the PCR reactions were carried out using Bio-RAD Thermal cycler (Bio-RAD, US). The amplified PCR products were separated on ABI3730 Genetic Analyzer (Applied Biosystem, USA), at M/S Eurofins facility Bengaluru. The obtained data were further analysed using Peak Scanner software (Applied Biosystems, USA) for determining the exact fragment size in base pair.

**Statistical analysis**

The fragment size in base pair of the PCR products were used for calculating the expected heterozygosity (He), observed heterozygosity (Ho), polymorphic information content (PIC) and number of alleles per locus employing Cervus 3.0 software (Kalinowski *et al*. 2007). And the dendrogram analysis was performed using neighbour-joining method (NJ) employing Darwin Software (Perrier *et al*. 2003; http://darwin.cirad.fr/darwin).

## RESULTS

**Sequencing and *de novo* assembly**

A total of 3.8Gb raw data were obtained using Illumina-Hiseq platform from comprehensive okra transcriptome analysis (root, stem, leaf, bud, flower, different parts of developing pods, drought stress, heat stress and salt stress plantlets). Quality control analysis was performed in order to filter out the reads containing adaptors, low quality reads and the unknown nucleotides. The total number of generated transcripts was 112597 with maximum transcripts length of 20701bp and minimum transcripts length of 201bp and total length of transcripts generated was 72314062bp. The size distributions of the transcripts are given in the Table 2. And the sequencing analysis of GC content was found to be 47.1% and AT content as 53.9%.

**Table 2. *De novo* assembly statistics**

| Transcriptome assembly | | |
|---|---|---|
| Transcripts Generated | : | 112597 |
| Maximum Transcript Length | : | 20701bp |
| Minimum Transcript Length | : | 201bp |
| Average Transcript Length | : | 642.2bp |
| Total Transcripts Length | : | 72314062bp |
| Transcripts > 100 bp | : | 112597 |
| Transcripts > 500 bp | : | 47905 |
| Transcripts > 1 Kbp | : | 21670 |
| Transcripts > 10 Kbp | : | 722369251 |
| Number of reads used | | |
| Total number of reads | : | 24885138 |
| Percentage of reads used | : | 89.9% |

### Assembly statistics and designing primers

All the obtained unigenes were screened for identification of SSRs using MISA software. The total number of examined sequence was 112597 with a total of 72314062bp and the total number of identified SSRs was found to be 10492 and the number of SSR designed using Primer 3.0 software were 2532 (Untergasser *et al*. 2012). Number of SSR containing sequence were 9849 and the number of sequence containing more than one SSR is 568 and the number of SSR present in compound formation is found to be 783 (Table 3). Further the identified SSRs were screened for di, tri, tetra, penta and hexa nucleotide repeat motifs (a total of 1285 di-repeats, 2112 tri-repeats, 149 tetra-repeats, 24 penta-repeats, 9 hexa-repeats and 783 complex repeats were observed). Tri-repeats were found to be more predominant class of microsatellite than any other classes like di, tetra, penta and hex- repeats (Fig 1). The kind of repeats observed in high frequency among tri was found to be (AGT)10 and (CCA)10 and di-repeats as (TA)22 and tetra as (TTTC)18 and among hexa all repeats were found to present once.

**Table 3. Assembly statistics of SSRs**

| | |
|---|---|
| Total number of sequences examined | 112597 |
| Total size of examined sequences (bp) | 72314062 |
| Total number of identified SSRs | 10492 |
| Number of SSR containing sequences | 9849 |
| Number of sequences containing more than 1 SSR | 568 |
| Number of SSRs present in compound formation | 783 |

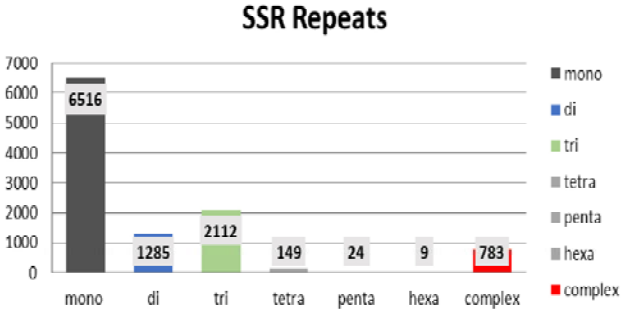


**Fig. 1. Distribution to different repeat type classes of SSR repeats**

### Genetic analysis

The allelic data regarding the expected heterozygosity, observed heterozygosity and number of alleles per locus were examined using Cervus 3.0 software. And the values for expected heterozygosity ranged from 0.125 to 0.971 with the mean value of 0.593; the values for observed heterozygosity ranged from 0.000 to 0.839 with a mean value of 0.203; the number of allele per locus ranged from 1 to 30 and the polymorphic information content (PIC) ranged from 0.119 to 0.955 with the mean PIC value of 0.554 and PI (Probability of identity) values ranged from 0.0036 to 1.0000 with a mean value of 0.263 (Table 4). Dendrogram analysis showed that the genotypes used in the study were classified into three major clusters (Fig 2).

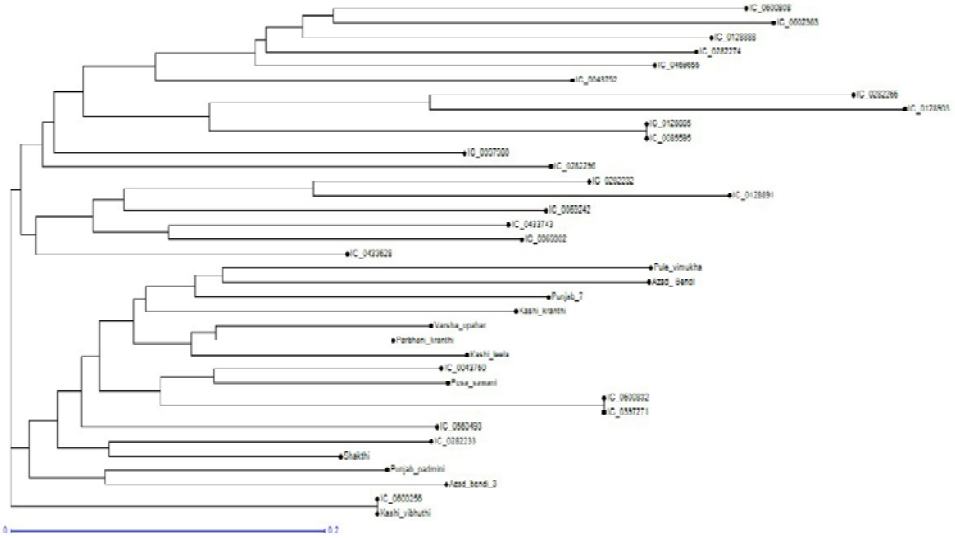

**Fig. 2. Dendrogram analysis showing the genetic relationship among *Abelmoschus esculentus* L. accessions using transcriptome SSR marker data**

**Table 4. Genetic analysis of microsatellite loci using 34 SSRs**

| Sl. No. | Primer name | Primers | *T*m | Allele size | No. of Allele/locus | Ho | He | PIC value | PI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | IIHR-2434 | F: AGCTTCCGTATATTTTGGATT<br>R: CCAAACTATCCAACTATGCTT | 55 | 160 | 18 | 0.156 | 0.884 | 0.861 | 0.0265 |
| 2 | IIHR-1877 | F: TGAGATTCGTTTGATCGTTTA<br>R: CTTTGGGTCAAAGCTGTC | 55 | 151 | 4 | 0.200 | 0.444 | 0.408 | 0.3464 |
| 3 | IIHR-817 | F: TAAATATGCTTCTCAGGCATT<br>R: CGTCTTGTTACGATTTATATGC | 55 | 163 | 1 | 0.000 | 0.324 | 0.307 | 1.0000 |
| 4 | IIHR-518 | F: TCCCTCGTACTAGATCATTCA<br>R: GTAACAAGGATGAGCAAAAGA | 55 | 150 | 5 | 0.143 | 0.508 | 0.457 | 0.2935 |
| 5 | IIHR-205 | F: GGGAAGATTTTGCTAAACTTATT<br>R: CCAATAGGATGTCTCAGTCAA | 57 | 151 | 5 | 0.200 | 0.414 | 0.386 | 0.3727 |
| 6 | IIHR-91 | F: TGATCTTCGATTCATCCTTAT<br>R: AGAATGGCAGCGCCAAAAG | 55 | 151 | 3 | 0.030 | 0.287 | 0.250 | 0.5472 |
| 7 | IIHR-30 | F: TAAAATTTTCCCATCAATCC<br>R: GGTGTTTGTTTTGTGGTGATA | 60 | 172 | 4 | 0.094 | 0.424 | 0.371 | 0.3861 |
| 8 | IIHR-18 | F: TCTCTTTAAAATCACCGCTAA<br>R: TTTAGCAAGGAAGGGAGAA | 57 | 152 | 19 | 0.152 | 0.908 | 0.887 | 0.0187 |
| 9 | IIHR-353 | F: TAAAAATCAGAGCCTTCCTTT<br>R: CAGATTTCTGAGAGCAAAGAG | 55 | 174 | 6 | 0.457 | 0.701 | 0.644 | 0.1429 |
| 10 | IIHR-343 | F: GATATGGGATGGTTGAAATC<br>R: GAGAAAACCAACGGATGAT | 57 | 152 | 5 | 0.171 | 0.472 | 0.439 | 0.3122 |
| 11 | IIHR-328 | F: TAGGAAAACTACAGCAAGGATT<br>R: GGACTTGGTTCTGCAATCT | 60 | 150 | 3 | 0.000 | 0.125 | 0.119 | 0.7731 |
| 12 | IIHR-319 | F: GCACTTGATATTGCATTACATT<br>R: CCAAATCATTATCAGGGAGT | 55 | 150 | 3 | 0.156 | 0.347 | 0.311 | 0.4641 |
| 13 | IIHR-303 | F: TAGGAGGACAATCACAGAAAA<br>R: GGTAACCCAAGTGTTGTTCTT | 57 | 151 | 22 | 0.176 | 0.921 | 0.902 | 0.0139 |
| 14 | IIHR-277 | F: GCTCAAGTAAGCATTAAAACAG<br>R: GTCGTGCAAAACTTGTCTAAG | 55 | 162 | 11 | 0.000 | 0.869 | 0.839 | 0.0370 |
| 15 | IIHR-267 | F: TAAGGAGTCCAAACTCCAACT<br>R: TGGTTGTTTAGGTTCCAATTT | 55 | 160 | 11 | 0.313 | 0.760 | 0.724 | 0.0881 |
| 16 | IIHR-254 | F: TGTCTGTAGTCTCGCAACTTT<br>R: ATACATTGACGGTACAAGTGG | 57 | 152 | 13 | 0.794 | 0.679 | 0.620 | 0.1590 |
| 17 | IIHR-244 | F: TGGGGCCTAAGTAAATACAAT<br>R: AAAGTTAGTTCAATGCAGTTTTC | 57 | 180 | 11 | 0.030 | 0.759 | 0.732 | 0.0792 |
| 18 | IIHR-221 | F: ACAGGTCCATAAATGCTATGA<br>R: CCCTAATATTATTGTTTTTACCC | 58 | 161 | 7 | 0.000 | 0.673 | 0.605 | 0.1713 |
| 19 | IIHR-195 | F: TCACTTAACCCCATGAAAAAT<br>R: GTTTCTGAGAATCCTTGCTG | 55 | 158 | 28 | 0.324 | 0.957 | 0.940 | 0.0060 |
| 20 | IIHR-165 | F: GGATGACCAAAACGAAGTG<br>R: CTGTCATTTTCTTTCCTTCTG | 57 | 151 | 2 | 0.000 | 0.507 | 0.375 | 0.3752 |
| 21 | IIHR-154 | F: CGCCGTAGTACCTCAATCTT<br>R: GCAATTAACGGTGACGAC | 55 | 153 | 30 | 0.333 | 0.971 | 0.955 | 0.0036 |
| 22 | IIHR-99 | F: TGAAAAGAACATGAAAGCCTA<br>R: CCTTCCTTCCTAGTCATCATC | 57 | 160 | 15 | 0.156 | 0.742 | 0.707 | 0.0958 |
| 23 | IIHR-94 | F: TATATTTGCAGCATTTGTCTGT<br>R: AACAGTCGGTACTTAGACAGC | 57 | 151 | 18 | 0.545 | 0.891 | 0.870 | 0.0228 |
| 24 | IIHR-68 | F: GAACTTTTGGAATTTGTGTCA<br>R: TTCTTGGAGTAGGAGCTTGAT | 60 | 153 | 11 | 0.061 | 0.822 | 0.791 | 0.0543 |
| 25 | IIHR-50 | F: GTTCAGGATCAGAGTCGAG<br>R: GCGGCCTCAATATTCACT | 55 | 150 | 8 | 0.032 | 0.589 | 0.544 | 0.2118 |
| 26 | IIHR-36 | F: GGGACAGAGTTGAAAATGAC<br>R: GGATCAGGAATGTTATCGACT | 55 | 150 | 7 | 0.065 | 0.396 | 0.377 | 0.3856 |
| 27 | IIHR-27 | F: GGAACTCCGGTGGAGAAG<br>R: AAGCTTTATCTCAAAAATCC | 57 | 150 | 7 | 0.188 | 0.624 | 0.589 | 0.1738 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 28 | IIHR-11 | F: TGGAAGAGAAGAAGAACAACA<br>R: TTCACGATGAACTGACC | 55 | 151 | 6 | 0.645 | 0.556 | 0.478 | 0.2741 |
| 29 | IIHR-02 | F: AACAACAACAACAACAGTCG<br>R: CATAAAAAGTGTTTGCGTCTC | 55 | 158 | 18 | 0.147 | 0.879 | 0.855 | 0.0295 |
| 30 | IIHR-1463 | F: TGACGATCTTCACAGGCTAGTA<br>R: AAGTGAACCAGGTAGCATGT | 57 | 153 | 4 | 0.219 | 0.584 | 0.521 | 0.2342 |
| 31 | IIHR-1506 | F: TTGAAACTCCCACTATCAAAA<br>R: TAATTATGGAGGTGGAGGTG | 55 | 150 | 4 | 0.839 | 0.543 | 0.447 | 0.3042 |
| 32 | IIHR-1896 | F: CAATGCCAGATTTCTTTGTAG<br>R: TTCCTTGCTTTAGTTTTCCTT | 55 | 163 | 3 | 0.029 | 0.140 | 0.132 | 0.7494 |
| 33 | IIHR-1835 | F: CCATTATATCTTATCCGTTCG<br>R: CATACACGTCAAAAACATCAA | 55 | 214 | 5 | 0.286 | 0.505 | 0.467 | 0.2825 |
| 34 | IIHR-1680 | F: GGTGGCAACATTATCCAT<br>R: GGAGGTGGCTATAACAGAAAT | 55 | 168 | 3 | 0.031 | 0.294 | 0.256 | 0.5386 |
| | **Mean** | | | **9.412** | | **0.2037** | **0.5933** | **0.5546** | **0.2639** |

## DISCUSSION

Okra is an important vegetable crop in India, Africa and other Asian countries and is considered as a minor crop at the genome studies until recently, very little attention was paid towards its genetic improvement and generation of genomic resources. The studies regarding the molecular marker development are very limited, and there is a still no comprehensive transcriptome data for this crop. This study presents the first comprehensive transcriptome data from different parts of okra such as from root, stem, leaf, bud, flower, different stages of developing pod and from twenty days old plantlets of heat, drought and salt stressed by RNA sequencing. RNA sequencing is considered as an effective way for obtaining the gene sequences of a non-model crop (Strickler *et al*. 2012) and for developing the SSR markers (Zhang *et al*., 2010; Guo *et al*., 2016& Ravishankar *et al*. 2015). The application and development of molecular marker technology as it detects the genetic differences at the DNA level and is commonly used in the evaluation of genetic diversity and mapping (Yoder *et al*. 2018; Niemandt *et al*. 2018 & Pan *et al*. 2017). SSRs are considered as an important marker for application in plant genetics and breeding studies, because of its high reproducibility, codominant, multi allelic nature and good genome coverage. Genic SSRs developed from transcriptome data are highly useful as they reflect functional variability.

On an average 112,597 unigenes were with an maximum length of 20,701 were obtained through comprehensive okra transcriptome which was little lesser than a study on combined leaf and pod transcriptome of okra which yielded a total of 150,000 unigenes (Schafleitner *et al*. 2013) and higher than studies on okra by NGS using RNA sequencing from the leaf samples which yielded a total 66,382 assembled unigenes (Priyavathi *et al*. 2018); 94,769 unigenes with an length of 1921bp were obtained through NGS of transcriptome sequencing in okra to drought stress (Shi *et al*. 2020) and 293971 unigenes with okra transcriptome sequencing of five organs (roots, stem, leaves, flower and fruits) (Zhang *et al*. 2018). In our present study though a large number of unigenes have been produced compound than a study by (Schafleitner *et al*. 2013) and higher than the other studies which indicates that the sequencing depth was not sufficient to represent the whole transcriptome. Deeper and increased sequencing would have reduced the redundancy of unigenes annotation. However, redundancy at certain level is also due to the allopolyploid of *Abelmoschus*, where the transcripts from different genomes with slightly different sequences are present in the transcriptome (Schafleitner *et al*. 2013).

The number of SSRs identified in this study are 10, 492 which is high compared to earlier studies on *Abelmoschus esculentus* 9,574 (Priyavathi *et al*. 2018) and *Clerodendrum trichotomum* which is 6,444 (Chen *et al*. 2019) and among the mined SSRs the tri repeats (2112) are more predominant followed by di (1285), tetra (149), penta (24) and hexa (9) this pattern is similar in earlier studies in *Abelmoschus esculentus* (Shi *et al*. 2020; Priyavathi *et al*. 2018 & Schafleitner *et al*. 2013). The frequency of tri repeats are higher in transcriptome sequencing (Schafleitner *et al*. 2013) because of the shortening or the extension of amino acid in proteins may not cause much alteration in

functions and other type of repeats cause frame shift mutation. But the mechanisms behind the evolution and origin of microsatellite repeats are not very clear. So, the relative dominant occurrence of repeats motifs may be due to their evolution through various selection pressures. It was assumed that replication slippage and unequal crossing over are the few common mutation mechanisms which might come addition or removal of motifs leading to the variation in length (Buschiazzo and Gemmell 2006; Sonah *et al*. 2011).

In the present study, we successfully identified 10,492 SSRs and 34 SSRs were standardized for PCR and screened over 36 okra genotypes and accessions. Among these 18 SSR primers were found to be highly polymorphic with the PIC values more than 0.5. And the overall statistical analysis revealed that expected heterozygosity ranged from 0.125 to 0.971 with the mean 0.593; the values for observed heterozygosity ranged from 0.000 to 0.839 with the mean of 0.203; the number of allele per locus ranged from 1 to 30 and the Polymorphic Information Content (PIC) ranged from 0.119 to 0.955 with the mean value of 0.554. Similar kind of work were performed on okra reported Polymorphic information content (PIC) across all 50 loci values ranged from 0.000 to 0.865 with a mean value of 0.519. The observed and expected heterozygosity ranged from 0.000 to 0.750, and 0.000 to 0.972, respectively. Alleles per locus ranged from 1 to 27 (Ravishankar *et al*. 2018). A study for the development and characterization of SSR in cotton with the mean PIC of 0.65 (John *et al*., 2012) and a genetic diversity in cotton with the mean PIC of 0.8 (Muhammad *et al*. 2013). Dendrogram analysis depicted that all the genotypes used in the study were classified into three major clusters with most of the accessions grouped to cluster I, and the Cluster II & III with the mixture of genotypes and accessions. The SSR markers developed here will help in genetic diversity studies, mapping, marker assisted breeding and helpful in gene discovery. Being gene based SSR markers, these markers would be great help in tagging genes for various traits.

## ACKNOWLEDGEMENTS

We thank the RKVY (Rashtriya Krishi Vikas Yojana) for the financial assistance.

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***(Received on 24.08.2021, Revised on 27.11.2021 and Accepted on 18.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 215-221, 2021*

***Original Research Paper***

# Generation mean analysis of important yield traits in bitter gourd (*Momordica charantia*)

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## ABSTRACT

**Generation mean analysis study in bitter gourd was undertaken using six basic generations viz. $P_1$, $P_2$, $F_1$, $F_2$, $B_1$ and $B_2$ population were developed from gynoecious (IIHRBTGy- 491) × monoecious (IIHR Sel -19 -1 and IIHR Sel-78-4) crosses. The gynoecious parent was superior for node for first female flowering, number of fruits and yield/plant whereas the monoecious parents were superior for fruit length, fruit diameter and fruit weight. $F_1$ showed superior performance over mid parent for number of fruits, fruit length, fruit weight and yield per plant. $F_2$ plants were significantly diverse. $B_1$ and $B_2$ population exhibited mean value closer to their recurrent parents. Significance of one or more scaling tests, i.e. A, B, C and D in most of the traits revealed the presence of epistasis in both the crosses except for node bearing 1st male flower. Days to 1st female flower opening, node bearing 1st female flower, fruit diameter and yield showed presence of duplicate epistasis whereas days to 1st male flower opening, number of fruits per plant, fruit length and fruit weight showed complimentary epistasis in IIHRBTGy - 491 × IIHR Sel -19 -1 cross. Node bearing 1st female flower, fruit length, fruit diameter and yield showed presence of duplicate epistasis whereas days to 1st female flower opening, days to 1st male flower opening, number of fruits and fruit weight showed complimentary epistasis in IIHRBTGy - 491× IIHR Sel-78-4 cross. Additive gene action may be predominant for inheritance of node bearing 1st male flower.**

**Key words**: Bitter gourd, epistatic interactions. gene action and scaling test.

## INTRODUCTION

Bitter gourd is an economically important vegetable crop and considered as one of the most nutritious gourds, grown for its fruit and leaves. It is a good source of phytonutrients like carbohydrates, minerals like iron, calcium, phosphorus and vitamin B, vitamin C, and also contains vitamin A (Behera *et al*., 2010). The primary centre of diversity is India, and China is considered as the secondary centre of diversity. It is grown widely throughout India. The primary breeding goal for bitter gourd is to increase fruit yield and quality. This gynoecious sex form has been commercially exploited worldwide in cucumber for increased number of fruits, earliness, uniformity and mechanical harvesting. It is mostly useful for hybrid development as it avoids manual emasculation and pollination. So simply by isolating from other genotypes and with a desirable parent we can go for hybrid development. Yield is a complicated trait influenced by polygenes with small but cumulative effects. Therefore, detailed understanding of the genetics and inheritance that underpins yield and its component traits is required in order to achieve the actual yield potential by adopting appropriate breeding and selection strategies. Generation mean analysis has proven to be a useful tool for estimating various genetic parameters. Hayman (1960) proposed the concept of generation mean analysis for estimating various genetic components. This method gives data on several genetic parameters as well as epistatic interactions. It is beneficial to have a precise understanding of the nature and magnitude of gene action of various characters to maximise the use of genetic potential by choosing of effective breeding methods.

## MATERIALS AND METHODS

The sib-mated seeds of gynoecious bitter gourd germplasm, IIHRBTGy–491 and two monoecious lines IIHR Sel -19 -1 and IIHR Sel-78-4 used as parents to develop, $F_1$, $F_2$ and back cross generations during 2018–2021 at Vegetable Research Block VIII of Division of Vegetable Crops, ICAR–Indian Institute of Horticultural Research, Bengaluru. The IIHRBTGy–491gynoecious plant was maintained by sib matting and through the pollens from silver nitrate 250 ppm induced hermaphrodite flowers in the gynoecious plant. The data was recorded on 10 competitive plants in parents and $F_1$, 100 plants in $F_2$ and 20 plants in backcrosses laid out in a randomized complete block design in three replications. The observations were recorded for 9 economical characters viz., days to first female flower opening,

**Table 1. Generation means for different characters**

| Character | Cross | $P_1$ | $P_2$ | MP | $F_1$ | $F_2$ | $B_1$ | $B_2$ |
|---|---|---|---|---|---|---|---|---|
| Days to 1st female flower opening | 1 | 29.10 ± 0.73 | 37.23 ± 0.93 | 33.16 | 37.96 ± 0.84 | 35.25 ± 0.43 | 33.66 ± 1.62 | 38.00 ± 0.90 |
| | 2 | 28.93 ± 0.67 | 38.13 ± 0.85 | 33.53 | 39.10 ± 0.71 | 34.47 ± 0.43 | 30.10 ± 0.67 | 36.66 ± 1.02 |
| Days to 1st male flower opening | 1 | 0.00 ± 0.00 | 29.23 ± 0.68 | 14.61 | 27.53 ± 0.75 | 24.25 ± 1.49 | 17.63 ± 5.71 | 31.06 ± 0.60 |
| | 2 | 0.00 ± 0.00 | 28.93 ± 0.83 | 14.46 | 25.20 ± 0.48 | 25.07 ± 1.46 | 15.56 ± 5.03 | 33.16 ± 0.75 |
| Node bearing 1st male flower | 1 | 0.00 ± 0.00 | 5.80 ± 0.47 | 2.90 | 4.33 ± 0.26 | 3.77 ± 0.24 | 2.50 ± 0.82 | 5.26 ± 0.32 |
| | 2 | 0.00 ± 0.00 | 6.96 ± 0.30 | 3.48 | 5.33 ± 0.24 | 4.25 ± 0.26 | 2.60 ± 0.85 | 5.83 ± 0.26 |
| Node bearing 1st female flower | 1 | 4.26 ± 0.31 | 12.40 ± 0.74 | 8.33 | 9.83 ± 0.72 | 9.40 ± 0.37 | 8.46 ± 1.09 | 13.00 ± 0.53 |
| | 2 | 4.26 ± 0.31 | 13.26 ± 0.86 | 8.76 | 11.86 ± 0.44 | 9.95 ± 0.38 | 7.73 ± 1.20 | 13.33 ± 0.50 |
| Number of fruits per plant | 1 | 42.10 ± 1.53 | 26.56 ± 1.03 | 34.33 | 37.73 ± 1.19 | 37.57 ± 0.77 | 41.96 ± 3.11 | 29.56 ± 0.85 |
| | 2 | 42.10 ± 1.53 | 24.49 ± 0.83 | 33.29 | 35.43 ± 1.09 | 38.01 ± 0.78 | 46.26 ± 1.83 | 30.56 ± 0.94 |
| Fruit length (cm) | 1 | 12.23 ± 0.33 | 22.91 ± 0.28 | 17.57 | 17.70 ± 0.26 | 14.42 ± 0.47 | 13.53 ± 0.21 | 21.15 ± 0.37 |
| | 2 | 12.23 ± 0.33 | 17.89 ± 0.30 | 15.06 | 16.57 ± 0.47 | 12.14 ± 0.46 | 13.13 ± 0.38 | 18.52 ± 0.30 |
| Fruit diameter (cm) | 1 | 4.18 ± 0.11 | 4.57 ± 0.15 | 4.37 | 4.31 ± 0.15 | 4.26 ± 0.04 | 4.09 ± 0.14 | 4.63 ± 0.15 |
| | 2 | 4.18 ± 0.11 | 5.02 ± 0.08 | 4.61 | 4.39 ± 0.12 | 4.39 ± 0.04 | 4.12 ± 0.11 | 4.72 ± 0.13 |
| Fruit weight (g) | 1 | 79.56 ± 1.38 | 106.00 ± 2.04 | 92.78 | 96.63 ± 3.55 | 98.19 ± 3.39 | 77.93 ± 1.82 | 103.46 ± 1.27 |
| | 2 | 79.56 ± 1.38 | 117.34 ± 3.18 | 98.36 | 109.43 ± 3.81 | 103.21 ± 3.41 | 83.69 ± 1.24 | 120.63 ± 1.42 |
| Yield/ plant (kg) | 1 | 3.54 ± 0.12 | 2.72 ± 0.13 | 3.13 | 3.26 ± 0.21 | 3.10 ± 0.16 | 3.28 ± 0.26 | 2.18 ± 0.11 |
| | 2 | 3.54 ± 0.12 | 2.87 ± 0.18 | 3.18 | 3.22 ± 0.21 | 3.06 ± 0.16 | 3.31 ± 0.35 | 2.37 ± 0.29 |

1: IIHRBTGy - 491× IIHR Sel -19 -1; 2: IIHRBTGy - 491× IIHR Sel -78-4

days to 1st male flower opening, node bearing 1st male flower, node bearing 1st female flower, number of fruits per plant, fruit length (cm), fruit diameter (cm), single Fruit weight (g) and fruit yield/ plant (g). Data from three replications was pooled to calculate mean values for all of the attributes investigated for the parents ($P_1$ and $P_2$), $F_1$'s ($P_1 \times P_2$), $F_2$'s ($F_1$'s selfed) and their first-generation backcrosses ($B_1$'s = $F_1 \times P_1$ and $B_2$'s = $F_1 \times P_2$). The ABCD scaling tests of Mather and Jinks (1982) were employed to detect the presence of non-allelic interactions before calculating the different parameters. In addition to scaling test data was further subjected to joint scaling (Deb and Khaleque 2009). The parameters for the various gene effects employed in this investigation are the same as those used by Hayman (1960) namely, mean (m), additive (d), dominance (h), additive × additive (i), additive × dominance (j) and dominance × dominance (l). The OPSTAT software was used to perform the generation mean analysis.

## RESULT AND DISCUSSION

The information regarding gene action, interaction and inheritance study is the key factor for designing appropriate breeding strategy for improvement of any crop. The gynoecious parent IIHRBTGy – 491 was superior for node for first female flowering, number of fruits and yield/plant whereas the monoecious parents were superior for fruit length, fruit diameter and fruit weight. The mean performance of $F_1$ surpassed the mid parent for number of fruits, fruit length, fruit weight and yield per plant (Table - 1) in both the crosses (IIHRBTGy - 491 × IIHR Sel -19 - 1 and IIHRBTGy - 491× IIHR Sel-78-4). The superior performance of $F_1$ over mid parent indicated that these traits can be exploited through heterosis breeding. These findings are consistent with the findings of Dey *et al*. (2012) and Mishra *et al* (2015). The reduction in mean performance of $F_2$ population than $F_1$ for fruit length and yield in both crosses was observed, implying influence of inbreeding depression. Rathod *et al*. (2021) also obtained similar results in bitter gourd.

### Days to 1st female flower opening

In IIHRBTGy - 491 × IIHR Sel -19 -1cross, C scale was significant (4.25) (Table - 2) and dominance component (h ) was also significant (7.12) (Table - 3). The opposite sign of h (7.12) and l (-3.38) indicates presence of duplicate epistasis. Mishra *et al* (2015) reported similar gene interaction for the trait days to first flowering in the cross of DBGy 201 × Pusa Do Mausami indicating selection at later generation. However, in IIHRBTGy - 491× IIHR Sel-78-4 all four scales were significant which indicate the inadequacy of simple additive-dominance model to estimate the gene effects. The similar sign of h (2.20) and l (16.09) indicates presence of complementary epistasis. Kumari *et al*. (2015) reported additive gene effect and Rani *et al*. (2014) reported presence of dominance and epistasis for the trait.

### Days to 1st male flower opening

In IIHRBTGy - 491 × IIHR Sel-19-1 cross, B (2.63) and C (3.27) scale and dominance component (21.29) were significant. The similar sign of h (21.29) and l (2.52) indicates presence of complementary epistasis. Similarly, in IIHRBTGy - 491× IIHR Sel-78-4 cross, A (4.06) and B (-2.20) scales were significant which indicate the inadequacy of simple additive-dominance model to estimate the gene effects. The similar sign of h (17.89) and l (4.70) indicate presence of complementary epistasis. Kumari *et al*. (2015) and Thangamani (2016) reported additive gene effect for days to 1st male flowering.

### Node bearing 1st male flower

In both the crosses all the scaling tests, namely, A, B, C and D were insignificant for node bearing 1st male flower. It was determined that the additive–dominance model is sufficient to explain the effects. The sufficiency of the simple additive–dominance model implies that nonallelic interaction is absent and generation means are solely dependent on the additive–dominance effect of the gene. Additive gene action may be predominant for inheritance and selection should be delayed to later generations for this trait. Similar result reported by Thangamani (2016).

### Node bearing 1st female flower

In IIHRBTGy - 491 × IIHR Sel -19 -1 cross, C (4.72) and D (-2.66) scale and dominance (9.82) component were significant. Non-additive component has a significant role in the inheritance of this trait. The opposite sign of h (9.82) and l (-5.92) indicates presence of duplicate epistasis. Similarly, in IIHRBTGy - 491× IIHR Sel-78-4 cross, C (3.44) and D (-2.15) scales were significant. The opposite sign of h (8.40) and l (-5.17) indicates presence of duplicate

**Table 2. Scaling test**

| Character | Cross | A | B | C | D |
|---|---|---|---|---|---|
| Days to 1st female flower opening | 1 | -0.26 ± 1.98 | -0.80 ± 1.27 | 4.25 ± 1.54** | -1.16 ± 1.18 |
| | 2 | 7.83 ± 0.96** | 3.90 ± 1.34** | 7.37 ± 1.44** | 2.18 ± 0.86** |
| Days to 1st male flower opening | 1 | 0.26 ± 6.61 | 2.63 ± 0.91** | 3.27 ± 3.57** | -0.18 ± 3.73 |
| | 2 | 4.06 ± 5.81** | -2.20 ± 1.03** | -0.97 ± 3.47 | 1.42 ± 3.39 |
| Node bearing 1st male flower | 1 | -0.66 ± 0.96 | -0.40 ± 0.48 | -0.61 ± 0.70 | -0.22 ± 0.58 |
| | 2 | 0.13 ± 0.99 | 0.63 ± 0.37 | 0.63 ± 0.69 | 0.06 ± 0.59 |
| Node bearing 1st female flower | 1 | 0.16 ± 1.34 | -0.76 ± 0.86 | 4.72 ± 1.29** | -2.66 ± 0.82* |
| | 2 | -0.33 ± 1.42 | -0.53 ± 0.81 | 3.44 ± 1.14** | -2.15 ± 0.87* |
| Number of fruits per plant | 1 | 8.90 ± 3.76** | 8.16 ± 1.34** | 13.82 ± 2.50** | 1.62 ± 2.06 |
| | 2 | 1.00 ± 2.38 | 11.76 ± 1.35** | 14.36 ± 2.42** | -0.80 ± 1.49 |
| Fruit length (cm) | 1 | 2.75 ± 0.34* | 6.19 ± 0.48** | 12.63 ± 1.17** | -2.84 ± 0.60* |
| | 2 | 2.54 ± 0.55* | 1.52 ± 0.47 | 10.79 ± 1.22** | -3.36 ± 0.60** |
| Fruit diameter (cm) | 1 | -0.02 ± 0.20 | -2.10 ± 0.21* | -3.15 ± 0.23** | 0.01 ± 0.13 |
| | 2 | 0.04 ± 0.16 | 2.49 ± 0.17* | 4.66 ± 0.19** | -0.06 ± 0.11 |
| Fruit weight (g) | 1 | 47.32 ± 3.04** | -11.63 ± 2.88** | 24.03 ± 8.95** | 5.82 ± 4.13** |
| | 2 | 43.12 ± 3.15** | -5.00 ± 3.30** | 30.41± 9.25** | 3.85 ± 4.16** |
| Yield/ plant (kg) | 1 | 2.61 ± 0.34* | 0.63 ± 0.20 | 2.79 ± 0.46* | 0.73 ± 0.25 |
| | 2 | 2.44 ± 0.34* | 0.80 ± 0.21 | 3.93 ± 0.46** | 0.65 ± 0.25 |

*, ** significant at 5 and 1% probability respectively

1: IIHRBTGy - 491 × IIHR Sel - 19 - 1; 2: IIHRBTGy - 491 × IIHR Sel - 78 - 4

epistasis. Similar result obtained by Mishra *et al*. (2015) and additive gene action for the trait reported by Thangamani (2016).

**Number of fruits per plant**

In both the crosses, B and C scales were significant and dominance component, dominance × dominance components were significantly higher compared to additive component which indicate the inadequacy of simple additive-dominance model to estimate the gene effects. The similar sign of h and l indicates presence of complementary epistasis in both the cross. Similar result reported by Mishra *et al*. (2015) in DBGy 201 × Pusa Do Mausami cross and complementary epistasis observed in DBGy 201 × S-2 cross. Shukla *et al*. (2014) reported insignificant $\chi^2$ value for number of fruits/plant, internodal length, seeds/fruit and yield/ plant in Gy333 × DRAR-1 cross indicating the absence of non-allelic interaction.

**Fruit length**

In IIHRBTGy - 491 × IIHR Sel -19 -1 cross, all the scaling tests, namely, A, B, C and D were significant and dominance component was higher compared to additive component. The similar sign of h (3.71) and l (5.26) indicates presence of complementary epistasis. However, in IIHRBTGy - 491× IIHR Sel-78-4cross, A, C and D scales were significant and dominance, additive × additive components were in positive direction indicating their significant role in inheritance

**Table 3. Estimates of components of generation mean for different yield related character in bitter gourd**

| Character | Cross | m | d | h | i | j | l | Epistasis |
|---|---|---|---|---|---|---|---|---|
| Days to 1st female flower opening | 1 | 35.25 ± 0.24 | -4.33 ± 1.07** | 7.12 ± 2.44** | 2.32 ± 2.36* | -0.53 ± 2.25 | -3.38 ± 4.56** | D D |
| | 2 | 34.47 ± 0.25 | -6.56 ± 0.70** | 2.20 ± 1.81* | -4.36 ± 1.73** | -3.93 ± 1.55** | 16.09 ± 3.18** | C C |
| Days to 1st male flower opening | 1 | 24.25 ± 0.87 | -13.43 ± 3.31** | 21.29 ± 7.49** | 0.37 ± 7.43 | 2.36 ± 6.64* | 2.52 ± 13.74* | C C |
| | 2 | 25.07 ± 0.84 | -17.60 ± 2.93** | 17.89 ± 6.79** | -2.84 ± 6.78* | -6.26 ± 5.89** | 4.70 ± 12.24** | C C |
| Node bearing 1st male flower | 1 | 3.77 ± 0.14 | 2.77 ± 0.50* | 1.89 ± 1.18 | 0.45 ± 1.16 | 0.27 ± 1.05 | -1.52 ± 1.09 | - - |
| | 2 | 4.25 ± 0.15 | 3.23 ± 0.51* | 1.72 ± 1.20 | -0.13 ± 1.19 | 0.50 ± 2.17 | 0.90 ± 2.15 | - - |
| Node bearing 1st female flower | 1 | 9.40 ± 0.21 | -4.53 ± 0.70** | 9.82 ± 1.72** | 5.32 ± 1.65** | -0.93 ± 1.48 | -5.92 ± 3.10** | D D |
| | 2 | 9.95 ± 0.21 | -4.60 ± 0.75** | 8.40 ± 1.78** | 4.30 ± 1.74** | -0.20 ± 1.59 | -5.17 ± 3.22** | D D |
| Number of fruits per plant | 1 | 37.57 ± 0.44 | 3.82 ± 1.86** | 10.40 ± 4.22** | -3.24 ± 4.13** | -0.73 ± 3.87 | 20.30 ± 7.85** | C C |
| | 2 | 38.01 ± 0.45 | 4.25 ± 1.19** | 15.70 ± 3.10** | 1.60 ± 2.99 | 10.76 ± 2.59** | 11.16 ± 5.35** | C C |
| Fruit length (cm) | 1 | 14.42 ± 0.27 | -3.62 ± 0.24** | 3.71 ± 1.22** | 3.68 ± 1.21** | 3.44 ± 0.55** | 5.26 ± 1.53** | C C |
| | 2 | 14.14 ± 0.26 | -5.39 ± 0.28** | 6.19 ± 1.24** | 6.73 ± 1.20** | -1.02 ± 0.62 | -2.66 ± 1.66* | D D |
| Fruit diameter (cm) | 1 | 4.26 ± 0.02 | 2.05 ± 0.12* | 2.26 ± 0.28* | -1.02 ± 0.26 | -2.07 ± 0.27* | -5.10 ± 0.54** | D D |
| | 2 | 4.35± 0.71 | -1.20 ± 0.10 | -3.15 ± 0.24** | 2.13 ± 0.23* | 4.44 ± 0.21** | 11.40 ± 0.44** | D D |
| Fruit weight (g) | 1 | 108.19 ± 1.95 | -48.21 ± 1.33** | 7.19 ± 8.55** | -11.65 ± 8.27** | -58.96 ± 3.03** | 47.34 ± 10.43** | C C |
| | 2 | 107.21 ± 1.97 | -54.70 ± 1.33** | 1.52 ± 8.67 | -7.71 ± 8.32** | -48.12 ± 3.34** | 45.84 ± 10.68** | C C |
| Yield/ plant (kg) | 1 | 4.10 ± 0.09 | -0.90 ± 0.17 | -4.48 ± 0.52** | -1.46 ± 0.50 | -1.97 ± 0.35 | 4.71 ± 0.82** | D D |
| | 2 | 4.06 ± 0.09 | -0.90 ± 0.17 | -3.67 ± 0.52** | -1.31 ± 0.50 | -1.63 ± 0.36 | 4.56 ± 0.82** | D D |

*, ** significant at 5 and 1% probability respectively
1: IIHRBTGy - 491× IIHR Sel -19 -1; 2: IIHRBTGy - 491× IIHR Sel -78-4
C: Complementary epistasis, D: Duplicate epistasis

of the trait. Presence of duplicate epistasis is noticed. Similar result obtained by Mishra *et al*. 2015 for fruit length in both DBGy 201 × S-2 and DBGy 201 × Pusa Do Mausami) whereas incomplete dominance effect for fruit length reported by Kumari *et al*. (2015).

**Fruit diameter**

In both the crosses, B and C scale were significant. In IIHRBTGy - 491× IIHR Sel -19 -1 cross additive (2.05) and dominance (2.26) components were significant while in IIHRBTGy - 491× IIHR Sel -78-

4 cross dominance × dominance (11.40) component was significant. The opposite sign of h and l indicates presence of duplicate epistasis in both the crosses. In such circumstances, available populations must be carried to future generations in order to arrive at the best-fit model (Mather and Jinks 1982). The opposite signs of h and l neutralize each other, resulting in reduced heterosis for the trait. Similar result obtained by Mishra *et al*. (2015).

**Fruit weight**

In both the crosses, all the scaling tests, namely, A, B, C, D were significant and dominance × dominance (l) component was significantly higher. Non-additive component has significant role in the inheritance of this trait. The similar sign of h and l indicates presence of complementary epistasis. In contrary to the result, duplicate epistasis with predominance of additive × dominance gene action reported by Mishra *et al*. (2015) in both the crosses *i.e* DBGy 201 × S-2 and DBGy 201 × Pusa Do Mausami and Thangamani (2016) reported presence of additive gene action for fruit weight.

**Yield/plant**

In both the crosses, A and C scales were significant and dominance × dominance (l) component was higher and in positive direction. Non-additive component has a significant role in the inheritance for yield per plant. The opposite sign of h and l indicates presence of duplicate epistasis. Similar result obtained by Mishra *et al*. (2015) in both the crosses namely DBGy 201 × S-2 and DBGy 201 × Pusa Do Mausami and Shukla *et al*. (2014) in Cross Gy323 × DRAR-1. The opposite signs neutralize each other. It also shows reduced variability in segregating generations, which prevents the selection and makes them challenging to use in breeding programmes (Parihar *et al*. 2016).

## CONCLUSION

The mean performance of $F_1$ surpassed the mid parent for number of fruits, fruit length, fruit weight and yield per plant in both the crosses indicating that these traits can be exploited through heterosis breeding. The reduction in mean performance of $F_2$ population than $F_1$ for fruit length and yield in both crosses was observed which apparently indicated influence of inbreeding depression. Significance of one or more scaling tests, i.e. A, B, C and D in most of the traits revealed the presence of epistasis in both the crosses except for node bearing 1st male flower where additive gene action was predominant. Characters showing complimentary epistasis have the possibility of considerable amount of heterosis for the trait and characters showing duplicate epistasis have the possibilities of obtaining transgressive segregants in later generations in the particular cross.

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***(Received on 05.11.2021, Revised on 06.01.2022 and Accepted on 18.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 222-233, 2021*

***Original Research Paper***

# Influence of phenophase based irrigation and fertigation schedule on vegetative performance of chrysanthemum (*Dendranthema grandiflora* Tzelev.) var. Marigold

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## ABSTRACT

**The vegetative performance of chrysanthemum var. Marigold with respect to phenophase based irrigation and fertigation schedule was evaluated. In the vegetative phase, the maximum plant height (62.44 cm), number of secondary branches per plant (42.65), number of primary branches per plant (10.85), leaf area (3793.81 cm$^2$) was recorded in the treatment combination. Whereas, the maximum average plant spread (47.98 cm) was in $I_1F_4$, number of leaves per plant (217.76) was in $I_3F_1$. Scheduling irrigation regime $I_3$-(0.8 ER each at vegetative, bud and flowering phases) in combination with weekly application of ($F_4$) 75:112.5:75 kg NPK/ha in three splits 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (bud phase) 30:40:40% NPK (flowering phase) through fertigation recorded maximum loose flower yield (26.27 t/ha) and this can be correlated with increased values for most of the vegetative parameters that directly influence the yield of the crop. Hence the above was observed best treatment over other treatment combinations with respect to vegetative parameters of chrysanthemum var. Marigold.**

**Key words:** Chrysanthemum var. Marigold, fertigation, irrigation, phenophase and vegetative performance.

## INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Tzvelev.) is one of the important commercial flower crops in India as well as in the world. It is native of the Northern hemisphere, chiefly Europe and Asia. It belongs to family Asteraceae and is commonly called as the "Queen of the East". Its flowers are valued for its long keeping quality, wide array of colours and different forms, which make it suitable for use in floral bouquets, flower arrangements and decorations. Chrysanthemum is the second most important flower crop after rose in India. The area under flower crops is 339000 ha with an overall production of 19.91 lakh tonnes. The leading chrysanthemum growing state is Karnataka with an area of 5453 ha and production of 59.54 thousand tonnes of loose flowers in 2017-18 after Tamil Nadu. Water and fertilizer are the two vital inputs for crop production. Apart from the economic considerations, it is also well known that the injudicious use of water and fertilizer can have far reaching deleterious implications on the environment. Therefore, the need arises for technological options, which will help in sustaining the precious resources and maximizing crop production without any pernicious impact on the environment. Optimum plant nutrition is very essential in plant growth and development, if it is not in sufficient amount then it reduces the vigor of the plant and affects yield of flower crops by producing small leaves, light green or off-color foliage, fewer branches and poor flowering (Melvin and James, 2001). Excessive application of nutrients can cause adverse effects on plant growth, increase the potential for environmental contamination through leaching and waste of resources. Method of nutrient application to plants is also a key issue to get the optimum potential of the crop. Fertigation helps in reducing the wastage of nutrients through enhanced use efficiency of fertilizer besides providing flexibility in timing of

fertilizer application in relation to crop demand based on phenological stages of growth (Papadopoulos, 1992). It also determines quantity of nutrients, timing of application and most important component of water distribution (Ahmad and Khan, 2017). The amount of nutrient and water requirement of a plant varies according to its phenophase and dispensation of water and nutrients can be scheduled accordingly. The fertigation scheduling should be based on plant, soil-air, plant water relations and growth stage of plant (Sankaranarayanan, 2007).

It is essential to work out an economically feasible and technologically efficient fertigation scheduling for optimum use of water and nutrients for enhanced water productivity with reference to different growth and developmental stages. Hence, it is important to evaluate under phenophase based irrigation and fertigation treatments for improving vegetative performance of chrysanthemum var. Marigold under open field condition.

## MATERIAL AND METHODS

The present investigation conducted during two seasons *i.e.* 2018 & 2019, at the Division of Flowers and Medicinal Crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The experimental site is situated in eastern dry zone of Karnataka state at $13^0$ 7′ north latitude, $77^0$ 29′ east longitudes and at an altitude of 890 meters above the mean sea level. The experiment was laid out in split plot design with fifteen treatment combinations along with three replications. The treatment consists of three main plot treatments at phenophases of vegetative phase i.e. $I_1$ – (0.8, 1.0 and 1.2 ER at vegetative, bud and flowering phases, respectively), $I_2$ - (0.6, 0.8 and 1.0 ER at vegetative, bud and flowering phases, respectively) and $I_3$- (0.8 ER each at vegetative, bud and flowering phases) and five sub plot treatments ($F_1$: 33.3:33.3:33.3 % NPK (vegetative phase), 33.3:33.3:33.3 % NPK (Bud phase) 33.3:33.3:33.3 % NPK (Flowering phase) @ 100:150:100 Kg NPK/ha (RDF), $F_2$: 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (Bud phase ) 30:40:40% NPK (Flowering phase) @ 100:150:100 Kg NPK/ha (RDF), $F_3$: 33.3:33.3:33.3 % NPK (vegetative phase), 33.3:33.3:33.3 % NPK (Bud phase ) 33.3:33.3:33.3 % NPK (Flowering phase @ 75:112.5:75 Kg NPK/ ha (75% RDF), $F_4$: 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (Bud phase) 30:40:40% NPK (Flowering phase) @ 75:112.5:75 Kg NPK/ha (75% RDF), $F_5$: Soil application of recommended dose of fertilizer (100:150:100 Kg NPK/ha) and $F_1$-$F_4$: 25% of fertilizer dose i.e. 100:150:100 and 75:112.5:75 kg NPK/ha was applied as basal dose. The previous day open pan evaporimeter observation was considered for scheduling the irrigation as per the treatment. The

$$\text{Evaporation replenishment (ER)} = \frac{\text{Bed Size (m}^2\text{)} \times \text{pan evaporation rate (mm)}}{\text{Discharge capacity of drip per minute (ml)}}$$

irrigation schedule was calculated by using following formula.

The organic manure *i.e.* farmyard manure (20 t/ha) and basal application (Urea, DAP and MOP) was applied as per the treatments as earlier to transplanting. Transplanting was followed with a spacing of 60 cm × 45 cm. The dose of fertilizers was applied based on treatments through fertigation in the form of water-soluble fertilizers (Urea, MAP and SOP). The fertigation was given at weekly intervals from thirty days after transplanting to 120 days.

## RESULTS AND DISCUSSION

The vegetative parameters *viz.*, plant height (cm), number of primary and secondary branches per plant, average plant spread (cm) at flowering and leaf area ($cm^2$) as influenced by phenophase based different irrigation and fertigation regimes are discussed below.

The plant height (cm) of chrysanthemum was significantly influenced by different levels of phenophase based irrigation and fertigation. Among interactions effects the maximum plant height (61.19 cm) was recorded in $I_3F_4$ and it was on par with $I_2F_4$ (59.19 cm) and $I_2F_3$ (59.10 cm) whereas, the minimum (41.10 cm) was recorded in the treatment combination $I_2F_2$ during the first year. The maximum plant height (65.30 cm), was recorded in $I_3F_1$ and it was on par with the treatments, $I_1F_4$ (64.50 cm), $I_2F_4$ (64.43 cm) and $I_3F_4$ (63.68 cm) whereas, the minimum (44.60 cm) was recorded in $I_1F_2$ during the second year. In pooled interaction, the maximum plant height (62.44 cm) was recorded in $I_3F_4$ and it was on par with the treatment $I_2F_4$ (61.81 cm) and the minimum (46.91 cm) was recorded in $I_1F_2$. (Table 1 & 2) (Fig.1).

**Table 1. Influence of phenophase based irrigation and fertigation scheduling on plant height (cm) and number of primary branches of chrysanthemum var. Marigold**

| Treatments | Plant height (cm) | | | Number of primary branches per plant | | |
|---|---|---|---|---|---|---|
| | I year | II year | Pooled mean | I year | II year | Pooled mean |
| $I_1$ | 51.42 | 54.48 | 52.95 | 9.64 | 9.96 | 9.80 |
| $I_2$ | 52.32 | 56.30 | 54.31 | 9.74 | 9.14 | 9.44 |
| $I_3$ | 48.88 | 58.97 | 53.92 | 9.43 | 9.20 | 9.32 |
| SE. d | 0.65 | 0.40 | 0.38 | 0.03 | 0.08 | 0.05 |
| CD (P=0.05) | 1.83 | 1.11 | 1.07 | 0.10 | 0.23 | 0.14 |
| $F_1$ | 51.70 | 57.50 | 54.60 | 8.71 | 9.30 | 9.00 |
| $F_2$ | 44.14 | 52.40 | 48.27 | 9.77 | 9.50 | 9.63 |
| $F_3$ | 55.33 | 54.76 | 55.04 | 10.13 | 9.57 | 9.85 |
| $F_4$ | 58.83 | 64.20 | 61.52 | 10.61 | 10.83 | 10.72 |
| $F_5$ | 44.36 | 54.05 | 49.21 | 8.80 | 7.96 | 8.38 |
| SE. d | 0.66 | 0.58 | 0.40 | 0.11 | 0.11 | 0.08 |
| CD (P=0.05) | 1.14 | 1.20 | 0.83 | 0.22 | 0.23 | 0.17 |

**Table 2. Interaction effect of phenophase based irrigation and fertigation scheduling on plant height (cm) of chrysanthemum var. Marigold**

| Treat-ments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 51.90 | 49.21 | 56.79 | 56.10 | 43.09 | 51.42 | 53.71 | 44.60 | 55.60 | 64.50 | 54.00 | 54.48 | 52.81 | 46.91 | 56.20 | 60.30 | 48.55 | 52.95 |
| $I_2$ | 55.10 | 41.10 | 59.10 | 59.19 | 47.10 | 52.32 | 53.50 | 56.70 | 51.27 | 64.43 | 55.60 | 56.30 | 54.30 | 48.90 | 55.19 | 61.81 | 51.35 | 54.31 |
| $I_3$ | 48.10 | 42.10 | 50.10 | 61.19 | 42.90 | 48.88 | 65.30 | 55.90 | 57.40 | 63.68 | 52.56 | 58.97 | 56.70 | 49.00 | 53.75 | 62.44 | 47.73 | 53.92 |
| Mean | 51.70 | 44.14 | 55.33 | 58.83 | 44.36 | | 57.50 | 52.40 | 54.76 | 64.20 | 54.05 | | 54.60 | 48.27 | 55.04 | 61.52 | 49.21 | |
| | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | |
| I | 0.65 | | | 1.83 | | | 0.40 | | | 1.11 | | | 0.38 | | | 1.07 | | |
| F | 0.66 | | | 1.14 | | | 0.58 | | | 1.20 | | | 0.40 | | | 0.83 | | |
| I at F | 1.22 | | | 2.77 | | | 0.99 | | | 2.15 | | | 0.73 | | | 1.66 | | |
| F at I | 1.14 | | | 2.37 | | | 1.01 | | | 2.09 | | | 0.69 | | | 1.43 | | |

**Table 3. Interaction effect of phenophase based irrigation and fertigation scheduling on number of primary branches per plant of chrysanthemum var. Marigold**

| Treat-ments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 8.79 | 9.20 | 10.80 | 10.50 | 8.90 | 9.64 | 10.60 | 9.80 | 10.20 | 11.20 | 8.00 | 9.96 | 9.70 | 9.50 | 10.50 | 10.85 | 8.45 | 9.80 |
| $I_2$ | 8.93 | 10.40 | 10.00 | 10.77 | 8.60 | 9.74 | 8.10 | 9.60 | 9.60 | 10.19 | 8.19 | 9.14 | 8.52 | 10.00 | 9.80 | 10.48 | 8.40 | 9.44 |
| $I_3$ | 8.40 | 9.70 | 9.60 | 10.56 | 8.89 | 9.43 | 9.20 | 9.10 | 8.90 | 11.10 | 7.70 | 9.20 | 8.80 | 9.40 | 9.25 | 10.83 | 8.30 | 9.32 |
| Mean | 8.71 | 9.77 | 10.13 | 10.61 | 8.80 | | 9.30 | 9.50 | 9.57 | 10.83 | 7.96 | | 9.00 | 9.63 | 9.85 | 10.72 | 8.38 | |
| | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | |
| I | 0.03 | | | 0.10 | | | 0.08 | | | 0.23 | | | 0.05 | | | 0.14 | | |
| F | 0.11 | | | 0.22 | | | 0.11 | | | 0.23 | | | 0.08 | | | 0.17 | | |
| I at F | 0.17 | | | 0.36 | | | 0.19 | | | 0.42 | | | 0.14 | | | 0.30 | | |
| F at I | 0.19 | | | 0.39 | | | 0.19 | | | 0.40 | | | 0.14 | | | 0.30 | | |

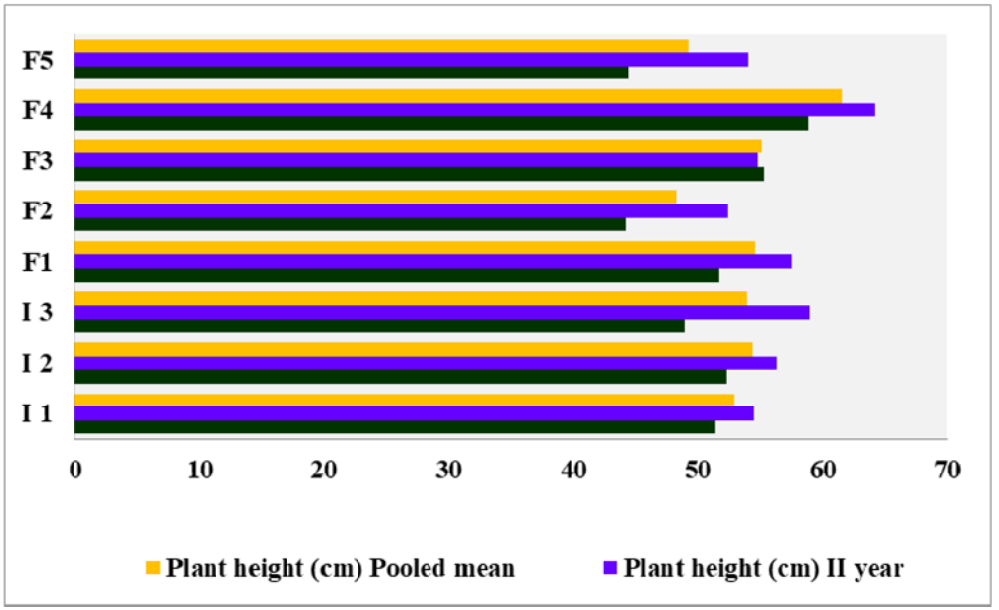


**Fig. 1. Influence of phenophase based irrigation and fertigation scheduling on plant height (cm)**

The irrigation treatment $I_3$- (0.8 ER each at vegetative, bud and flowering phases) in combination with $F_4$ fertigation at 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (bud phase) 30:40:40% NPK (flowering phase) @ 75:112.5:75 kg NPK/ha2 recorded the maximum plant height (62.44 cm) in chrysanthemum var. Marigold. The increase in plant height with irrigation at $I_3$ might be due to adequate moisture provided in the soil throughout the crop period. Adequate soil moisture resulted in greater development of meristematic tissues leading to higher rate of photosynthesis and assimilation in the plant system in marigold (Chawla, 2008).

In the fertigation treatment $F_4$, higher proportion of nitrogen fertilizer at vegetative phase might have increased the plant height because of the synergistic interaction of nitrogen with available endogenous auxin resulting in enhanced cell wall plasticity and increased cell elongation thus resulting in increase in the height of the plant. Further, during the bud and flowering phases, the sustained growth of the plant might have been the result of optimum application of nitrogen. The results from the present investigation could hence be attributed to the frequent and constant application of optimum levels of fertilizers at appropriate intervals at crop phenophases, which increases the available nutrient status in the root rhizosphere at constant levels during all the phases thus increasing the uptake of nutrients rapidly, and further influencing the growth of the plant. Similar observations were earlier reported by Mamata *et al*. (2017) in marigold, Parya *et al.* (2017) in gerbera, Priyanka *et al.* (2017) in gladiolus and Satapathy *et al*. (2016) in marigold, Jamil *et al*. (2016), Zawadzisnka and Janicka (2007) in amaryllis and viola respectively.

The treatment $I_1F_4$ was on par with $I_3F_4$ for maximum (10.83), number of primary branches per plant (Table 1 & 3) and the maximum number of secondary branches per plant (42.65) was recorded in the treatment combination $I_3F_4$ and it was on par with $I_1F_4$ (41.44) and the minimum (17.75) was recorded in $I_1F_5$. The treatment $I_3F_4$ recorded the maximum number of secondary branches per plant (42.65) in chrysanthemum var. Marigold. This increase in number branches might be mainly due to the increased irrigation scheduled favoring longer availability of soil moisture which leads to better growth and development of vegetative part of the plant. The greater availability of nutrient at optimum proportions at critical growth stages in the present fertigation treatment might have resulted in production of more number of branches per plant as observed by Siraj Ali (1998) in bird-of-paradise. Polara *et al.* (2015) recorded similar results in African marigold. These findings are in conformation with the earlier results of Jawaharlal and Ganesh (2020) in chrysanthemum and Nagaraju *et al*. (2003) in rose (Table 4 & 5).

The average plant spread was significantly influenced and showed linear increase with irrigation regime and with optimum dosage of water-soluble fertilizers through fertigation. Among interactions effect the maximum average plant spread (53.23 cm) was recorded in the treatment combination $I_1F_4$ followed by the treatment $I_1F_3$ (45.76 cm) and the minimum (31.60 cm) was recorded in the treatment combination of $I_1F_5$ during the first year. The maximum average plant spread (49.33 cm) was recorded in the treatment combination $I_3F_1$ followed by $I_2F_3$ (44.87 cm) and the minimum (30.80 cm) was recorded in the treatment combination $I_1F_2$ during the second year. In pooled interaction, the maximum average plant spread (47.98 cm) was recorded in the treatment combination $I_1F_4$ followed by the treatment $I_1F_3$ (43.61 cm) and the minimum (32.23 cm) was recorded in the treatment combination of $I_3F_2$ (Table 4 & 6).

It was recorded that irrigation regime $I_1$- (0.8, 1.0 and 1.2 ER at vegetative, bud and flowering phases, respectively) in combination with fertigation at 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (bud phase) 30:40:40% NPK (flowering phase) @ 75:112.5:75 kg NPK/ha registered maximum average plant spread (47.98 cm). This result clearly showed that higher amount of nitrogen supplied at vegetative phase along with higher soil moisture levels leads to increased vegetative growth of chrysanthemum var. Marigold. According to Paul *et al*. (1996) the plant spread could be attributed to the frequent application of fertilizers with constant supply of nutrients, at regular intervals for better growth which would have resulted in reduced nutrient losses by leaching and efficient use of nutrients through fertigation compared to soil application. This is in accordance with the findings of Deshmukh and Wavhal (1998) in china aster and Ahirwal *et al.* (2012) in African marigold.

The maximum number of leaves (235.03) was recorded in the treatment combination $I_1F_4$ and it was

**Table 4. Influence of phenophase based irrigation and fertigation scheduling on vegetative parameters of chrysanthemum var. Marigold**

| Treatments | Number of secondary branches per plant | | | Average plant spread (cm) | | | Number of leaves per plant | | |
|---|---|---|---|---|---|---|---|---|---|
| | I year | II year | Pooled mean | I year | II year | Pooled mean | I year | II year | Pooled mean |
| $I_1$ | 29.18 | 30.65 | 29.91 | 42.32 | 39.67 | 41.00 | 221.93 | 136.89 | 179.40 |
| $I_2$ | 32.42 | 27.27 | 29.85 | 36.71 | 41.04 | 38.88 | 220.26 | 141.43 | 180.82 |
| $I_3$ | 30.97 | 29.29 | 30.13 | 35.69 | 40.48 | 37.43 | 218.84 | 156.34 | 187.59 |
| SE. d | 0.78 | 0.61 | 0.06 | 0.34 | 0.13 | 0.58 | 1.23 | 1.99 | 3.20 |
| CD (P=0.05) | 1.41 | 1.20 | 0.12 | 0.95 | 0.26 | 1.62 | 2.60 | 4.02 | 6.98 |
| $F_1$ | 30.06 | 34.06 | 32.06 | 37.13 | 43.87 | 40.50 | 224.07 | 159.64 | 191.86 |
| $F_2$ | 26.32 | 23.44 | 24.88 | 38.26 | 35.26 | 36.76 | 220.74 | 136.21 | 178.47 |
| $F_3$ | 31.50 | 27.30 | 29.40 | 40.54 | 40.92 | 40.73 | 214.97 | 143.18 | 178.57 |
| $F_4$ | 42.12 | 39.86 | 40.99 | 42.98 | 41.81 | 42.39 | 225.88 | 154.83 | 190.36 |
| $F_5$ | 24.30 | 20.68 | 22.49 | 34.30 | 38.45 | 36.37 | 217.97 | 130.57 | 173.77 |
| SE. d | 0.89 | 0.60 | 0.55 | 0.53 | 0.96 | 0.79 | 0.26 | 0.27 | 0.05 |
| CD (P=0.05) | 1.54 | 1.19 | 1.02 | 1.10 | 2.03 | 1.64 | 0.45 | 0.55 | 0.10 |

**Table 5. Interaction effect of phenophase based irrigation and fertigation scheduling on number of secondary branches per plant of chrysanthemum var. Marigold**

| Treatments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 26.82 | 26.59 | 31.78 | 42.04 | 18.69 | 29.18 | 37.24 | 28.42 | 29.93 | 40.84 | 16.81 | 30.65 | 32.03 | 27.50 | 30.85 | 41.44 | 17.75 | 29.91 |
| $I_2$ | 36.48 | 27.05 | 31.99 | 39.91 | 26.67 | 32.42 | 29.15 | 29.17 | 17.28 | 37.84 | 22.91 | 27.27 | 32.82 | 28.11 | 24.64 | 38.88 | 24.79 | 29.85 |
| $I_3$ | 26.88 | 25.31 | 30.72 | 44.40 | 27.55 | 30.97 | 35.78 | 12.74 | 34.70 | 40.89 | 22.33 | 29.29 | 31.33 | 19.02 | 32.71 | 42.65 | 24.94 | 30.13 |
| Mean | 30.06 | 26.32 | 31.50 | 42.12 | 24.30 | | 34.06 | 23.44 | 27.30 | 39.86 | 20.68 | | 32.06 | 24.88 | 29.40 | 40.99 | 22.49 | |
| | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | |
| I | 0.78 | | | 1.41 | | | 0.61 | | | 1.20 | | | 0.06 | | | 0.17 | | |
| F | 0.89 | | | 1.54 | | | 0.60 | | | 1.19 | | | 0.55 | | | 1.02 | | |
| I at F | 1.34 | | | 2.68 | | | 1.01 | | | 2.07 | | | 0.89 | | | 1.76 | | |
| F at I | 1.33 | | | 2.66 | | | 1.00 | | | 2.06 | | | 0.90 | | | 1.75 | | |

**Table 6. Interaction effect of phenophase based irrigation and fertigation scheduling on average plant spread (cm) of chrysanthemum var. Marigold**

| Treatments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 38.40 | 42.63 | 45.76 | 53.23 | 31.60 | 42.32 | 40.46 | 30.80 | 41.46 | 42.73 | 42.90 | 39.67 | 39.43 | 36.71 | 43.61 | 47.98 | 37.25 | 41.00 |
| $I_2$ | 37.50 | 35.23 | 38.73 | 37.20 | 34.90 | 36.71 | 41.82 | 41.47 | 44.87 | 39.23 | 37.83 | 41.04 | 39.66 | 38.35 | 41.80 | 38.21 | 36.36 | 38.88 |
| $I_3$ | 35.50 | 30.93 | 37.13 | 38.50 | 36.40 | 35.69 | 49.33 | 33.53 | 36.43 | 43.47 | 33.10 | 40.48 | 42.42 | 32.23 | 36.78 | 40.99 | 34.75 | 37.43 |
| Mean | 37.13 | 38.26 | 40.54 | 42.98 | 34.30 | | 43.87 | 35.27 | 40.92 | 41.81 | 38.45 | | 40.50 | 36.76 | 40.73 | 42.39 | 36.38 | |
| | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | |
| I | 0.34 | | | 0.95 | | | 0.13 | | | 0.26 | | | 0.58 | | | 1.62 | | |
| F | 0.53 | | | 1.10 | | | 0.96 | | | 2.03 | | | 0.79 | | | 1.64 | | |
| I at F | 0.89 | | | 1.93 | | | 2.53 | | | 5.59 | | | 1.36 | | | 2.99 | | |
| F at I | 0.92 | | | 1.90 | | | 2.54 | | | 5.24 | | | 1.37 | | | 2.84 | | |

on par with $I_1F_1$ (229.61) and the minimum number of leaves per plant (205.01) were recorded in $I_1F_5$ during the first year. The maximum number of leaves per plant (215.50) was recorded in the treatment combination $I_3F_1$ and it was on par with $I_2F_2$ (192.21), $I_1F_3$ (171.61) and $I_3F_4$ (175.90) whereas, the minimum (89.61) was recorded in $I_1F_2$ during the second year. In pooled interaction the maximum number of leaves per plant (217.76) were recorded in the treatment combination $I_3F_1$ and it was on par with $I_2F_2$ (208.41), $I_1F_3$ (195.96), $I_1F_4$ (197.22) and $I_3F_4$ (198.75) whereas, the minimum (154.61) was recorded in $I_1F_2$ (Table 4 & 7).

The treatment $I_3F_4$ registered maximum number of leaves per plant and maximum leaf area (2404.74 $cm^2$) was recorded in $I_1F_4$ and it was on par with $I_3F_4$ (2352.18 $cm^2$) and the lowest (1308.31 $cm^2$) was recorded in $I_3F_1$ during the vegetative phase (Tables 8 & 9) (Fig. 2a, 2b & 2c). In the present study, the increase in number of leaves and leaf area could be

**Fig. 2. Influence of phenophase based irrigation and fertigation scheduling on leaf area ($cm^2$) at vegetative phase**

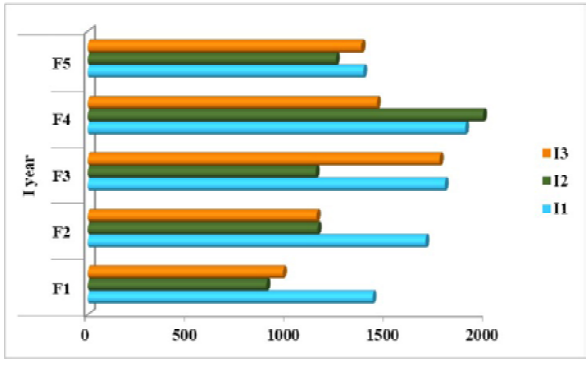


**Fig. 2.a. Influence of phenophase based irrigation and fertigation scheduling on leaf area ($cm^2$) at vegetative phase during first year**

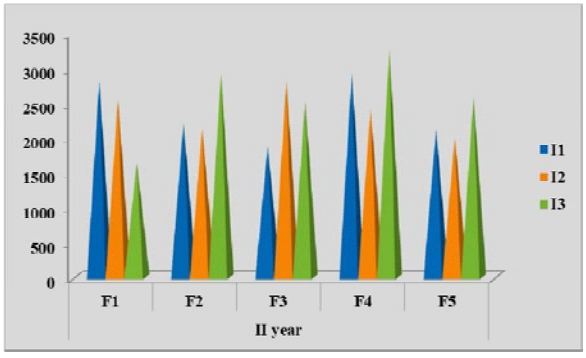


**Fig. 2.b. Influence of phenophase based irrigation and fertigation scheduling on leaf area ($cm^2$) at vegetative phase during second year**

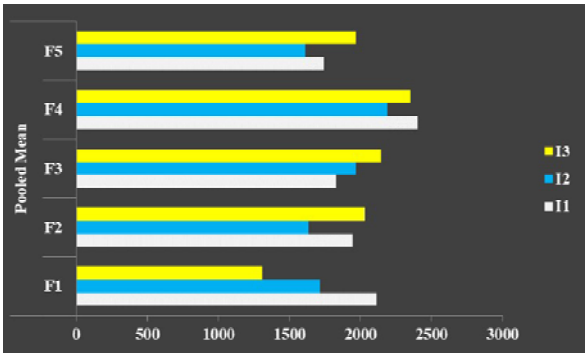


**Fig. 2.c. Pooled influence of phenophase based irrigation and fertigation scheduling on leaf area ($cm^2$) at vegetative phase**

**Table 7. Interaction effect of phenophase based irrigation and fertigation scheduling on number of leaves per plant of chrysanthemum var. Marigold**

**Leaf area (cm²) at vegetative phase**

| Treatments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 229.61 | 219.60 | 220.31 | 235.03 | 205.01 | 221.93 | 124.21 | 89.61 | 171.61 | 159.40 | 139.60 | 136.89 | 176.91 | 154.61 | 195.96 | 197.22 | 172.31 | 179.40 |
| $I_2$ | 222.60 | 224.61 | 208.60 | 221.00 | 224.30 | 220.26 | 139.21 | 192.21 | 104.21 | 129.20 | 142.30 | 141.43 | 180.91 | 208.41 | 156.41 | 175.10 | 183.30 | 180.82 |
| $I_3$ | 220.01 | 218.00 | 213.00 | 221.60 | 221.60 | 218.84 | 215.50 | 126.80 | 153.71 | 175.90 | 109.80 | 156.34 | 217.76 | 172.40 | 183.36 | 198.75 | 165.70 | 187.59 |
| Mean | 224.07 | 220.74 | 214.97 | 225.88 | 217.97 | | 159.64 | 136.21 | 143.18 | 154.83 | 130.57 | | 191.86 | 178.47 | 178.57 | 190.36 | 173.77 | |
| | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | | SE. d | | | CD (P=0.05) | | |
| I | 1.23 | | | 2.60 | | | 1.99 | | | 4.02 | | | 3.20 | | | 6.98 | | |
| F | 0.26 | | | 0.45 | | | 0.27 | | | 0.55 | | | 0.05 | | | 0.10 | | |
| I at F | 4.27 | | | 9.08 | | | 23.26 | | | 52.41 | | | 12.39 | | | 27.79 | | |
| F at I | 4.58 | | | 9.45 | | | 22.28 | | | 45.98 | | | 11.99 | | | 24.76 | | |

**Table 8. Influence of phenophase based irrigation and fertigation scheduling on leaf area (cm²) at vegetative phase of chrysanthemum var. Marigold**

| Treatments | I year | II year | Pooled mean |
|---|---|---|---|
| $I_1$ | 1640.80 | 2373.74 | 2007.27 |
| $I_2$ | 1286.42 | 2362.97 | 1824.68 |
| $I_3$ | 1345.88 | 2575.28 | 1960.58 |
| SE. d | 8.89 | 45.52 | 5.30 |
| CD (P=0.05) | 24.68 | 101.36 | 10.50 |
| $F_1$ | 1102.67 | 2319.68 | 1711.18 |
| $F_2$ | 1334.65 | 2408.43 | 1871.54 |
| $F_3$ | 1569.73 | 2388.75 | 1979.24 |
| $F_4$ | 1778.79 | 2851.51 | 2315.15 |
| $F_5$ | 1336.00 | 2218.27 | 1777.14 |
| SE. d | 20.81 | 26.44 | 135.35 |
| CD (P=0.05) | 42.96 | 59.02 | 279.33 |

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**Table 9. Interaction effect of phenophase based irrigation and fertigation scheduling on leaf area ($cm^2$) at vegetative phase of chrysanthemum var. Marigold**

| Treat-ments | I year | | | | | | II year | | | | | | Pooled Mean | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mean |
| $I_1$ | 1430.34 | 1698.00 | 1794.19 | 1896.34 | 1385.15 | 1640.80 | 2792.27 | 2198.03 | 1857.80 | 2913.13 | 2107.47 | 2373.74 | 2111.31 | 1948.02 | 1826.00 | 2404.74 | 1746.31 | 2007.27 |
| $I_2$ | 897.36 | 1154.90 | 1145.80 | 1986.50 | 1247.54 | 1286.42 | 2530.47 | 2118.10 | 2794.03 | 2390.57 | 1981.67 | 2362.97 | 1713.92 | 1636.50 | 1969.92 | 2188.54 | 1614.61 | 1824.68 |
| $I_3$ | 980.32 | 1151.04 | 1769.20 | 1453.53 | 1375.31 | 1345.88 | 1636.30 | 2909.17 | 2514.43 | 3250.83 | 2565.70 | 2575.28 | 1308.31 | 2030.11 | 2141.82 | 2352.18 | 1970.51 | 1960.58 |
| Mean | 1102.67 | 1334.65 | 1569.73 | 1778.79 | 1336.00 | | 2319.68 | 2408.43 | 2388.75 | 2851.51 | 2218.27 | | 1711.18 | 1871.54 | 1979.24 | 2315.15 | 1777.14 | |
| | SE.d | | | CD (P=0.05) | | | SE.d | | | CD (P=0.05) | | | SE.d | | | CD (P=0.05) | | |
| I | 8.89 | | | 24.68 | | | 45.52 | | | 101.36 | | | 5.30 | | | 10.50 | | |
| F | 20.81 | | | 42.96 | | | 26.44 | | | 59.02 | | | 135.35 | | | 279.33 | | |
| I at F | 33.45 | | | 70.72 | | | 37.86 | | | 65.41 | | | 23.49 | | | 50.01 | | |
| F at I | 36.05 | | | 74.41 | | | 35.78 | | | 71.36 | | | 23.51 | | | 57.89 | | |

attributed to Application of higher proportion of nitrogen fertilizer and optimum irrigation regimes at vegetative phase might have increased the number of leaves and leaf area. It may be due to the fact that the vegetative growth increased with nitrogen application and hence nitrogen is an essential part of nucleic acid, which plays a vital role in promoting vegetative growth. The present results were also in line with the reports of Maharnor *et al.* (2011) and Polara *et al.* (2014) in African marigold, Karam *et al*. (2007) in sunflower and Jaleel *et al*. (2009) in *Catharanthus*. Rawat and Mathpal (1984), Paul *et al*. (1996) and Khan *et al*. (1996) in various crops.

## CONCLUSION

In the vegetative phase of chrysanthemum var. Marigold, the irrigation treatment $I_3$-(0.8 ER each at vegetative, bud and flowering phases) in combination with fertigation treatment $F_4$ at 40:20:20 % NPK (vegetative phase), 30:40:40 % NPK (bud phase) 30:40:40% NPK (flowering phase) @ 75:112.5:75 kg NPK/ha was found adequate to cater the demand of water as well as nutrient requirement for vegetative phase of chrysanthemum var. Marigold. This can be correlated with the maximum loose flower yield (26.27 t/ha) registered by the same treatment. Further better plant growth as recorded during the investigation is indicative of better uptake of nutrients which in turn are involved in basic reaction of photosynthesis and in synthesis of metabolites required for plant growth with above irrigation and fertigation schedule. Hence it is concluded that the above treatment combination $I_3F_4$ was registered as the best treatment to improve the vegetative growth of chrysanthemum var. Marigold.

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***(Received on 05.09.2021, Revised on 23.09.2021 and Accepted on 15.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 234-240, 2021*

***Original Research Paper***

# Performance evaluation of double type tuberose IIHR-4 (IC-0633777) for flower yield, quality and biotic stress response

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## ABSTRACT

**An experiment was carried out to evaluate an advance breeding line of tuberose double type IIHR-4 along with check for flowering, yield and resistance to root knot nematode and *Alternaria polianthi* leaf blight disease. The hybrid selection IIHR-4 was developed through hybridization by crossing Mexican Single x Pearl Double, followed by selection. Double type tuberose IIHR-4 was found to be novel with better flowering and quality traits such as relatively shorter spike (62.00 cm) and rachis length (25.59cm) and favourable diameter of floret (4.47cm) and number of florets per spike (50.75), more number of florets (7.10) open at a time on the spike and shorter internodal length between the florets (3.45cm). The florets are with shorter length (5.22cm) arranged very compactly on the spike making IIHR-4 ideal as cut flower. Added to this, the advanced breeding line IIHR-4 was found to be highly resistant to root knot nematode *Meloidogyne incognita* under field conditions and tolerant to *Alternaria polianthi* leaf blight disease.**

**Keywords:** Advanced breeding line, cut flower, flowering, double type, tuberose and yield

## INTRODUCTION

Tuberose (*Polianthes tuberosa* Linn.) is an important tropical bulbous ornamental plant belonging to the family 'Asparagaceae' and is native to Mexico (Bailey, 1919). There are two types of tuberose namely, Single and Double which are commercially cultivated across the globe for their exquisite flowers. Single types are used as loose flowers for garland purpose and perfumery industry whereas double varieties are highly preferred for cut flower and bouquets because of the longer keeping quality of the flower spikes. Double tuberose flowers have high demand in both local and international markets and are being exported to gulf countries. The increasing demand for superior and novel double type tuberose necessitates the development of varieties of this category. Tuberose is commercially cultivated in India in an area of about 16,190 ha, with a loose flower production of 1, 07, 910 metric tonnes and cut flower production of 89.29 lakh numbers of cut stems (Anon, 2016). Root knot nematode infects tuberose and leads to 10-14% of crop loss (Khan and Reddy, 1992). Leaf blight disease caused by *Alternaria polianthi* is extensive in tuberose growing regions of the country. Development of tolerant and resistant varieties to these biotic stresses is the need of the hour to help the tuberose growers. Keeping these objectives in view, an advance breeding line of double type tuberose (IIHR-4) developed by ICAR-IIHR was evaluated for flowering, yield, quality and reaction to root knot nematode and A.*polianthi* leaf blight disease.

## MATERIALS AND METHODS

The investigation was carried out at the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during 2015 - 2018. The advance breeding line of tuberose IIHR-4 with double flowers was evaluated along with the commercial check varieties Arka Vaibhav, Arka Suvasini, local checks Hyderabad Double and Pearl Double. Randomized block design was followed for the experiment with three replications. Uniform size of bulbs (2.5 cm diameter) were planted on raised bed

with the spacing of 30 x 30 cm. Standard cultural practices were followed throughout the experimental period. The growth, yield and quality parameters *viz*., days to spike emergence, days to opening of first floret, spike length, rachis length, number of florets per spike, length of floret, diameter of floret, bud length, matured bud weight, single flower weight, number of spikes per clump, vase life, number of bulbs per clump, number of bulblets per clump, internodal length between the florets and number of florets open at a time on the rachis were observed. The tuberose lines/cultivars were screened for the tolerance/ resistance against root knot nematode *Meloidogyne incognita* for three consecutive years. Gall Index (GI) was recorded in the roots in a 0-5 scale as per Taylor and Sasser (1978) at the time of bulb harvest. Per cent disease index and host reaction of tuberose genotypes against leaf blight disease caused by A. *polianthi* under field condition was recorded thrice at15 day interval using 0-5 scale (Narayanappa and Chandra, 1984). The pooled data of three years were statistically analyzed as per Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

The results of the study revealed significant differences among the tuberose lines for flowering and yield parameters (Tables 1 and 2). Days to spike emergence ranged from 133.73 (Arka Suvasini) to 198.00 (Pearl Double) with the general mean of 161.53 days. The advanced breeding line IIHR-4 recorded 154.15 days to spike emergence. Early spike appearance in tuberose cultivar Arka Suvasini was reported by Safeena *et al*. (2019) who noticed wide range of variation in days taken to flowering due to variation in genetic makeup and prevailing environmental conditions. Spike length ranged from 62.00 cm (IIHR-4) to 86.36 cm (Pearl Double) with the mean of 78.64 cm. The advanced breeding line IIHR-4 recorded spike length of 62.00 cm with upright stalk categorized into short spike group suitable for cut flower. Varietal differences for spike length was earlier reported by Madhumati *et al*. (2018), Prashanta *et al*. (2016), Safeena *et al*. (2019) and Dogra *et al*. (2020) in tuberose. Rachis length varied from 25.59cm (IIHR-4) to 33.71cm (Arka Vaibhav) with the general mean of 29.06 cm. The number of florets per spike was recorded the maximum in Arka Suvasini (55.33) and minimum in Pearl Double (48.78) with the mean of 50.91. The results are in line with findings of Ranchana *et al.* (2013), Rao and Sushma (2015), Bharathi *et al*. (2018) in tuberose and Rani and Singh (2005) in gladiolus. The variation observed in spike length and rachis length might be attributed to the inherent genetic characters of the individual cultivars and environmental factors.

The line IIHR-4 recorded least floret length of 5.22 cm and Arka Suvasini recorded the highest floret length of 6.22 cm with the general mean of 5.69 cm. Similar results on highest floret length of tuberose cultivar Arka Suvasini was stated by Ranchana *et al*. (2013). The florets of IIHR-4 are short and arranged closely without any gap between the internodes, making the spike very attractive and rendering the line highly suitable as cut flower. Such variation might be due to the varietal characters and similar observations were made by Bharathi and Umamaheswari (2018).

Diameter of floret varied from 4.44 cm (Pearl Double) to 4.77 cm (Arka Suvasini) with the general mean of 4.56cm. This may be due to varied growth rates and genetic make-up. The results are in line with the findings of Rao and Sushma (2015) and Gandhi *et al.* (2017) and Safeena *et al*. (2019) in tuberose. Bud length ranged from 4.86 cm (Hyderabad Double) to 5.68 cm (Arka Suvasini) with the mean of 5.40 cm. Single flower weight was varied from 2.29g (Arka Vaibhav) to 3.57g (Arka Suvasini) with the mean of 2.73g. The variation in floral parameters might be primarily governed by the genetic makeup of the varieties and these results were also experimentally supported by the findings of Andrew *et al.* (2017).

Number of florets open at a time on the spike is an important trait for cut flower spike since it depicts the exquisiteness of the cut flower. The line IIHR-4 recorded the highest number of florets (7.10) open at a time on the spike. The lowest was observed in Arka Vaibhav (2.40) with the general mean of 4.50. The advanced breeding line IIHR-4 was very appealing with highest number of florets open at a time on the spike and this character makes the line IIHR-4 highly suitable as cut flower, especially for flower arrangement and bouquet (Fig.1). The line IIHR-4 was found to be superior over the commercial check for the above character. The variations in number of florets open at a time on the spike might be due to different genetic make-up of the different cultivars and prevailing environment conditions of the experimental area. The results are in conformity with the findings of Kusum (2010) in tuberose who also reported the variation among the tuberose cultivars for the maximum open florets per spike.

**Fig. 1. The field view of tuberose line IIHR-4 and flower spikes**

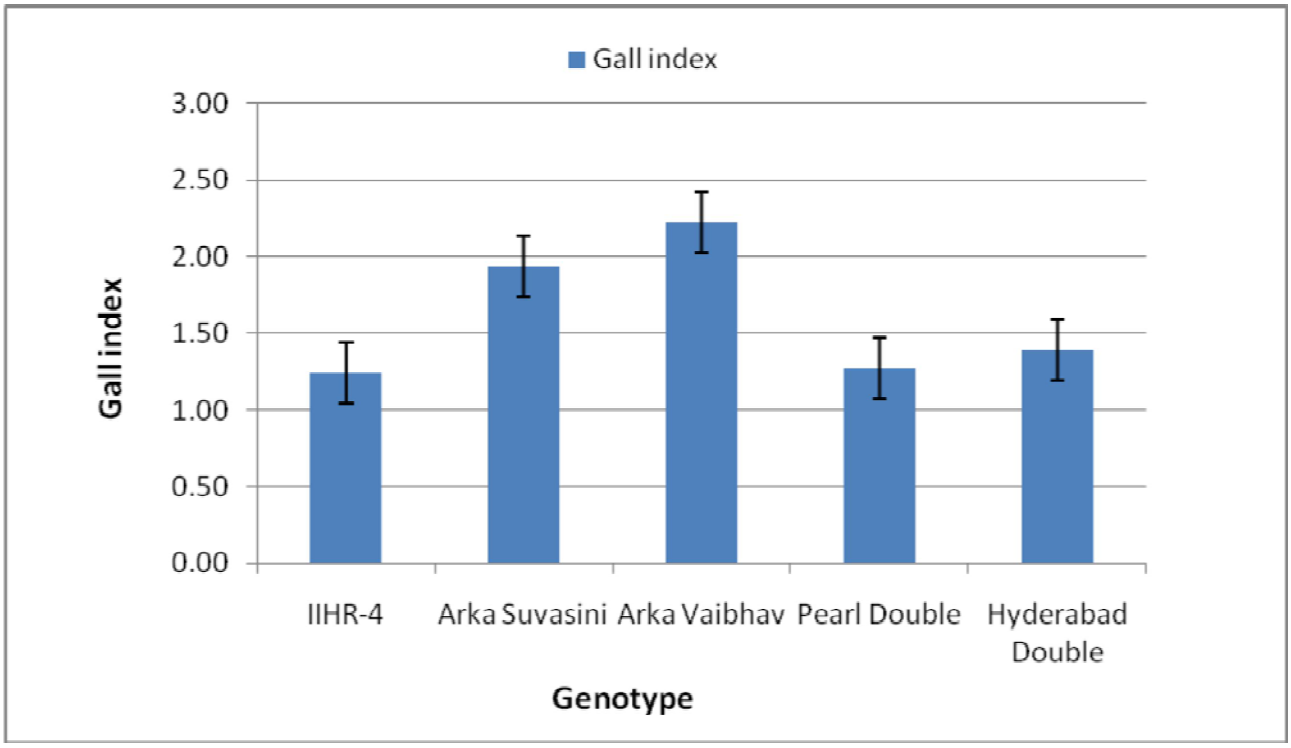


**Fig. 2. Reaction of tuberose cultivars to root knot nematode**

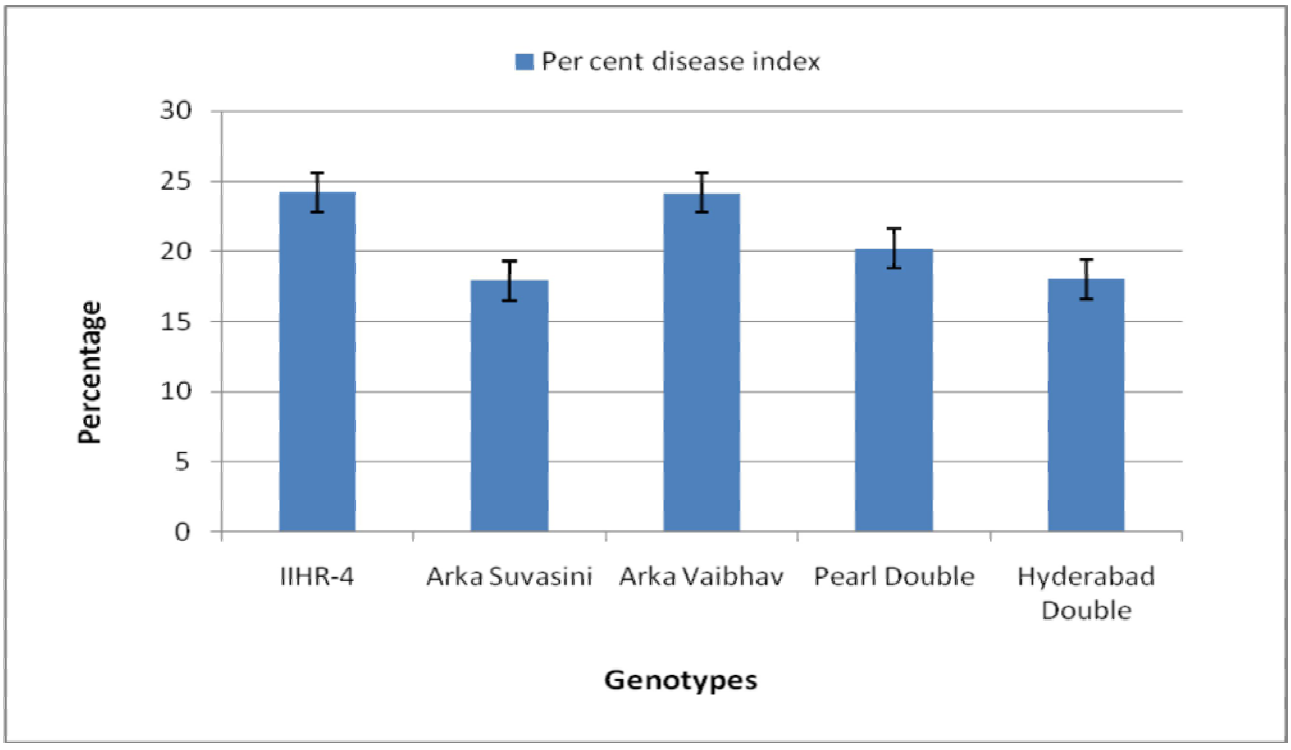


**Fig. 3. Per cent disease index and host reaction of different tuberose varieties to leaf blight disease caused by *Alternaria polianthi* under field condition**

**Table 1. Performance evaluation of advance breeding line of tuberose double type for flowering parameters**

| Genotype | Days to spike emergence | Spike length (cm) | Rachis length (cm) | No. of florets per spike | Length of floret (cm) | Diameter of floret (cm) | Bud length (cm) | Single flower weight (g) | No. of florets open at a time on spike | Internodal length (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| IIHR-4 | 154.15 | 62.00 | 25.59 | 50.75 | 5.22 | 4.47 | 4.98 | 3.16 | 7.10 | 3.45 |
| Arka Suvasini | 133.73 | 79.66 | 31.41 | 55.33 | 6.20 | 4.77 | 5.68 | 3.57 | 3.70 | 4.45 |
| Arka Vaibhav | 137.60 | 80.43 | 33.71 | 52.35 | 6.02 | 4.49 | 5.49 | 2.29 | 2.40 | 5.79 |
| Pearl Double | 198.00 | 86.36 | 28.24 | 48.78 | 5.48 | 4.44 | 5.28 | 2.94 | 5.45 | 4.83 |
| Hyderabad Double | 184.18 | 84.77 | 26.35 | 50.10 | 5.51 | 4.71 | 4.86 | 3.05 | 3.85 | 4.17 |
| Mean | 161.53 | 78.64 | 29.06 | 51.46 | 5.69 | 4.58 | 5.26 | 3.00 | 4.50 | 4.54 |
| Range | 133.73-198.00 | 62.00-86.36 | 25.59-33.71 | 48.78-55.33 | 5.22-6.20 | 4.44-4.77 | 4.86-5.68 | 2.29-3.57 | 2.40-7.10 | 3.45-5.79 |
| CV% | 3.49 | 5.77 | 4.82 | 5.45 | 3.32 | 3.23 | 3.84 | 5.83 | 7.27 | 9.67 |
| CD (P=0.05) | 8.69 | 6.99 | 2.16 | NS | 0.29 | 0.23 | 0.31 | 0.27 | 0.50 | 0.68 |

**Table 2. Performance evaluation of advance breeding line of tuberose double type for flower, bulb yield and vase life**

| Genotype | No. of spikes per clump | No. of spikes per $m^2$ | No. of spikes per ha/year | No. of bulbs per clump | No. of bulblets per clump | Vase life (days) | Nature of spike | Tinge on flower bud | Type of flower opening |
|---|---|---|---|---|---|---|---|---|---|
| IIHR-4 | 4.43 | 33.94 | 398781.25 | 4.25 | 44.63 | 6.50 | Straight | Green | Wide |
| Arka Suvasini | 2.91 | 22.82 | 261593.75 | 2.69 | 32.81 | 7.25 | Slightly bent | Pink | Wide |
| Arka Vaibhav | 4.00 | 33.77 | 360125.00 | 8.19 | 54.81 | 7.25 | Straight | Green | Wide |
| Pearl Double | 2.14 | 19.24 | 192750.00 | 5.00 | 71.00 | 7.00 | Slightly bent | Pink | Shy |
| Hyderabad Double | 2.66 | 22.83 | 239156.25 | 5.56 | 63.13 | 7.13 | Straight | Pink | Wide |
| Mean | 3.23 | 26.52 | 290481.25 | 5.14 | 53.28 | 7.03 | - | - | - |
| Range | 2.14-4.43 | 19.24-33.94 | 192750.00-398781.25 | 2.69-8.19 | 32.81-71.00 | 6.50-7.25 | - | - | - |
| CV% | 6.26 | 7.74 | 6.28 | 15.03 | 10.44 | 7.63 | - | - | - |
| CD (P=0.05) | 0.31 | 3.16 | 28095.99 | 1.19 | 8.57 | NS | - | - | - |

According to Bharathi and Umamaheswari (2018), the trait internodal length indicates compactness of the florets arranged on rachis, which is the ideal character for the selection of suitable cut flower. In the present investigation, internodal length between florets ranged from 3.45 (IIHR-4) to 5.79 cm in Arka Vaibhav with the general mean of 4.54 cm. Among the double types evaluated, the line IIHR-4 recorded the shortest internodal length and the florets are arranged very densely on the spike. In agreement with findings of the present study, the highest internodal length in Arka Suvasini was reported by Singh and Singh (2013) in tuberose under Delhi condition. Variation in the internodal length might be due to the genetic makeup of the cultivars under study and similar observations were reported by Bharathi and Umamaheswari (2018) in single type tuberose.

Number of spikes per clump ranged from 2.14 (Hyderabad Double) to 4.43 (IIHR-4) with the mean of 3.23. Number of spikes per $m^2$ varied from 19.24 (Hyderabad Double) to 33.94 (IIHR-4) with the mean of 26.52. This variation in spikes per clump is in line with the findings of Rao and Sushma (2015), Ranchana *et al*. (2013), Gandhi (2017) and Safeena *et al*. (2019) in tuberose. Number of spikes per hectare ranged from 1,92,750.00 (Hyderabad Double) to 3,98,781.25(IIHR-4) with the mean of 2,90,481.25. The advanced breeding line IIHR-4 was found to be superior in flower yield than the commercial check Arka Vaibhav. This variation in the production of spikes/plant and spikes per plot might be due to the genetically controlled factor and also due to the hereditary traits of different cultivars under prevailing environment.

The vase life ranged between 6.50 and 7.25 days for the genotypes evaluated. Significant differences were not noticed among the double genotypes for vase life indicating that the advanced breeding line IIHR-4 has good vase life and it is on par with commercial cultivars interms of vase life.

Arka Suvasini recorded minimum number of bulbs per clump (2.69) and maximum number of bulbs per clump was recorded in Arka Vaibhav (8.19) with the mean of 5.14. Number of bulblets per clump ranged from 32.81 (Arka Suvasini) to 71.00 (Pearl Double) with the mean of 53.28. The variations in bulb parameters might be due to the presence of genetic variability of the cultivar and the results are in line with the findings of Madhumathi *et. al*. (2018) in tuberose. With respect to straightness of spike, the line IIHR-4, Arka Vaibhav and Hyderabad Double were found to bear straight spikes, while the cultivars Arka Suvasini and Pearl Double produced slightly bent spikes. The tinge on flower bud was recorded to be green in the line IIHR-4 and Arka Vaibhav and all the

other cultivars recorded pink tinge on flower bud. The type of flower opening was found to be shy in Pearl Double while all the other cultivars recorded wide flower opening. Differences in nature of spike, flower opening and tinge on flower bud was earlier reported by Bharathi and Umamaheswari (2018) in tuberose and these are due to the distinguished generic make up of the genotypes.

The advanced breeding line IIHR-4 was screened for the tolerance/ resistance against root knot nematode *M. incognita* for three consecutive years and the pooled analysis revealed that it was highly resistant under field conditions with least gall index of 1.24 (Fig. 1). Variations of tuberose genotypes for root knot nematode tolerance and resistance were reported earlier by Gandhi *et al*. (2018) who stated that this might be due to the inherent genetic character. Per cent disease index and host reaction of tuberose genotypes against leaf blight disease caused by *Alternaria* under field conditions were recorded and the results indicated that the breeding line IIHR-4 has better field tolerance to *Alternaria* leaf blight as compared to the other tuberose genotypes evaluated (Fig. 3).

## CONCLUSION

It is concluded from the above study for three consecutive years that among the cultivars evaluated for flowering, yield, quality and biotic stresses, the advanced breeding line IIHR-4 with superior flowering and quality parameters namely the double type florets on shorter spike and rachis, more number of florets open at a time on the spike, shorter intermodal length between the florets with compact floret arrangement, straight spikes with wide open florets and green tinge on flower buds makes the IIHR-4 as most ideal cut flower cultivar. It was also found to be highly resistant to root knot nematode *M. incognita* under field conditions with better field tolerance to *Alternaria* leaf blight disease.

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***(Received on 15.01.2021, Revised on 24.07.2021 and Accepted on 27.07.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 241-250, 2021*

***Original Research Paper***

# Anti-fungal activity of *Trichoderma atroviride* against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease of tomato

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## ABSTRACT

***Fusarium oxysporum* f. sp. *lycopersici* causing tropical wilt of tomato is a destructive phytopathogen. To study bio efficacy of fungal bio agents, fifteen isolates of *Trichoderma* species were isolated from rhizosphere soil of healthy tomato plants. Among the isolates, TA12 showed higher antagonistic efficacy against the pathogen. Upon analysis of the rDNA of internal transcribed spacers (ITS) and molecular data, the isolate was identified as *Trichoderma atroviride*. The *in vitro* antagonistic assessment indicated that the *T. atroviride* isolate caused significant inhibition of *F. oxysporum* f. sp. *lycopersici*. *Trichoderma atroviride* (TA12) showed antagonistic activity against *F. oxysporum* with mycelial inhibition of 71.25%. The culture filtrates of *Trichoderma atroviride* exhibited its antifungal activity against *F. oxysporum* with a suppression of 77.77%. Moreover, the ethyl acetate extracts of *T. atroviride* TA12 showed the highest antifungal potency against *F. oxysporum* f. sp. *lycopersici*. The main bioactive constituents of *T. atroviride* were 6-pentyl - 2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3-pyridazinyl) and heptadecane. In conclusion, the isolate TA12 could be exploited to develop an effective biocontrol management practice for combating Fusarium wilt disease of *Fusarium oxysporum* f.sp. *lycopersici* in tomato.**

**Keywords:** Anti-fungal activity, *Fusarium oxysporum* f. sp. *lycopersici,* tomato and *Trichoderma* and GC-MS.

## INTRODUCTION

Tomato (*Lycopersicum esculentum*) belonging to solanaceae family, ranks first and second in processing crops and vegetables respectively in India (Fatima and Anjum, 2017). It is commercially cultivated globally in both indoor and outdoor conditions. It contains lycopene, a rich source of anti-oxidant property (Miller *et al*., 2002). Cultivation of tomato becomes limited due to invasion of wide pests *viz.,* insects, diseases, weeds and nematodes which accounts for major yield loss. *Fusarium oxysporum* f. sp. *lycopersici* (Fol) infecting tomato is a destructive pathogen, causing severe economic losses all over the world. Major symptoms include yellowing of lower leaves, stunted growth, wilting of leaves and finally death of plant (Prihatna *et al*., 2018). Chemical control of plant disease management is commonly employed approach (Hirooka and Ishii, 2013). The efficiency of fungicides chiefly depends on the timing of application, method of application, disease intensity, the efficiency of disease forecasting systems and the rate of emergence of fungicide resistant strains (Skamnioti and Gurr, 2009). Since, plant disease management using fungicides have constraints on environment and paves for evolution of resistant in pathogen, biological control using potential antagonists play a key approach in managing tomato wilt disease (Horinouchi *et al*., 2010, Zhao *et al*., 2011). *Trichoderma* spp. (*Hypocrea*) have found to be the most effective antagonists as they have mechanisms like mycoparasitism, antibiosis, competition and induced systemic resistance in host plants (Rodriguez *et al.,*2020). Numerous *Trichoderma* isolates secreted many volatile and non-volatile substances that one anti-fungal in nature against soil borne pathogens (Nagamani *et al.,* 2017). Besides disease control, *Trichoderma harzianum* also associated with

enhancing soil fertility (Liton *et al.,* 2019). This study exploits the anti-fungal efficiency of *Trichoderma atroviride* against wilt disease of tomato.

## MATERIALS AND METHODS

### Isolation and identification of pathogen

The various isolates of the pathogen tomato wilt were collected from infected tomato plants in different places of Madurai district. The isolate FO (Maa)-5 was found highly virulent. This isolate was identified as *Fusarium oxysporum* f.sp. *lycopersici* based on sequencing of ITS region (Accession number: MZ043720). The pathogen was maintained on PDA slants and used for further studies.

### Isolation of *Trichoderma*

Soil samples from rhizosphere region (3 cm) of healthy tomato plants were collected from 15 different locations of Madurai district, Tamil Nadu. The collected samples were dried and subjected to serial dilution (up to $10^{-4}$). The biocontrol agent was isolated using the selective medium of *Trichoderma* (TSM) and incubated for 7 days at 25±3°C (Awad *et al.,* 2018). Later the putative colonies were purified by single hyphal tip method. General biochemical tests were done to confirm the biocontrol agent. Later these cultures were preserved in PDA slants for further studies.

### Antifungal assay using *Trichoderma* spp. against pathogen

Antifungal assay was carried out to evaluate the antimicrobial efficacy of the potential isolates of *Trichoderma* spp. against the pathogen.

### (i) Dual culture assay

The dual culture described by Yassin *et al.* (2021) was followed to test the antagonistic ability of *Trichoderma* species against the pathogen. Small block (5 mm disc) of *Fusarium* cut from the periphery was placed at one cm away from the periphery of the Petri dish previously poured with PDA. Similarly, the *Trichoderma* isolate was placed one cm away from the edge of the same Petri plate aseptically on the opposite end and plates were incubated at room temperature for 5 days. The experiment was replicated thrice and per cent growth inhibition was calculated by using the following formula,

$$I = \frac{(A-B)}{A} \times 100$$

Where A is mycelial growth of pathogen in control plate, B is mycelial growth of pathogen in treatment plate and I is the percent inhibition of mycelial growth.

### (ii) Effect of culture filtrates on inhibition of pathogen

Mycelial plugs were taken from the freshly grown *Trichoderma* cultures and inoculated into conical flask containing fresh 100 ml potato dextrose broth and incubated for 7 days at 150 rpm at 28°C (You *et al*., 2016). Supernatant of the cultures were collected and centrifuged at 9000 rpm for 10 min. Then the cell free filtrates were sterilized through a 0.22 µm millipore filters and mixed with unsolidified PDA medium at 10% (v/v) concentration. Uninoculated PDB was added to PDA with same ratio for control. Mycelial disc of the pathogen was placed in all PDA plates and kept for incubation at 28°C for 5 days. Reduction in mycelial growth of the pathogen was measured and per cent inhibiton over control was arrived by the formula of Sreedevi *et al*. (2011),

$$I = \frac{(C-R)}{C} \times 100$$

Where,

C - Mean linear growth of pathogen in control ,

R - Mean linear growth of pathogen in treatments

### Extraction of *Trichoderma* DNA and PCR amplification

The potent cultures were inoculated in conical flask containing 100 ml of potato dextrose broth and incubated in shaker 150 rpm for 7 days. The mycelial mat was sieved and pierced into powder using liquid nitrogen (Liu *et al*., 2020). DNA extraction of virulent isolates was done by using the procedure of Zhang *et al*. (2010). Genomic DNA was isolated by using CTAB method. PCR amplification was carried out using universal primers – internal transcribed spacer ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990). Polymerase chain reaction was performed in a reaction mixture of 50 µl with 35 cycles including 63 ng of genomic DNA, 50 pmol of each primer, 500 µM concentrations of dNTPs and 1.25 units of *Taq* DNA polymerase in an eppendorf

thermal Cycler. The PCR programme was performed with initial denaturation (95°C for 2 mins), followed by the repeated cycles of denaturation (94°C 1min), annealing (56°C for 30 sec) and extension (72°C for 1 min), and final extension of 72°C for 10 min. Amplicons were detected by 2% (w/v) agarose gel electrophoresis. Sequencing of purified PCR product was done at Eurofins Genomics India Pvt. Ltd. Bangalore.

**Identification of the *Trichoderma* sp. and phylogenetic relationships**

ITS region of potential isolate was sequenced and BLAST searched with sequences in the NCBI, GenBank. Phylogenetic dendrogram was constructed by the neighbor-joining method in MEGA 10.0 software depending over multiple sequence alignment with an evolutionary distance of 0.05. The tree topologies were evaluated by performing analysis of 1000 data sets. The sequence was submitted to GenBank for obtaining accession number.

**Preparation of crude extracts**

Mycelial disc from an actively growing colony of *Trichoderma* isolate was inoculated into fresh potato dextrose broth and incubated for seven days. The culture filtrates were collected by filtering using Whatmann no.1 filter paper followed by centrifugation at 9000 rpm for 15 min and finally the metabolites were extracted using ethyl acetate as solvent (Jantarach and Thanaboripat, 2010). Further concentration of extracts and elimination of solvents were done using rotary evaporator (Sharma *et al.,* 2016).

**Gas chromatography mass spectrometry (GCMS)**

The extract possessing high antimicrobial property has been subjected to GCMS analysis. The antibiotics, volatiles and secondary metabolites present in the sample were detected by injecting one microlitre of sample in Capillary Standard Non – Polar Column of GC - MS in which Helium was used as carrier gas. The analytical conditions were adjusted by following the procedures given by Yassin *et al.,* (2020). The m/z peaks representing mass to charge ratio, characteristic of the antimicrobial fractions were compared with those of the corresponding organic compounds in the NIST library (Manigundan *et al.,* 2020).

**Thin layer chromatography (TLC)**

Thin Layer Chromatography was performed to identify the presence of antifungal compounds in crude extract of *Trichoderma* isolate. TLC tank was filled with acetone and chloroform solvents in the ratio of 3:1 and sealed the tank immediately (Vivek *et al.,*2013). Desired size of TLC plate (60 F254, Merck, India) was taken and marked 0.5 cm above the bottom corner of plate. Samples were spotted at 1 cm distance and labelled. Spotted TLC plate was allowed to run in TLC tank. Then the plate was removed and visualized in laminar under UV fluorescence light (254 nm) and marked the dark purple fluorescence with pencil. The $R_f$ value was calculated based on the distance covered (Fried and Sherma, 1982),

$R_f$= Distance travelled by substance / Distance travelled by solvent

**Statistical analysis**

Statistical analysis were performed using analysis of variance (ANOVA) by SPSS software version 16 (SPSS.Chicago). The data were tabulated as mean of triplicates ± standard error and will be considered significant when the $P < 0.05$ and the means were compared by Duncan's Multiple Range Test (DMRT).

## RESULTS

**Antifungal assay**

The results of antifungal assay revealed that all the *Trichoderma* isolates possessed certain amount of antifungal activity both in dual and culture filtrate assays.

**(a) Dual culture assay**

A total of fifteen isolates of *Trichoderma* spp. were isolated from the rhizosphere soil of healthy tomato plants. Among the isolates, *Trichoderma* isolate TA 12 was found superior against *Fusarium oxysporum* with 71% mycelial inhibition over control (Fig 1). The next best isolate was TA 2 with mycelial inhibition of 68.75%. Isolate TA 5 recorded minimum inhibition percentage of 46.22 (Table 1).

**(b) *Trichoderma* culture filtrate assay against Fol**

The experimental results revealed that all the isolates inhibited the mycelial growth of pathogen at significant level.. Among the isolates tested, TA12 showed the maximum mycelial inhibition of 77.77% (Fig 2).This was followed by the isolate TA 2 (75.65%). The least

**Table 1. Antifungal assay of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici* in dual culture assay**

| S.No. | Treatments | Mycelial growth (cm)* | Per cent mycelial inhibition |
|---|---|---|---|
| 1. | TA – 1 | 4.2$^{ij}$ | 47.50(43.57) |
| 2. | TA – 2 | 2.5$^{b}$ | 68.75(56.01) |
| 3. | TA – 3 | 3.9$^{h}$ | 51.23(45.70) |
| 4. | TA – 4 | 3.3$^{e}$ | 58.75(50.04) |
| 5. | TA – 5 | 4.3$^{j}$ | 46.22(42.83) |
| 6. | TA – 6 | 3.5$^{f}$ | 56.23(48.58) |
| 7. | TA – 7 | 2.7$^{c}$ | 66.25(54.48) |
| 8. | TA – 8 | 3.2$^{e}$ | 60.00(50.77) |
| 9. | TA – 9 | 4.1$^{i}$ | 48.77(44.30) |
| 10. | TA – 10 | 3.5$^{f}$ | 56.21(48.57) |
| 11. | TA – 11 | 3.0$^{d}$ | 62.50(52.24) |
| 12. | TA – 12 | 2.3$^{a}$ | 71.25(57.58) |
| 13. | TA – 13 | 4.1$^{i}$ | 48.74(44.28) |
| 14. | TA – 14 | 2.9$^{d}$ | 63.76(52.99) |
| 15. | TA – 15 | 3.7$^{g}$ | 53.73(47.14) |
| | Control | 8.0 | 0.00(0.00) |
| | CD (P=0.05) | 1.37 | |

*Mean of three replications
Values with different superscripts are significantly differ from each other at p<0.05
Values in the parenthesis are arc sine transformed values


**Fig. 1. *In vitro* dual confrontation assay of antagonistic *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici***


**Fig. 2. Anti-mycotic potency of culture filtrate of *Trichoderma* spp (TA12) against *Fusarium oxysporum* f.sp. *lycopersici***

mycelial growth was observed in TA 5 at the rate of 52.20% (Table 2).

### Molecular confirmation of potential *Trichoderma* isolate TA12

PCR of *Trichoderma* isolate with ITS-1 and ITS-4 primer pairs resulted in amplification of a fragment of size 636 bp (Fig 3).

**Table 2. Antifungal assay of culture filtreates of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici***

| S.No. | Treatments | Mycelial growth (cm)* | Per cent mycelial inhibition |
|---|---|---|---|
| 1. | TA – 1 | 4.2$^{j}$ | 53.33(46.91) |
| 2. | TA – 2 | 2.2$^{b}$ | 75.65(60.43) |
| 3. | TA – 3 | 3.7$^{hi}$ | 58.88(50.11) |
| 4. | TA – 4 | 3.0$^{de}$ | 66.67(54.74) |
| 5. | TA – 5 | 4.3$^{j}$ | 52.20(46.26) |
| 6. | TA – 6 | 3.1$^{ef}$ | 65.55(54.06) |
| 7. | TA – 7 | 2.3$^{bc}$ | 74.45(59.64) |
| 8. | TA – 8 | 2.8$^{d}$ | 68.88(56.09) |
| 9. | TA – 9 | 4.0$^{ij}$ | 55.54(48.18) |
| 10. | TA – 10 | 3.3$^{fg}$ | 63.33(52.73) |
| 11. | TA – 11 | 2.5$^{c}$ | 72.22(58.19) |
| 12. | TA – 12 | 2.0$^{a}$ | 77.77(61.87) |
| 13. | TA – 13 | 3.8$^{i}$ | 57.76(49.46) |
| 14. | TA – 14 | 2.5$^{c}$ | 72.22(58.19) |
| 15. | TA – 15 | 3.4$^{gh}$ | 62.20(52.06) |
| | Control | 9.0 | 0.00(00.00) |
| | CD (P=0.05) | 1.97 | |

*Mean of three replications
Values with different superscripts are significantly differ from each other at p<0.05
Values in the parenthesis are arc sine transformed values

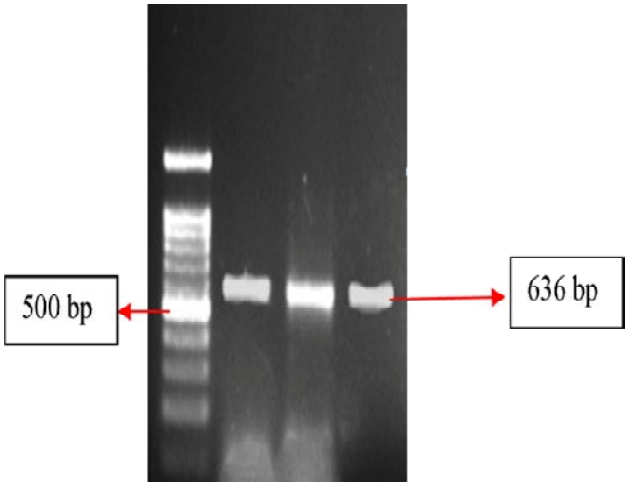



**Fig. 3. The results of ITS amplification in potent *Trichoderma* isolates**

Phylogenetic analysis of the sequence (TA 12) with existing sequences in the NCBI database showed 99% sequence similarity with *Trichoderma atroviride* (Fig 4). The sequence was deposited in Genbank and obtained accession number (MW984524; Fig 5).

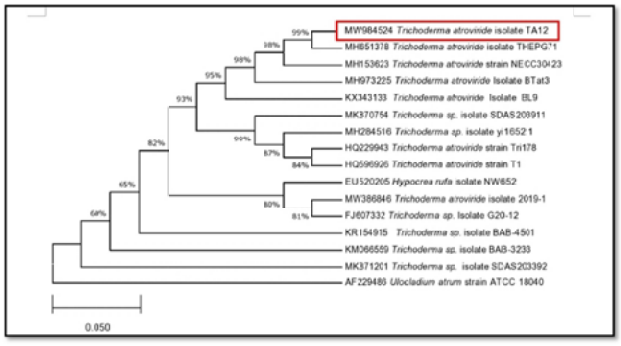



**Fig. 4. Phylogenetic analysis of *Trichoderma atroviride* using neighbor joining method. The numbers over branches indicates bootstrap coefficient.**

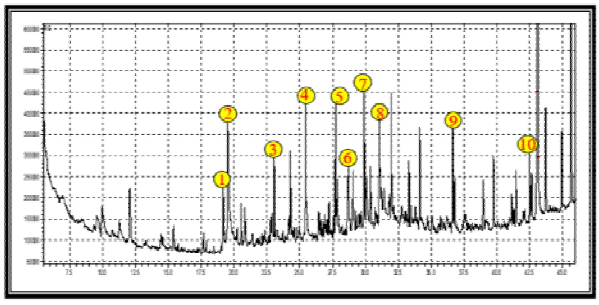


**Fig. 5. GCMS chromatogram for detection of secondary metabolites in *Trichoderma atroviride* using ethyl acetate as solvent.**

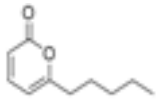
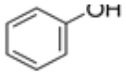
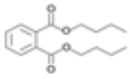
### GC- MS analysis of extracts of *Trichoderma*

The extracts of *T. atroviride* were analyzed to determine its active chemical constituents. Active constituents of *T. atroviride* extract were demonstrated in Fig 5 and Table 3. The results showed that a numerous compounds produced by *Trichoderma atroviride,* possessing high antimycotic property.

### TLC of *Trichoderma* spp.

The TLC plate with the sample was observed under UV laminar fluoroscence. The spot was resolved without any smear or streak pattern in TLC plate. In case of chitinase (developed in acetone:chloroform (3:1)) distinct spots were visualized under UV light (254 nm) with $R_f$ value of 0.84 The distance travelled by the substance was 5.1 cm.

**Table 3. Identification of secondary metabolites from *Trichoderma atroviride* through GCMS analysis**

| Peak | Retention Time | Compound Name | Structure | Molecular Formula | Molecular Weight (g/mol) | Peak area % |
|---|---|---|---|---|---|---|
| 1 | 19.162 | Quinoline |  | $C_9H_7N$ | 129.16 | 1.92 |
| 2 | 19.518 | 2H-Pyran-2-one, 6-pentyl |  | $C_{10}H_{14}O_2$ | 166.22 | 6.25 |
| 3 | 23.056 | Nonadecane |  | $C_{19}H_{40}$ | 268.5 | 1.81 |
| 4 | 25.450 | Heptadecane |  | $C_{17}H_{36}$ | 240.5 | 3.20 |
| 5 | 27.725 | Heneicosane |  | $C_{21}H_{44}$ | 296.57 | 3.11 |
| 6 | 27.866 | Eicosane |  | $C_{20}H_{42}$ | 282.54 | 1.68 |
| 7 | 28.690 | Phenol |  | $C_6H_5OH$ | 94.11 | 1.77 |
| 8 | 31.062 | Dibutyl phthalate |  | $C_{16}H_{22}O_4$ | 278.34 | 2.79 |
| 9 | 36.633 | Hexadecane,1-iodo |  | $C_{16}H_{33}I$ | 352.34 | 3.54 |
| 10 | 42.653 | Benzene propanoic acid |  | $C_9H_{10}O_4$ | 182.17 | 1.78 |

## DISCUSSION

*Trichoderma* species have a global range of distribution and live in a variety of ecological niches, including decaying bark and wood, other fungus, soil, and healthy plant roots, stems, and leaves (Du Plessis *et al*., 2018; Mukherjee *et al*., 2013). The number of *Trichoderma* species used in biocontrol has drastically increased in modern era. Up to date, more than 290 *Trichoderma* species have been discovered (Bissett *et al*., 2015; Du Plessis *et al*., 2018; Zhu *et al*., 2017).

In this study, a survey was conducted and obtained fifteen isolates of *Trichoderma* after isolation. Among the isolates, *Trichoderma* isolate TA12

showed greater inhibition against the *Fusarium* strain than the other *Trichoderma* isolates. TA12 suppressed the mycelial growth of the pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) by 71%. The results were in accordance with Schoffen *et al.* (2020), who reported that *T. atroviride* strain suppressed the mycelial growth of *F. oxysporum* in the range of 52.37% – 70.56%. *Trichoderma virens* exhibited a mycelial inhibition percentage of 80 against Fusarium wilt (Banerjee *et al.,* 2020). Sallam *et al.* (2019) confirmed the antagonistic potency of *T. atroviride* strain against *Fusarium* wilt of tomato with a mycelial inhibition rate of 66.80%.

The metabolites produced by *Trichoderma* spp. inhibited *Fusarium* isolates. Among the 15 isolates tested, 12 were able to inhibit the growth of *Fusarium oxysporum* (>50%) within which six isolates showed relatively strong inhibitory effect (>60%). Further in vitro assay of *Trichoderma* culture filtrates against Fol confirmed the similar trend as the TA 12 isolate recorded the highest inhibition of the pathogen (77%). Our findings are consistent with those of Rudresh *et al.* (2005), who reported the antimicrobial efficiency of culture filtrates of *T. harzianum* against *F. oxysporum* strain, recording mycelial inhibition rates of 78.5%. Alvarez-Garcia *et al.* (2020) also reported the suppression of mycelial growth of *Fusarium* spp. by the culture filtrates of *T.harzianum* and recorded the inhibition rate of 76.27%. Findings of Tomah *et al.* (2020) proved that *Trichoderma citrinoviride* retarded the growth of fungal pathogen at 77.8%.

The potent antagonist (TA 12) was subjected to sequencing of ITS regions and phylogenetic analysis. The phylogenetic analyses indicated that the isolate shown 99% similarity with other *T. atroviride* isolates thus TA12 confirmed as *T. atroviride*.

The antifungal ability of *T. atroviride* was confirmed by performing GCMS. Previous studies indicated that these compounds inhibited the mycelial growth of different pathogenic fungal strains (Keszler *et al.,* 2000**,** Jelen *et al.,* 2014, Mallaiah *et al.,* 2016). The main constituents alone do not attribute to the antifungal activity but also the presence of other bioactive substances attributed to antifungal potency. The anti-fusarial potency of *T. atroviride* extract may be attributable to the presence of many

bioactive compounds such as 6-pentyl-2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3pyridazinyl), heptadecane, 17-methoxy-4-methyl-d-homo-18-norandrosta, nonadecane, heneicosane, eicosane, dibutyl phthalate, hexadecane and benzene propianoic acid. The antifungal efficacy of the extract may also be referred to the synergistic effect among the bioactive components (Khan *et al*., 2020).

Thin layer chromatography was done to separate and identify antifungal compound of *T. atroviride.* The $R_f$ value calculated was similar to the values obtained with separation of enzymes from *Trichoderma* isolates (Rabinal and Bhat, 2017). Vinale *et al.* (2008) also revealed the same range of values when isolated from *Trichoderma.* The $R_f$ value of 0.86 was identified in TLC separation of *T. harzianum* isolates (Kiss *et al.,* 2000).

Many studies indicated that *Trichoderma* spp. possess the multiple mechanisms, including mycoparasitism, extracellular enzymes such as cellulase, amylase, pectinase, protease and chitinase, antagonistic compounds and induced resistance, to inhibit pathogens and reduce diseases (Cherkupally *et al.,* 2017). Thus, the *Trichoderma atroviride* TA12 possibly uses multiple mode of action to inhibit pathogen, while antifungal compounds secreted by it could have played a major role in inhibiting pathogen and controlling fusarium wilt incidence of tomato.

The potent antagonist, *Trichoderma atroviride* isolate exhibited excellent antimycotic activity against Fusarial phytopathogen of tomato. Hence its antimicrobial potency of culture filtrates and organic solvent extracts against fusarial pathogen of tomato highlights the ability to employ novel and safe biofungicide in order to neglect the hazards of chemical fungicides on the human health and environment.

## ACKNOWLEDGEMENTS

This work was supported by Centre of Innovation, Department of Biotechnology, Agricultural College and Research Institute, Madurai.

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***(Received on 12.08.2021, Revised on 31.08.2021 and Accepted on 24.10.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 251-260, 2021*

***Original Research Paper***

# Seed transmission of bean common mosaic virus - blackeye cowpea mosaic strain (BCMV-BlCM) threaten cowpea seed health in the Ashanti and Brong-Ahafo regions of Ghana

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## ABSTRACT

**Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) were used to detect the presence and seed transmissibility of bean common mosaic virus-blackeye cowpea mosaic (BCMV-BICM) in farm- retained cowpea seed lots obtained from 46 locations, including markets and farms in major cowpea growing areas in the Ashanti and Brong Ahafo regions of Ghana. In the grow-out tests, virus symptomatic plants were observed in seedlings of 19 of the 46 seed lots tested under insect-proof screen-house conditions. All the symptomatic plants tested positive to polyclonal antiserum raised against BCMV-BICM in ACP-ELISA. The seed transmission rates based on symptoms ranged from 0 to 37.8 %. RT-PCR with primer pair designed to amplify the potyvirus Cylindrical Inclusion (CI) region resulted in an expected 720 bp DNA segment in 19 seed lots as a further confirmation of virus in the seed lots. The remaining 27 lots were asymptomatic and tested negative to BCMV-BlCM in both ACP-ELISA and RT-PCR. The findings of this study revealed seed as the source of primary inoculum in the farmers' fields and may aid in the implementation of control strategies such as discouraging farmers from retaining their own seeds for subsequent sowing and encouraging them to take appropriate measures in obtaining virus-free cowpea seeds from other sources.**

**Key Words:** Bean common mosaic virus-blackeye cowpea mosaic, Cowpea, vegetable legume, ELISA, Potyvirus, RT-PCR, virus detection virus-seed transmission

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is the most widely cultivated tropical vegetable legume in sub-Saharan Africa (SSA). It is predominantly produced by smallholder farmers because of its tolerance to drought and ability to thrive in zero or low input farming. It provides affordable protein for humans and animals in SSA, Asia, and Latin America (Bashir and Hampton 1993; Tarawali *et al*., 2002; Boukar *et al*., 2013) and also serves as a cover crop in soil nitrogen fixation and the control of erosion and weeds (Hutchinson and McGiffen, 2000). Cowpea has the potential to enhance food security and reduce poverty in West Africa, provided both socio-economic and biological constraints such as poor application of appropriate cultural technologies, infestation by weeds and insect pests, and infection by diseases are adequately tackled (Jackai and Adalla, 1997; Quin, 1997; Coulibaly and Lowenberg – DeBoer, 2002; Boukar *et al*., 2013).

In Ghana, cowpea is second to groundnut in terms of area under cultivation and quantity produced and consumed annually (Egbadzor *et al*., 2013). An average of 143,000 MT is produced annually on about 156,000 ha making Ghana the fifth-highest producer of cowpea in Africa (ICRISAT, 2012). The Guinea savannah zone of Ghana, which includes the Northern and Upper West regions, is the major production area in the country (Al-Hassan and Diao, 2007). Other

production areas include the Sudan savannah zone (Upper East region) and some districts in the transitional zones of Brong Ahafo and Ashanti regions (Haruna *et al*., 2018).

Bean Common Mosaic Virus – Blackeye Cowpea Mosaic (BCMV-BICM) is an important seed-borne virus reported in almost all cowpea growing areas worldwide (CABI/EPPO, 2010; Hema *et al*., 2014). Cowpea fields can suffer substantial yield losses from seed-borne pathogens (Bankole and Adebanjo, 1996). Sowing infected seeds increase germination failure, seedling mortality, and diseased plants, leading to lower yields. Additionally, diseased crops may increase seed infection levels in young plants (Manyangarirwa *et al*., 2009).

Seed transmission offers an effective means of introducing viruses into crop fields at an early stage, giving randomized foci of primary infections throughout the season, which serves as the primary inoculum source for further virus spread by insect vectors (Booker *et al*., 2005). Viruses may persist in cotyledons and embryo axes of matured seeds for long periods (Sekar and Sulochana, 1988), enabling scope for long distances virus spread through contaminated seed lots. The role of farmer-saved seeds in transmitting cowpea diseases was analyzed in northern Nigeria (Biemond *et al*., 2013), and seed to plant transmission of seed-borne pathogens in farmer-saved cowpea was investigated in Zimbabwe (Manyangarirwa *et al*., 2009). These studies have shown that farmer-saved cowpea seeds were heavily infected, with a range of seed- and soil-borne pathogens. The latter emphasizes the negative influence on germination and potential crop losses. Infections caused by seed-borne viruses reduce seed quality and the potential yield of crops. Booker *et al*. (2005) reported seed transmission rates from less than 1 to 100% depending on the virus and host. Yield reductions from expected 2500kg/ha to 50kg/ha were also reported in fields infected with BCMV-BICM in India (Puttaraju *et al*., 2000a). Further, cowpea varieties inoculated with BCMV-BICM at the primary leaf stage showed 92-100% infection at first trifoliate leaf (http://cropgenebank.sgrp.cgiar.org/ Date accessed: 16/07/2019). The virus is readily transmitted mechanically and in a non-persistent manner by the aphids *Aphis craccivora*, *A. gossypii,* and *Myzus persicae* (Orawu, 2007).

A survey conducted on cowpea fields in the Forest and Transitional zones of Ghana revealed the presence of BCMV-BlCM among other six viruses, namely, cowpea aphid-borne mosaic virus (CABMV, genus Potyvirus), cowpea mottle virus (CPMoV, genus Carmovirus), southern bean mosaic virus (SBMV, genus Sobemovirus), cowpea mild mottle virus (CPMMV, genus Carlavirus), cowpea yellow mottle virus (CYMV, genus Comovirus) and cucumber mosaic virus (CMV, genus Cucumovirus) with BCMV-BICM being the most prevalent (Adams *et al*., 2020). According to the study, farmers in the Forest and Transitional zones of the Brong-Ahafo and Ashanti regions adopt production practices such as high cropping density as a result of random sowing methods, recycling of seeds from season to season, the closeness of fields to each other with different planting and pesticide application periods as well as preference for and cultivation of susceptible local cowpea cultivars which increases the incidence and severity of viruses on fields in those areas (Amaza *et al*., 2010; Adams *et al*, 2016).

During the 2015 growing season, viral disease symptoms, similar to those caused by the BCMV-BlCM, were observed on cowpea fields in the Ashanti and Brong Ahafo regions of Ghana. Seeds collected from farmers in these areas were mostly shriveled. This study was conducted to confirm the virus identity in the symptomatic plants observed in the farmers' fields and virus seed transmission in the seeds lots harvested from the 46 farmers' fields and seed markets in Ghana.

## MATERIALS AND METHODS

### Seed sample collection

A total of forty-six (46) cowpea seed lots were collected from randomly selected farms and markets in the Amantin-Atebubu (17 lots), Ejura-Sekyeredumasi (13 lots), and Nkoranza (16 lots) districts. Seed lots were obtained from 24 farm locations (15 in Amantin-Atebubu, and 9 in Ejura-Sekyeredumasi) and 22 market locations (16 in Nkoranza, 2 in Amantin-Atebubu, and 4 in Ejura-Sekyeredumasi) (Table 1). Seeds sourcing from farmers was done by selecting cowpea farms separated by at least 0.5 Km in each district. In each farm, seed lots were obtained by collecting and bulking seeds from 30 plants randomly selected in an 'X' transect,

with 15 plants per diagonal axis. For market-sourced seeds, lots were obtained by randomly collecting seeds from different market women during the main market days in each district. Seed samples were kept in labeled sample bags with naphthalene balls. A GPS device was used to record coordinates and altitudes of the field and market locations.

**Grow-out test**

From each sampled seed lot, 100 seeds were sown in trays filled with two liters of steam-sterilized topsoil in an insect-proof screen house. Cowpea seedlings were visually examined for any symptoms. The total number of plants germinated and the number of symptomatic plants was counted in each try to estimate the percent symptomatic plants. At the three-week stage, apical leaves of both symptomatic and asymptomatic plants were sampled for BCMV-BlCM indexing by antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA). Symptomatic and asymptomatic plants were tested separately. In the case of asymptomatic plants, ten apical leaves, one from each plant, were collected, and they were together as one composite sample for virus indexing. This was repeated for all the seed lots.

**ACP-ELISA for BCMV-BICM detection**

To test each plant, a sterile cork borer was used to obtain 5 mm diameter pieces of all leaves in each of the 46 groups of leaf samples. About 100 mg of leaf tissue from each sample was grounded in the carbonate coating buffer (0.015 M $Na_2CO_3$ and 0.0349 M $NAHCO_3$) with DIECA at 100 mg/ml buffer (1:10 w/v). One hundred microlitres of the extract were added to each well of a microtitre plate. Infected, healthy plant sap and buffer were used as controls. Plates were incubated in a humid chamber for 1 hour at 37°C and then washed with three changes of phosphate-buffered saline with Tween 20 (PBS-Tween 20), allowing three minutes for each wash. Plates were emptied and tapped dry on a layer of paper towel. Wells were blocked with 200 µl of 3% dried skimmed milk in PBS-Tween 20. Plates were incubated at 37°C for 30 minutes, and then tapped dry. Healthy cowpea leaf extract in PBS-TPO (1:10 w/v) was used to cross-adsorption of the BCMV-BICM antiserum at 1:5000 µl. The mixture was incubated at 37°C for 30 minutes. One hundred microlitres of the cross-adsorbed antisera was dispensed in each well and plates were incubated at 37°C for 1 hour. Plates were washed and tapped dry as described above. One hundred microlitres of goat anti-rabbit alkaline phosphatase (ALP) conjugate diluted in conjugate buffer (Ovalbumin, Polyvinyl Pyrrolidone and PBS-Tween 20) (1: 15,000) were dispensed into each well and incubated for 1 hour at 37°C. Plates were washed and tapped dry.

One hundred microlitres of 0.5 mg $ml^{-1}$ p-nitrophenyl phosphate substrate in substrate buffer (diethanolamine and distilled water) were added to each well and incubated in a dark room for 1 hour. Absorbance values were measured, and plates were kept in a refrigerator at 4°C overnight. Quantitative measurements of the p-nitrophenyl substrate conversion resulting in yellow colour were made by determining the absorbance at 405 nm (A405) in an ELISA plate reader at 1 and 6 hours. The mean absorbance readings of negative controls were determined, and twice the values were used as the positive thresholds.

**Reverse-transcription polymerase chain reaction (RT-PCR)**

The RT-PCR protocol described by Gillaspie *et al*. (2001) was used for the detection of BCMV-BlCM in the 46 seed lots to confirm the ACP-ELISA result. Total nucleic acid was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method described by Dellaporta *et al*. (1983). Cylindrical inclusions forward (CI-F; 5'- CGI VIG TIG GIW SIG GIA ART CIA C-3') and reverse (CI-R; 5'-ACI CCR TTY TCD ATD ATR TTI GTI GC-3') primers designed by Ha *et al*. (2008) were used for RT-PCR amplification and the RT-PCR products were resolved on a 1.5% agarose gel along with 100 bp DNA ladder as a size marker (Cat Nos N0467S, Quick-load, Biolabs Inc., Ipswich, MA, USA). The gel was viewed under a UV trans-illuminator (BioRad Gel Doc XR, California, USA), and the virus-specific band in the samples were identified based on the presence of an expected amplicon size of 720bp.

## RESULTS

Among the 46 seed lots of cowpea subjected to a grow-out test in the screen-house, 19, made up of six lots obtained from Atebubu-Amantin, five from Ejura-Sekyeredumasi, and eight from Nkoranza, showed mottling and mosaic (Fig. 1) on leaves.


**Fig. 1. Mottle mosaic symptoms of seed-borne BCMV-BlCM on grow-out cowpea plants in a screen house**

All the symptomatic plants of 19 seed lots also gave positive reactions to BCMV-BlCM in ACP-ELISA (Table 1). BCMV-BlCM transmission based on symptoms among the lots ranged from 0 to 37.8 % (Table 2). Some of the infected seed lots recorded low germination rates. For instance, of the 100 seeds of each seed lot planted, 51 of "Nkoranza-14" and 30 of "Amantin-15," which were positive to BCMV-BICM, germinated. All asymptomatic plants tested negative to BCMV-BlCM in ACP-ELISA (Table 1).

All the 19 seed lots that had symptomatic plants and tested positive to BCMV-BlCM have also tested positive to the virus in RT-PCR (amplified a 720 bp amplicon) (Fig. 2). Amplification was not detected in

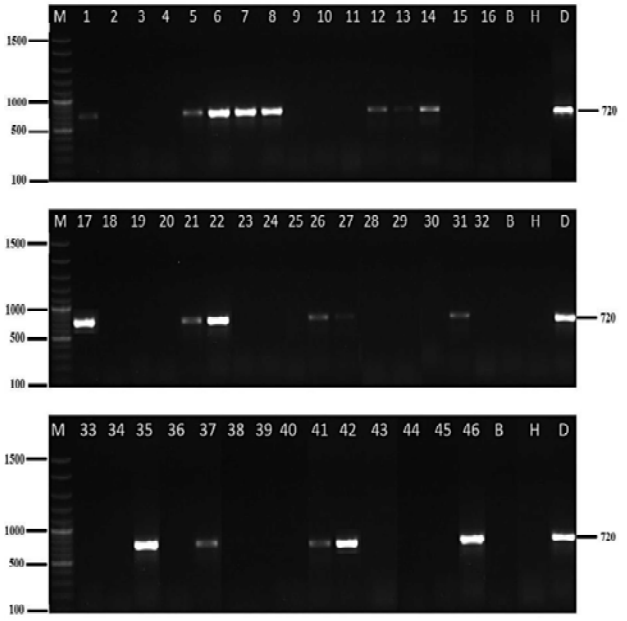


**Fig. 2. Agarose gel electrophoresis showing amplification of RT-PCR products**

Key; M = DNA marker, H = Healthy control, B = Buffer, D = Positive control Nkoranza samples: 1-16; Amantin samples: 17-33; Ejura samples: 34-46

the remaining 27 asymptomatic seed lots (Fig. 4), confirming the results obtained using BCMV-BlCM antiserum in ACP-ELISA.

## DISCUSSION

Grow-out tests, ACP-ELISA and RT-PCR have confirmed BCMV-BlCM seed transmission in the 19 of 46 seed lots assessed in this study. Aliyu *et al*. (2012) previously detected BCMV-BICM among other seed-borne viruses infecting cowpea in Nigeria, using ACP-ELISA. Like the results obtained in this study, several authors (Hampton *et al*., 1997; Shanker *et al*., 2009; Ittah and Binang, 2012) have at various times proved seed transmission of the virus. Shanker *et al*. (2009) reported BCMV-BlCM as a serious pathogen on cowpea worldwide, to which field plants succumb to infections from virulent strains. Booker *et al*. (2005) also reported the detrimental effect of the virus on cowpea production, causing stunting and plant deformation in the early growth stage and not allowing the plants to reach their full potential. Mottling and interveinal chlorosis observed on the primary leaves of the plants in the grow-out test were consistent with symptoms reported to be associated with infections caused by BCMV-BlCM (Aliyu *et al*., 2012).

The BCMV-BlCM incidence in farmers' fields and the corresponding seed transmission rates were given in Table 3. Some seed lots obtained from markets recorded seed transmission rates as high as 36.3% (Nkoranza-6) in the grow-out test. Some seed lots collected from farmers' fields with high BCMV-BlCM incidences recorded zero seed-transmission (Amantin-2, -7, -13, -14 and Ejura-3) while a few other lots recorded very low seed transmission values (Amantin-1, -11, Ejura-8 and -13). Amantin-6, Ejura-9, Amantin-5, and Ejura-4 recorded 100, 90, 87 and 83% field incidences, respectively, with corresponding seed transmission rates of 21.3, 37.8, 16.7 and 16.3%, respectively (Table 3).

Low germination rates recorded in some infected seed lots may be attributed to infection by the BCMV-BICM. Ittah *et al*. (2010) reported in a previous study that seed-borne viruses such as BCMV-BICM, CABMV, CMeV, and SBMV may cause some infected cowpea lines to lose their

**Table 1. ACP-ELISA result for BCMV-BlCM seed transmission**

| Samples | BCMV-BlCM | Samples | BCMV-BlCM | Samples | BCMV-BlCM |
|---|---|---|---|---|---|
| Nkoranza 1 | 3.175* | Amantin 1 | 2.658* | Ejura 1 | 0.287 |
| Nkoranza 2 | 0.253 | Amantin 2 | 0.349 | Ejura 2 | 2.658* |
| Nkoranza 3 | 0.225 | Amantin 3 | 0.367 | Ejura 3 | 0.204 |
| Nkoranza 4 | 0.222 | Amantin 4 | 0.288 | Ejura 4 | 3.370* |
| Nkoranza 5 | 3.438* | Amantin 5 | 3.383* | Ejura 5 | 0.345 |
| Nkoranza 6 | 3.446* | Amantin 6 | 2.851* | Ejura 6 | 0.282 |
| Nkoranza 7 | 3.645* | Amantin 7 | 0.247 | Ejura 7 | 0.281 |
| Nkoranza 8 | 3.445* | Amantin 8 | 0.416 | Ejura 8 | 3.457* |
| Nkoranza 9 | 0.139 | Amantin 9 | 0.373 | Ejura 9 | 3.285* |
| Nkoranza 10 | 0.249 | Amantin 10 | 3.396* | Ejura 10 | 0.224 |
| Nkoranza 11 | 0.193 | Amantin 11 | 2.962* | Ejura 11 | 0.282 |
| Nkoranza 12 | 3.174* | Amantin 12 | 0.568 | Ejura 12 | 0.283 |
| Nkoranza 13 | 3.381* | Amantin 13 | 0.316 | Ejura 13 | 3.322* |
| Nkoranza 14 | 3.275* | Amantin 14 | 0.471 | | |
| Nkoranza 15 | 0.517 | Amantin 15 | 3.140* | | |
| Nkoranza 16 | 0.285 | Amantin 16 | 0.410 | | |
| | | Amantin 17 | 0.419 | | |
| Positive control | OUT | | OUT | | OUT |
| Negative control | 0.268 | | 0.36 | | 0.36 |
| Buffer | 0.21 | | 0.28 | | 0.28 |

*Absorbance value (A405 nm) is >2x of negative control regarded as the virus positive.
"OUT" indicates an out-of-range value (A405 >4)

**Table 2. Seed transmission rates of BCMV-BlCM among accessions**

| Seed transmission rate (%) | Number of seed lots |
|---|---|
| 0 | 27 |
| 0.1 - 5.0 | 6 |
| 5.1 - 10 | 0 |
| 10.1 - 20 | 4 |
| 20.1 - 30 | 4 |
| 30.1 - 37.8 | 5 |

ability to germinate. Fawole *et al*. (2006) also analyzed the effect of seed-borne fungi infection of cowpea seed on germination rate and found reduced germination rate because of infection by the fungi. Further, Manyangarirwa *et al*. (2009) reported that farmer-produced cowpea seeds were heavily infected with a range of seed- and soil-borne pathogens in Zimbabwe, emphasizing the negative influence on germination. However, in contrast to the above findings, Biemond *et al*. (2013) found that natural infection of cowpea seeds with some seed-borne pathogens increased germination.

Although BCMV-BICM has been previously detected in cowpea seeds in Ghana (Zettler and Evans, 1972), according to the literature available, most previous detections were limited to grow-out test, host range, and reactivity to polyclonal antibodies. Ojuederie *et al*. (2009) suggested stringent screening methods such as RT PCR to be used in screening for the presence of seed-borne viruses in addition to ELISA, which employs reactivity to polyclonal antibodies since samples which appear negative with the latter could be positive when tested with RT PCR. The study conforms with the above recommendation as BCMV-BlCM was assessed with ACP-ELISA, and the results were confirmed with RT-PCR.

BCMV-BlCM was identified to be seed-borne in cowpea collected from farms and markets in

**Table 3. BCMV-BlCM incidence in farmers cowpea fields and respective seed transmission rates observed in grow-out test**

| Cowpea Seed lots | Seed source | Total sown | Total germinated | Total symptomatic | % Field incidence | Transmission rate (%) |
|---|---|---|---|---|---|---|
| Nkoranza 1 | Market | 100 | 53 | 18 | * | 34 |
| Nkoranza 2 | Market | 100 | 42 | 0 | * | 0 |
| Nkoranza 3 | Market | 100 | 93 | 0 | * | 0 |
| Nkoranza 4 | Market | 100 | 58 | 0 | * | 0 |
| Nkoranza 5 | Market | 100 | 63 | 14 | * | 22.2 |
| Nkoranza 6 | Market | 100 | 80 | 29 | * | 36.3 |
| Nkoranza 7 | Market | 100 | 91 | 28 | * | 30.8 |
| Nkoranza 8 | Market | 100 | 65 | 10 | * | 15.4 |
| Nkoranza 9 | Market | 100 | 36 | 0 | * | 0 |
| Nkoranza10 | Market | 100 | 71 | 0 | * | 0 |
| Nkoranza11 | Market | 100 | 89 | 0 | * | 0 |
| Nkoranza12 | Market | 100 | 87 | 18 | * | 20.7 |
| Nkoranza13 | Market | 100 | 80 | 19 | * | 23.8 |
| Nkoranza14 | Market | 100 | 51 | 16 | * | 31.4 |
| Nkoranza15 | Market | 100 | 68 | 0 | * | 0 |
| Nkoranza16 | Market | 100 | 50 | 0 | * | 0 |
| Amantin 1 | Farm | 100 | 60 | 1 | 63 | 1.7 |
| Amantin 2 | Farm | 100 | 68 | 0 | 50 | 0 |
| Amantin 3 | Farm | 100 | 52 | 0 | 30 | 0 |
| Amantin 4 | Farm | 100 | 42 | 0 | 33 | 0 |
| Amantin 5 | Farm | 100 | 78 | 13 | 87 | 16.7 |
| Amantin 6 | Farm | 100 | 94 | 20 | 100 | 21.3 |
| Amantin 7 | Farm | 100 | 63 | 0 | 70 | 0 |
| Amantin 8 | Farm | 100 | 69 | 0 | 38 | 0 |
| Amantin 9 | Farm | 100 | 82 | 0 | 38 | 0 |
| Amantin 10 | Farm | 100 | 96 | 12 | 87 | 12.5 |
| Amantin 11 | Farm | 100 | 100 | 4 | 60 | 4 |
| Amantin 12 | Farm | 100 | 73 | 0 | 45 | 0 |
| Amantin 13 | Farm | 100 | 76 | 0 | 87 | 0 |
| Amantin 14 | Farm | 100 | 65 | 0 | 57 | 0 |
| Amantin 15 | Market | 100 | 30 | 1 | * | 3.3 |
| Amantin 16 | Farm | 100 | 83 | 0 | 30 | 0 |
| Amantin 17 | Market | 100 | 33 | 0 | * | 0 |
| Ejura 1 | Farm | 100 | 42 | 0 | 43 | 0 |
| Ejura 2 | Market | 100 | 86 | 2 | * | 2.3 |
| Ejura 3 | Farm | 100 | 62 | 0 | 63 | 0 |
| Ejura 4 | Farm | 100 | 80 | 13 | 83 | 16.3 |
| Ejura 5 | Farm | 100 | 82 | 0 | 30 | 0 |
| Ejura 6 | Market | 100 | 72 | 0 | * | 0 |
| Ejura 7 | Farm | 100 | 71 | 0 | 17 | 0 |
| Ejura 8 | Farm | 100 | 92 | 1 | 50 | 1.1 |
| Ejura 9 | Farm | 100 | 90 | 34 | 90 | 37.8 |
| Ejura 10 | Market | 100 | 66 | 0 | * | 0 |
| Ejura 11 | Market | 100 | 57 | 0 | * | 0 |
| Ejura 12 | Farm | 100 | 63 | 0 | 40 | 0 |
| Ejura 13 | Farm | 100 | 80 | 1 | 53 | 1.3 |

***Denotes unknown (seed lots sourced from markets)**

Nkoranza, Amantin, and Ejura. A study conducted by Biemond *et al*. (2013) showed that farmer-produced cowpea seeds were heavily infected with a range of seed- and soil-borne pathogens. Transmission rates based on symptoms ranged from 0 to 37.8 %. Ladipo (1977) and Ng and Hughes (1998) estimated that the rate of seed-transmission of virus in cowpea may range from 0 to 90%, which aligns with the seed transmission rates observed in this study and a previous study by Zettler and Evans (1972) that showed the frequency of seed transmission of BCMV-BlCM at about 30.9% in cowpea. Seed transmission rates of BCMV-BlCM did not necessarily correspond with infection levels observed in fields from which the collections were made. Although some lots obtained from fields with high disease incidence recorded correspondingly high transmission rates in the grow-out test, others recorded either zero or very low rates.

Low transmission rates of BCMV-BlCM in seed lots obtained from fields with high disease incidences could be due to several reasons, including infection after flowering to the presence of virus in seed coat but not in the embryos (Gupta *et al*., 1985). Shanker *et al*. (2009) showed that sowing cowpea seeds with the incidence of BCMV-BlCM as low as less than 1% might result in significant virus spread with a major influence on grain yield. Puttaraju *et al*. (2004b) also reported a 65-100% BCMV-BlCM transmission resulting from sowing cowpea seeds with about 4-10% infection rate. Thus, even with the relatively low seed transmission rates observed in the current study, there is cause for concern. According to Shanker *et al*. (2009), a threshold level below 2% infection for cowpea seeds is recommended as suitable to avoid the risk of economic losses due to the spread of BCMV-BlCM in cowpea.

Seed-borne viruses can present a challenge to managing viral diseases in the fields and complicate the transfer of seeds by trade and other methods of seed exchange between farmers (Manyangarirwa *et al*., 2009; Ittah *et al*., 2010; Biemond *et al*., 2013). Recycling farmers' seeds for subsequent planting, as in the present study areas, may result in high virus incidence and significant yield loss (Owolabi *et al*., 1988).

In conclusion, this study demonstrated a high risk of seed-borne virus threat in the farmer-saved seed. It showed a need to improve awareness among farmers and extension agents about the risk of seed-borne virus infections and discourage farmers from reusing their seeds for long periods, particularly those harvested from infected fields. This study also calls for an increase in the supply of certified seed production that will serve as a sustainable solution to reduce the risk of BCMV-BlCM threat to cowpea production in Ghana.

## ACKNOWLEDGEMENTS

Authors acknowledge the West African Agriculture Productivity Program (WAAPP-Ghana) who funded the research work in Ghana, and the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, for supporting virus diagnostics research. Authors appreciate funding from CGIAR Research Program on Dryland Cereals and Legumes.

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***(Received on 24.11.2021, Revised on 09.12.2021 and Accepted on 11.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 261-270, 2021*

***Original Research Paper***

# Effect of container size and types on the root phenotypic characters of *Capsicum*

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## ABSTRACT

***Capsicum*** **genus comprised of several cultivars is considered as an important spice crop worldwide. Roots play a vital role in a plant to mine water from the deeper layers of the soil. Although, characterisation for root traits have been made using different containers in many crops, such efforts for phenotyping root characteristics in *Capsicum* species are limited. Therefore, the experiment was initiated to find out the influence of container size on root characteristics and also to identify the appropriate container for high throughput phenotyping of *Capsicum* species for desirable root characteristics. Nine genotypes belonging to different *Capsicum* spp. were grown in three types of containers having different dimensions. Among the three types of containers, the bucket type container with dimension of 32 cm height 30 cm diameter with 23 kg soil media capacity was most suitable for phenotyping root characteristics compared to PVC pipe and pot type. Subsequently, 18 genotypes were phenotyped for plant growth and root characteristics in the bucket type container. The genotypes IHR 4517, IHR 3529, IHR 4501, IHR 4550, IHR 4491 and IHR 3241 with better root characteristics were identified.**

**Key words**: Capsicum, container, root characteristics and plant growth

## INTRODUCTION

The genus *Capsicum* comprises several cultivars that are grown worldwide. In addition to their use as spices and food vegetables, *Capsicum* species have also been used in pharmaceutical industries. The genus *Capsicum* has five domesticated species, *Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz and Pav. However, among them, *Capsicum annuum* L. is distributed world over with greatest economic importance and is part of many dishes mainly because of its spicy taste, pungency, appealing colour and flavor. India is the world's largest producer and exporter of chilli, contributing about 25% of world's chilli production (National Horticultural Board, 2017).

Several abiotic stresses during critical stages of crop growth and development severely affect the productivity of *Capsicum* sp. inadequate water availability is a major abiotic stress which adversely affects growth and productivity of chilli crop (Bhutia *et al*., 2018). The major growing areas in India experience water limiting conditions due to limited water resources. In India in some parts, chilli is grown under rainfed conditions. The sporadic water stress is a common feature that causes considerable reduction in productivity of chilli, through modification in various morpho-physiological and bio-chemical processes (Singh, 1994). The antagonistic effects of water deficit stress have been studied by several workers in chilli (Cantore *et al.,* 2000; Kirnak *et al*., 2003; Antony and Singandhupe, 2004; Khan *et al*.,2008; Gunawardena and De-Silva 2014; R'Him and Radhouane, 2015; George and Sujatha, 2019).

Some of the plant's adaptive strategies under deficit water stress situations are; deep root system, higher water use efficiency (WUE) and tissue water retention through modifications in leaf, stomatal and cuticular characteristics (Basu *et al*., 2016). These adaptive features help plants to maintain higher tissue water content under deficit moisture stress and facilitate them to delay the imminent adverse effects of water stress. Roots play a major role under water deficit conditions by acquiring water from the deeper layers of the soil.

They also communicate with above ground parts through signaling pathways. The growth and development of plants is controlled through the alterations in root morphology and physiology. Modifications were noticed in root to shoot transport of signaling molecules including hormones, proteins, RNAs and mineral nutrients (DoVale and Neto, 2015).

The restricted growth and development of plants by limited water availability could be overcome through root morphological plasticity at different soil moisture levels (Forde 2009). Under water limited conditions, roots improve the ability of crop plants to maintain water relations by exploring available water in the soil profile. Identification of root characteristics that enhance the plant's capability to mine soil water and sustain productivity is very essential.Several workers have attempted studies on various root characteristics and have elucidated the role of root characteristics like deep root system (Sashidhar *et al*.,2000; Sinclair and Muchow 2001; Venuprasad *et al*., 2002), thick root system (Chang *et al*., 1986), root to shoot ratio (Fukai and Cooper 1995), enhanced root system (Price and Tomos, 1997), root penetrating ability (Ray *et al*., 1996) and higher number of roots in the crown region (Kinyua *et al*., 2003).

Understanding the role of roots in improving tolerance and maintenance of water relations under water limiting conditions is very important. In this direction quantification of the root characteristics and their role in enhancing water stress tolerance is of primary relevance. Conventional crop improvement approaches have played a principal role in many crops for enhancing drought tolerance (Sreenivasulu *et al*., 2007). The desirable root characteristics like, deeper root length, large root volume, high root dry weight, and higher root-to-shoot ratio coupled with thick lateral roots were observed to confer water stress tolerance in chilli germplasm IIHR 4502 (*Capsicum chinense*) (Naresh *et al*., 2017). Since, phenotyping root characteristics under field conditions are highly cumbersome and challenging, researchers have been relying on assessing the desirable root characteristics in container grown plants. Studies have also shown relationships between controlled-environment root vigor and field root vigor, indicating that evaluations at early stage are predictive of future root performance (Wasson *et al*., 2012). Using containers for measurement of root systems reduces the growing medium volume and enables proper removal of the root system as compared to plants grown in field (Neumann, 2009). There is a need for identification of suitable container type and size that provide congenial growing conditions for expression of genetic potential and also enable easy extraction of root system to phenotype root characteristics.

Though studies have been conducted to characterize root characteristics using different containers in many crops, such efforts for phenotyping root characteristics in *Capsicum* species are very much limited (Kulkarni and Phalke, 2009; Naresh et *al*., 2017). Hence, the objective of the study was to identify appropriate container and size for high throughput phenotyping of root characteristics which facilitate selection of genotypes having desirable root characteristics for water mining.

## MATERIAL AND METHODS

Experiment was carried out during 2018-2019 at the Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The experimental site is located at 13°58' N latitude, 78°E longitude and 890 m above mean sea level. Seeds of *Capsicum* sp. genotypes used in the study were obtained from the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru.

In order to achieve objectives of the study, two experiments were conducted. First experiment was carried out using three different containers to identify appropriate container for high throughput phenotyping of root characteristics. Second experiment was conducted to phenotype for desirable root characteristics using 18 genotypes belonging to different *Capsicum* sp. in the suitable container identified in the first experiment.

### Identification of appropriate container for high throughput phenotyping of root characteristics

In order to identify appropriate container for high throughput phenotyping of root characteristics, nine genotypes belonging to different *Capsicum* sp. IHR 3226, IHR 3455, IHR 3575, IHR 4517, IHR 3476 (*C. annuum*) IHR 3240, IHR 3241, IHR 4491(*C. baccatum*) and IHR 3529 (*C. chinense*) were selected. The genotypes were evaluated in three types of containers having different dimensions and soil media holding capacity. The containers used were: (i) bucket type container (Empty paint container, 30 cm diameter,

32 cm height having capacity to hold 23 kg soil), (ii) PVC pipe container (20 cm diameter, 64 cm height having capacity to hold 26 kg soil) and (iii) pot type container (18 cm diameter, 27 cm height having capacity to hold12 kg soil). The containers were filled with soil, Farm Yard Manure (FYM) and sand (2:1:1 v/v). The experiment was laid out in a factorial completely randomized block design with five replications.

**Phenotyping of *Capsicum* sp. genotypes in appropriate container for desirable root characteristics**

Eighteen genotypes belonging to different *Capsicum* sp. were evaluated for root characteristics in the bucket type container (30 cm diameter, 32 cm height having capacity to hold 23 kg soil). The experiment was laid out in a completely randomized block design with five replications.

**Seedling raising and crop care:** The seeds of genotypes used in both the experiments were sown in pro trays filled with coco peat as a growing medium. The seedlings were maintained in the shade net nursery for 45 days and recommended cultural practices were adopted to maintain plant health status and population. Forty-five-day old seedlings were transplanted into the containers. The plants were provided with recommended dose of fertilizer and crop protection measures. The plants were irrigated regularly to maintain 100 per cent field capacity.

**Growth parameters:** The observations in both the experiments were recorded at peak flowering stage (50 DAT). Plant height was measured using graduated scale and expressed in centimeters. The number of primary branches were counted manually at the point of initiation. The plant shoot parts were excised and the leaf and stem portions were separated. The entire root portion was carefully extracted from the soil medium using water jet to clean the soil. Soon after extracting the roots, observations on root parameters like root length (using graduated scale), root volume (water displacement method), number of primary roots and fresh and dry weights were recorded. Fresh weights of the root and shoot samples were measured immediately after extraction by using a Sartorius BSAZZAS-CW balance. The root, stem and leaf parts were dried in oven separately at 80°C for 72 h to achieve stable weight. The dry weight was recorded as total biomass accumulated and expressed as gram per plant.

To quantify the leaf area, representative sample of 20 leaves from each plant was taken and the leaf area was determined using leaf area meter (Biovis, PSM-L2000, India). Then the leaves were kept in oven at 70ºC for five days and leaf dry weight was measured using Sartorius BSAZZAS-CW balance. The ratio of leaf area to the leaf dry weight was computed as specific leaf area (SLA). The leaf dry weight of each plant was multiplied with SLA to arrive at the total plant leaf area (TLA).

**Root: shoot ratio:** It was arrived by dividing root dry matter with shoot dry matter.

**Statistical analysis**

ANOVA: The data obtained in different experiments was analyzed in factorial completely randomized block design and completely randomized block design for first and second experiment, respectively using two factors statistical package OPSTAT developed by CCSHAU (Sheoran *et al*.,1998).

## RESULTS AND DISCUSSION

Plants manifest physiological and morphological modifications in response to change with soil volume. The container size and type influence root volume and in turn determine the dry matter distribution between above and below ground parts. Studies have shown that with doubling in pot size there is an average increase of 43% plant mass (Poorter, 2012). Container size is known to influence morphological and physiological changes in crops like tomato (Oagile *et al*., 2016), bell pepper (Weston, 1988), squash (Nesmith, 1993) and cabbage (Csizinszky and Schuster, 1993). Alterations in container size leads to changes in available rooting volume which subsequently affects plant growth.

**Identification of appropriate container for high throughput phenotyping of root characteristics**

The container size plays a major role in plant root and shoots growth. The root length was not significantly influenced by the container type. However, among the three containers, higher root length was observed in PVC pipe container compared to bucket type and pot type containers. The root volume in bucket type container was 35.8% and 72.4% higher compared to pot type and PVC pipe containers, respectively (Figure 1). The studies conducted in bell pepper have shown that the container size has influence on the root volume and plant growth (Weston, 1988; Nesmith *et al*.,1992). In this experiment, among the three types of

containers, the plants grown in bucket type container produced significantly a greater number of primary roots (44.8) compared to pot type (33.1) and PVC pipe (25.4) containers (Figure 1). Studies conducted by Cantliffe, (1993) and Kharkina *et al*., (1999) have shown that there is a strong positive correlation between container size and root biomass. In the present study, significantly higher root fresh weight and dry weights were observed in bucket type container compared to other two types of containers (Figure 1). The genotypes IHR 4491, IHR 3241, IHR 4517 and IHR 3529 produced significantly higher root fresh weight as compared to remaining genotypes (Figure 1). Plants grown in bucket type container recorded 73.14 % (4.32 g) and 40.86% (5.31 g) higher root dry weight compared to PVC pipe and pot type containers (Table 1).

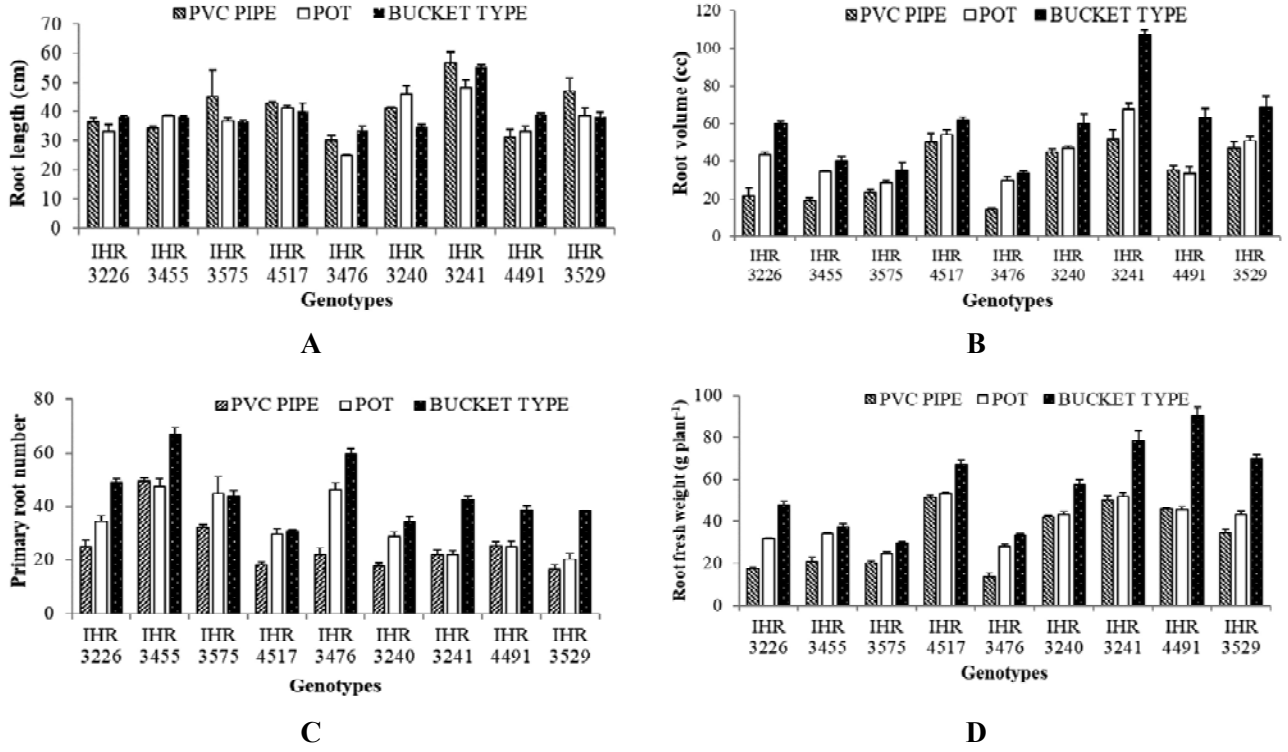


**Figure 1: Influence of containers on root length (A), root volume (B), primary root number (C) and root fresh weight (D) of *Capsicum* sp.**

Healthy root system growth promotes better above ground canopy growth. Hence, providing appropriate space for adequate root growth is essential. It is observed that the shoot growth is greatly impacted by varying container size and root restriction (Poorter, 2012). The plant height was significantly higher in bucket type container compared to remaining types of containers. Genotypes, IHR 3241 (68.1 cm) and IHR 3226 (57.2 cm) recorded significantly higher plant height compared to rest of the genotypes (Table 2). Tomato plants when grown in containers with low volume showed reduction in shoot height and biomass (Peterson *et al*., 1991). Hence, providing better rooting space helps the plants to produce higher above ground biomass with increased shoot height. Among the three types of containers, plants grown in bucket type produced significantly a greater number of branches compared to remaining two types of containers (Table 2). In bell pepper (*Capsicum annum* L.), root restriction caused reduction in number of branches (Nesmith *et al*.,1992). In container grown bell pepper plant, reduction in leaf area was observed mainly due to smaller and fewer leaves per plant (Weston, 1988; Nesmith *et al.,* 1992). With the increase in container size, the leaf area and shoot biomass has increased (Cantliffe, 1993). In this experiment, the leaf area was significantly higher in plants grown in bucket type container (5690 $cm^2$) as compared to pot (3797 $cm^2$) and PVC pipe (2690$cm^2$) containers (Table 2).

**Table 1: Influence of containers on root dry weight and shoot dry weight in *Capsicum* sp.**

| Genotype | Root dry weight (g plant$^{-1}$) | | | Shoot dry weight (g plant$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | PVC PIPE | POT | BUCKET TYPE | PVC PIPE | POT | BUCKET TYPE |
| IHR 3226 | 1.91 | 3.47 | 5.28 | 9.6 | 15.3 | 33.0 |
| IHR 3455 | 3.06 | 5.49 | 6.26 | 15.6 | 32.9 | 45.0 |
| IHR 3575 | 3.30 | 3.89 | 5.02 | 18.2 | 21.4 | 26.6 |
| IHR 4517 | 6.92 | 7.11 | 8.60 | 34.3 | 39.5 | 51.1 |
| IHR 3476 | 1.71 | 4.04 | 4.43 | 6.2 | 17.1 | 28.3 |
| IHR 3240 | 5.14 | 5.19 | 6.82 | 26.2 | 18.7 | 49.9 |
| IHR 3241 | 6.49 | 7.21 | 10.44 | 31.1 | 44.7 | 53.5 |
| IHR 4491 | 5.52 | 5.31 | 10.81 | 27.4 | 27.1 | 54.1 |
| IHR 3529 | 4.79 | 6.10 | 9.63 | 14.8 | 33.5 | 51.7 |
| Mean | 4.32 | 5.31 | 7.48 | 20.4 | 27.8 | 43.7 |
| Factors | G | C | GxC | G | C | GxC |
| C.D@0.05 | 0.65 | 0.38 | 1.13 | 3.2 | 1.85 | 5.54 |
| SE (m) | 0.23 | 0.13 | 0.4 | 1.12 | 0.65 | 1.95 |
| CV (%) | 10.8 | | | 11 | | |

**Table 2. Influence of containers on plant height, leaf area and number of branches in *Capsicum* sp.**

| Genotype | Plant height (cm plant$^{-1}$) | | | Leaf area (cm$^2$ plant$^{-1}$) | | | Branch number (no. plant$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | PVC PIPE | POT | BUCKET TYPE | PVC PIPE | POT | BUCKET TYPE | PVC PIPE | POT | BUCKET TYPE |
| IHR 3226 | 67.7 | 57 | 57.3 | 1363 | 2221 | 4526 | 12 | 9 | 13 |
| IHR 3455 | 40 | 44.3 | 54.7 | 2186 | 4510 | 6015 | 7 | 9 | 10 |
| IHR 3575 | 45.7 | 41.7 | 53.3 | 2706 | 3179 | 3977 | 9 | 10 | 12 |
| IHR 4517 | 49.3 | 38.3 | 47 | 4656 | 5263 | 6737 | 8 | 5 | 8 |
| IHR 3476 | 29.3 | 25 | 41.7 | 1161 | 3092 | 4797 | 3 | 4 | 7 |
| IHR 3240 | 46 | 52.7 | 52.3 | 2901 | 2209 | 5240 | 9 | 10 | 10 |
| IHR 3241 | 54.3 | 51.5 | 84.7 | 4389 | 6058 | 8365 | 9 | 9 | 11 |
| IHR 4491 | 32.3 | 29 | 71.3 | 2074 | 2040 | 4089 | 5 | 7 | 10 |
| IHR 3529 | 25.7 | 27 | 55.7 | 2773 | 5600 | 7467 | 4 | 5 | 6 |
| Mean | 43.4 | 40.7 | 57.6 | 2690 | 3797 | 5690 | 7 | 8 | 10 |
| Factors | G | C | GxC | G | C | GxC | G | C | GxC |
| C.D. (0.05) | 3.17 | 1.83 | 5.49 | 669 | 386 | 1159 | 0.88 | 0.5 | 1.52 |
| SE (m) | 1.11 | 0.64 | 1.93 | 136 | 235 | 408 | 0.31 | 0.18 | 0.53 |
| CV (%) | 6.8 | 17.4 | 10.8 | | | | | | |

**Table 3. Variability in root and shoot growth characteristics among 18 *Capsicum* sp. genotypes**

| Genotype | RL (cm plant$^{-1}$) | RV (cc plant$^{-1}$) | PRN (no. plant$^{-1}$) | RFW (g plant$^{-1}$) | RDW (g plant$^{-1}$) | SDW (g plant$^{-1}$) | Root: Shoot ratio | PH (cm plant$^{-1}$) | BN (no. plant$^{-1}$) | LA (cm$^2$ plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| IHR 3240 | 38.0 | 50.0 | 32 | 54.0 | 6.90 | 60.6 | 0.118 | 54.7 | 10 | 6284 |
| IHR 3241 | 55.0 | 100.0 | 43 | 69.2 | 10.63 | 51.2 | 0.211 | 79.7 | 10 | 8837 |
| IHR 4491 | 39.3 | 73.3 | 36 | 88.6 | 11.81 | 48.2 | 0.249 | 75.6 | 10 | 4455 |
| IHR 4550 | 54.3 | 110.0 | 45 | 127.7 | 15.38 | 42.9 | 0.412 | 68.0 | 6 | 6583 |
| IHR 4501 | 65.0 | 125.0 | 33 | 110.8 | 12.56 | 44.2 | 0.29 | 68.0 | 8 | 7630 |
| IHR 3529 | 48.7 | 71.3 | 36 | 76.0 | 10.23 | 36.9 | 0.268 | 54.7 | 7 | 7012 |
| IHR 4658 | 42.3 | 38.3 | 34 | 49.9 | 5.80 | 47.1 | 0.122 | 69.3 | 9 | 4724 |
| IHR 3982 | 33.3 | 18.3 | 14 | 24.6 | 2.01 | 19.7 | 0.346 | 48.3 | 13 | 3872 |
| IHR 3983 | 45.7 | 31.7 | 29 | 38.6 | 7.55 | 46.1 | 0.169 | 95.3 | 15 | 3302 |
| IHR 3226 | 35.0 | 38.3 | 47 | 30.5 | 3.78 | 40.0 | 0.096 | 58.0 | 12 | 5176 |
| IHR 3455 | 37.0 | 32.7 | 63 | 32.4 | 5.17 | 57.7 | 0.091 | 54.3 | 11 | 6922 |
| IHR 3575 | 33.3 | 30.0 | 41 | 29.5 | 4.13 | 25.2 | 0.164 | 52.0 | 12 | 3689 |
| IHR 4517 | 44.0 | 61.7 | 30 | 75.1 | 8.93 | 52.0 | 0.176 | 44.7 | 10 | 7936 |
| IHR 3476 | 30.7 | 35.0 | 60 | 32.2 | 4.45 | 29.8 | 0.15 | 41.3 | 7 | 4463 |
| IHR 3447 | 28.0 | 16.7 | 25 | 20.1 | 2.30 | 8.7 | 0.264 | 38.0 | 10 | 1854 |
| IHR 4108 | 42.7 | 44.0 | 46 | 35.1 | 3.40 | 54.1 | 0.065 | 71.7 | 10 | 4955 |
| Chikkaballapur Local | 42.0 | 30.0 | 19 | 28.6 | 3.13 | 15.8 | 0.206 | 52.0 | 11 | 1517 |
| Guntur Local | 42.3 | 29.3 | 31 | 41.0 | 6.70 | 53.6 | 0.125 | 72.7 | 14 | 6865 |
| C.D. (0.05) | 4.6 | 18.5 | 6.5 | 14.5 | 1.79 | 12.4 | 0.06 | 8.79 | 2.35 | 2914 |
| SE (m) | 1.6 | 6.4 | 2.2 | 5 | 0.6 | 4.3 | 0.021 | 3.05 | 0.82 | 1012 |
| CV (%) | 6.5 | 21.3 | 10.6 | 16.1 | 15.35 | 18.2 | 18.6 | 8.63 | 13.8 | 32.9 |

RL: Root length, RV: Root volume, PRN: Primary root number, RFW: Root fresh weight, RDW: Root dry weight, SDW: Shoot dry weight, PH: Plant height, BN: Branch number and LA: Leaf area Phenotyping of *Capsicum* genotypes for desirable root characteristics

Shoot growth is greatly impacted by varying container size and root restriction in tomato (Kemble *et al*., 1994) and soybean (Krizek *et al*.,1985). In this study, among the three types of containers, plants grown in bucket type container produced significantly higher amount of shoot biomass compared to remaining two types of containers. Plants in bucket type container produced 57.1% (15.9 g) and 114.2% (23.3 g) higher shoot biomass than plant grown in pot type and PVC pipe containers, respectively (Table 1). Therefore, the bucket type container with higher soil volume and area enabled the *Capsicum* spp. genotypes to express their genetic potential with higher shoot and root growth.

Roots, stems and leaves are functionally interdependent and these three systems maintain a dynamic balance in biomass production and distribution. It is clearly evident from the study that the bucket type container provided enough rooting space for *Capsicum* spp. genotypes to express their genetic potential in terms of shoot and root biomass production. Hence, the bucket type container was chosen for further studies on phenotyping *Capsicum* spp. genotypes for desirable root characteristics.

The importance of plant phenotyping based on specific root characteristics like root length, number of primary roots and root volume are of practical value for crop improvement (Garcia, 2015). Genetic potential of a genotype for root characteristics plays a critical role during growth and metabolic aspects of the plants. In this study, to know the genetic potential and behavior of each genotype under optimal moisture condition *Capsicum* sp. genotypes were evaluated for desirable root characteristics and shoot growth. The results clearly indicated that genotypes, IHR 4501, IHR 4491, IHR 3241, IHR 4550, IHR4517, IHR 3529 exhibited desirable root characteristics such as root length, root volume, primary root number, root fresh and dry weight. The genotypes, IHR 3982 and IHR 3447 showed poor root characteristics (Table 3). Studies have indicated that root length, root volume and root dry weight have strong positive correlation with total dry matter production (Lakshmamma *et al*., 2014). The genotypes which showed higher root length and volume also produced higher biomass because of adequate water and nutrients uptake from deeper layers of the soil and maintained the tissue water potential (Khan *et al*., 2008).

Under ample supply of water and nutrient, the plant height, leaf area, branch number and shoot biomass production are dependent on the size of the root system (Zakaria *et al*., 2020). Our results clearly demonstrated that genotypes, IHR 3241, IHR 4501, IHR 4491, IHR4517 and Guntur Local exhibited better shoot growth in terms of plant height, number of branches, leaf area and shoot biomass. The genotypes, Chikkaballapur Local, IHR 3447 and IHR 3982 showed poor shoot growth (Table 3). In fact; leaf area determines the light interception capacity of a crop and is often used as a surrogate for plant growth and above ground biomass. From the results it is clear that the genotypes having higher leaf area showed better shoot biomass. Concurrently, our results suggested that number of branches in a plant is independent with plant height. The branching pattern in a plant depends on the genetic makeup of each genotype and it is not linked with plant height and other characteristics. Similar observations were made in chilli (Bijalwan *et al*., 2018) and tomato (Malaker *et al*., 2016).

At optimal moisture condition, shoot and root dry weights are interred linked (Brdar-Jokanovic *et al*., 2014). Root to shoot ratio is an important index and it reflects the plant health status. In this regard our results confirms that genotypes, IHR 4550, IHR 4501, IHR 3529 and IHR 4491 recorded significantly higher root to shoot ratio compared to other genotypes. The genotypes, IHR 4108, IHR 3455 and IHR 3226 showed significantly lower root shoot ratio (Table 3).

Though enough rooting space was available in the bucket type container only few genotypes had higher shoot and root growth. This could be due to the genetic potential of the genotypes exhibiting higher root and shoot biomass (Chowdary *et al*., 2015). Based on the growth pattern with respect to root and shoot characteristics, six genotypes, IHR 4517 (*C. annuum*), IHR 3241 (*C. baccatum*), IHR 4491 (*C. baccatum*), IHR 4550 (*C. chinense*), IHR 3529 (*C. chinense*), IHR 4501 (*C. chinense*) were identified having desirable root characteristics and IHR 3447 (*C. annuum*) and IHR 3982 (*C. chacoense*) were identified having poor root characteristics.

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***(Received on 30.00.2020, Revised on 28.05.2021 and Accepted on 29.05.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 271-279, 2021*

***Original Research Paper***

# Physio-morphological and mechanical properties of chillies for mechanical harvesting

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## ABSTRACT

**The plants and its produce characteristics are the basis to design a crop specific harvester. The objective of this study was to determine the physical, morphological and mechanical properties of chilli plant and fruits, that can be used in the design of harvester machine. The observations and data were collected by taking measurements at harvesting stage of three chilli cultivars. The fruit bearing behavior of plants was solitary with fruit position erect in Demon $F_1$ and pendent in Arka Meghana and Mahyco Tejaswini. The plant height ranged between 81.76 to 84.87 cm depending on cultivars number of fruits per plant were 170.25, 158.96 and 156.15 in Tehaswini, Arka Meghana and Menon respectively. It was observed that the length and diameter at shoulder of fruits was in the range of 4.97 to 10.44 cm and 0.8 to 1.25 cm, respectively. The moisture content reduced in leaves, stems and fruits as the maturation changed from matured green fruits bearing of plants to semi dry condition. The detachment force of fruits from plants increased as the fruits colour changed from matured green to fully ripened red and there after decreased.**

**Keywords**: Chillies, erect, detachment force, mechanical harvester and pendent

## INTRODUCTION

Chilli is a seasonal vegetable that is part of the spicy food culture in India. Chilli (*Capsicum annuum* L.) belongs to the Solanaceae family (Farhan *et al*., 2014). It is well known for its edible, colourful, juicy and crispy flesh, as well as for its nutritious contents. Red chilli is an important commercial crop used as a condiment, culinary supplement or as a vegetable, physiological matured greens, ripened red color and red dried fruits. In India, among the spices consumed, dried chilies contribute a major share and grown in different agro-ecological zones and is the largest producer in the world. During 2019-20, India produced approximately 17.52 lakh tonnes of chillies from an area of 7.03 lakh ha and the productivity was 2.49 tonnes $ha^{-1}$ as per the report of Spice Board of India.

Chilli harvesting is not mechanized in the country and it depends entirely on the manual work force prolonging the extended period of field operation. The chilli fruit harvesting period occurs during the hot summer season, and the labour costs are very high, because the population residing in rural areas is decreasing and it is difficult to supply sufficient workforce to harvest in a timely manner. Therefore, mechanization of chilli harvesting is an urgent requirement to reduce the cost due to labour employed partly, faster operations at reduced drudgery and other production difficulties (Nam *et al.,* 2018).

To reduce mechanical damage due to harvest and post-harvest operations requires studies on the morphological, physical and mechanical properties of plants as well as fruits to design a harvesting machine. To optimize machines design and development parameters for operations such as harvesting, handling, cleaning and conveying, the morphological, physical and mechanical attributes and their

relationships play a major role (Rokayya and Khojah, 2016). Physical characteristics of agricultural crops, products are the most important parameters to determine the proper standards of grader design, conveying, processing and packaging systems (Tabatabaeefar and Rajabour, 2005). Several studies were conducted on pepper varieties in different countries, like Turkey (Ozgur *et al*., 2011; Kadri and Murat, 2010), Nigeria (Ilori *et al*., 2010), Thailand (Toontom *et al.,* 2012), Germany (Romano *et al*., 2012), Spain (Vega-Galvez *et al*., 2008), India (Nidhi *et al*., 2016) and Malaysia (Noryati and Revathi, 2006). Previous studies on chilli varieties and cultivars revealed that great variations existed in plant growth, other qualitative attributes and yield under different agro-climatic zones. In country like India, a large diversity in chilli with different quality factors and other traits is expected due to different agro ecological zones. Any developments in chilli harvesters should consider domestic cultivars and cropping systems because these are entirely different from exotic chilli varieties. Hence, more studies are required to collect data and standardize the design parameters pertinent to harvesting and post-harvest operational machines. So, the aim of the present work was to study morphological, physical attributes and mechanical properties of chilli plants and fruits of three most popular cultivars (hybrids) grown in southern states at different stages pertinent to harvesting, cleaning and grading machines.

## MATERIALS AND METHODS

The chilli cultivars selected for the present study were Arka Meghana, Mycho Tejaswini and Demon $F_1$ and cultivated as per the recommended agronomical practices at ICAR- Indian Institute of Horticultural Research, Bangalore. The observations and pertinent data collection study were carried out between 125 to 150 days after transplanting, at which the crop reached to full growth, maximum fruiting and harvestable ripped red fruits were present in considerable number. In the identified crop rows, 50 randomly selected plants from each cultivar were tagged and from each plant 100 fruits were plucked covering all directions and from fruit bearing lower to top branches. The instruments used in this study to measure linear dimensions were steel rule, digital caliper with an accuracy of 0.01 mm and fruits weight using a digital electronic balance with an accuracy of 0.01 g.

### Plant growth and morphological attributes

The plant growth habits and fruit bearing characteristics qualitative information was collected from reliable secondary sources of literature and characterization descriptors of IPGRI (1995). The plant growth attributes were measured when 100 per cent of the plants had at least certain proportion of fully ripped fruits. These attributes include plant height (cm), plant canopy spread across (cm) and along the row (cm), stem diameter (cm), stem length (cm), height of the lower most (cm) and upper most chilli fruit (cm) from ground. Plant height was measured from the ground surface to the uppermost tip of the plant using the steel rule. Plant stem girth measured at ground level and stem length was measured from the soil surface to the first internode of primary branch. The total number of fruits per plant was calculated by noting down harvested fruits at every picking from selected 50 plants. The moisture contents of three major portions of plants namely leaves, stems and fruits at different stages of fruits ripeness was collected randomly and estimated as per the standard laboratory drying procedure.

### Geometrical and physical properties of fruits

The geometrical and physical properties of fruits measured were length, diameter just below the calyx part where fruit is maximum in diameter and weight of 1000 fruits.

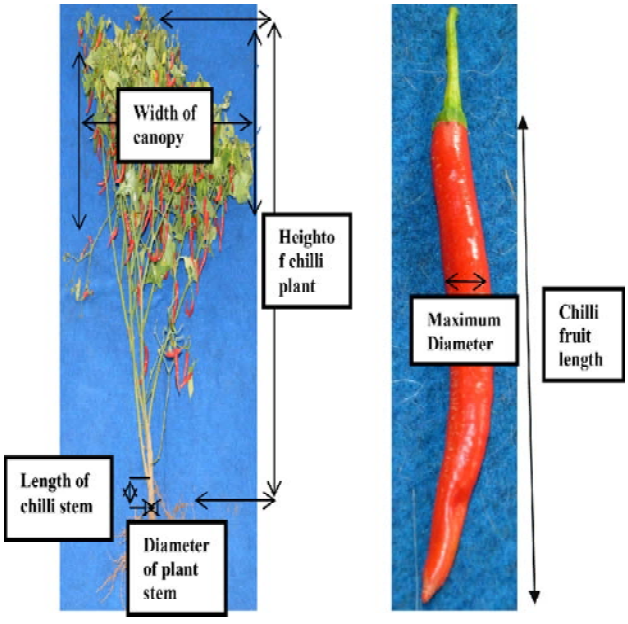


**Fig. 1. Measurement standard for chilli plant and fruits**

**Moisture content of plant parts**

As the fruits and vegetables changes from physiological maturity to full ripeness, the moisture content of various plant parts like leaves, stems and branches may change in addition to changes in textural property. This property plays a major role in harvesting, especially fruits moisture content has profound effect beyond harvesting operation. The moisture contents were measured using oven dry method at different ripeness stages of fruits by collecting samples from different plant parts randomly. The moisture content was determined by using standard procedure of AOAC (1970).

**Detachment force/ pulling force of chilli fruit**

The force of detachment or pulling force of fruit to separate from the chilli plants was measured using digital force gauge (Fig. 2), which can measure maximum 50 Newton (N). The digital force gauge used for the experiment was Model No. SF-50, maximum load 50N and the least count of the instrument was 0.01N. The push and pull type digital force gauge was held with one hand one side

**Fig. 2. Images showing fruit detachment force with digital force gauge**

hook and the other side hook was connected to the chilli fruit pedicle and the applied maximum force was noted from the display on the screen. For each cultivar detachment force of fruits was measured at four stages (i.e.) green, semi red, red/ fully ripped, partly dry and full dry condition (Fig.3).

The statistical analysis was carried out for the each observed character under the study using MS-Excel. The mean values of data were subjected to analysis of variance as described by (Gomez and Gomez, 1984).

Green Semi-red Red Semi-dry Dry

**Fig. 3. Image shows different stages of Arka Meghana cultivar**

## RESULTS AND DISCUSSION

### Plant growth characteristics

The three cultivars selected for the study were dual purpose (i.e.) useful as green vegetable and as well as dried red chilli. The Arka Meghana had branches spreading growth type and rest of two semi-spreading in nature either sparse or intermediate dense (Table 1). The stem shape was found to be round for all three. The fruits shape was determined based on comparison with the shapes proposed in the list of descriptors of the IPGRI (1995), the Arka Meghana and Mahyco Tejaswini possess elongate shaped fruits and Demon $F_1$ erect narrow fruits. Based on fruit position the cultivars fall in two groups *viz*., Demon $F_1$ erect position fruits and remaining two in pendent position. As per the fruit bearing characteristic all the three falls in solitary behaviour.

The plant growth, branching pattern, physical structure of fruits and other biological features have a significant impact on machine harvesting efficiency. Low plant structure and small branch angles make positive impact on machine harvesting efficiency. High canopy density vegetable crops need vigorous shaking of the branches by harvesting devices, which causes the high quantity of foreign material like tender branches, twigs in the harvested produce. This cause makes the quality produce separation process more energy intensive, because of necessary additional strength required for the mechanism to separate and transmit the unwanted material out of the harvesting machine.

**Table 1. Plant growth characteristics of selected three cultivars of chilli**

| Characteristics | Arka Meghana | Tejaswini | Demon $F_1$ |
|---|---|---|---|
| Utility - Green / Dried red / Dual purpose | Dual purpose | Dual purpose | Dual purpose |
| Plant growth habit | Medium height and spreading | Medium height and Semi spreading | Medium height and Semi spreading |
| Branching habit | Dense | Sparse | Intermediate |
| Stem Shape | Round | Round | Round |
| Fruit shape | Elongate | Elongated | Erect narrow |
| Fruit position | Pendent | Pendent | Erect |
| Fruit bearing | Solitary | Solitary | Solitary |

**Arka Meghana** **Tejaswini** **Demon $F_1$**

**Fig. 4. Fruit shape and fruit position of different cultivars of chilli**

**Chilli cultivars morphological attributes**

The distance from the ground level to the upper most tip of the plant is measured as the height of the plant. The average height of the plants was found as 82.20 cm, 84.87 cm and 81.76 cm for Arka Meghana, Mahyco Tejaswini and Demon $F_1$, respectively (Table 2). The height of the plants varied from 64 to 115 cm and maximum height 115 cm observed in Demon $F_1$ and minimum of 64 cm in Arka Meghana.

**Plant canopy width across and along the rows**

The minimum and maximum distances between the tips of the lengthiest branches spread in the tagged plant samples across the row (canopy width in East- West direction) ranged from 59 to 98 cm and along the row (canopy width in North - South direction) from 56 to 93 cm in Mahyco Tejaswini. Generally, in crops sown in rows, the harvesting machine being operated along the rows, so the spread width of canopy across the row plays a critical role in deciding the harvester head size to cover entire canopy for maximum harvesting efficiency.

**Plant stem length, stem diameter and number of fruits per plant**

The stem lengths of the chilli cultivars varied from 2 cm to 13 cm and the mean value of stem lengths recorded varied from 5.44 to 8.99 cm. The higher mean stem length to first bifurcation was recorded in Demon $F_1$. The stem diameter of the chilli cultivars varied from 1.82 to 2.16 cm. The plant stem diameter is higher 2.16 cm in Arka Meghana and lower in 1.82 cm in Demon $F_1$. The minimum and maximum number of fruits per plant ranged from 61-343 number for Demon $F_1$ with lowest mean value of 156.15 number of fruits per plant. Among the three cultivars, a maximum mean value 170.25 fruit per plant was recorded for the Mahyco Tejaswini.

**Table 2. Morphological characteristics of different chilli cultivars**

| Characteristics | Plant height (cm) | Across row EW (cm) | Along row NS (cm) | Plant stem length (cm) | Stem dia meter (cm) | Number of fruits per plant | Height of the lower most fruit (cm) | Height of the upper most fruit (cm) |
|---|---|---|---|---|---|---|---|---|
| **Arka Meghana** | | | | | | | | |
| Mean | 82.20 | 79.55 | 76.18 | 6.35 | 2.16 | 158.96 | 21.46 | 84.71 |
| Minimum | 64.00 | 63.00 | 59.00 | 3.00 | 1.60 | 76.00 | 10.00 | 62.00 |
| Maximum | 108.00 | 97.00 | 93.00 | 13.00 | 2.72 | 243.00 | 31.00 | 100.00 |
| Standard deviation | 9.28 | 7.46 | 7.90 | 1.90 | 0.63 | 39.91 | 4.24 | 10.43 |
| Standard error | 0.92 | 0.74 | 0.79 | 0.19 | 0.06 | 3.97 | 0.80 | 1.97 |
| **Mahyco Tejaswini** | | | | | | | | |
| Mean | 84.87 | 75.16 | 72.17 | 5.44 | 1.90 | 170.25 | 20.83 | 67.66 |
| Minimum | 69.00 | 59.00 | 56.00 | 2.00 | 1.06 | 73.00 | 12.00 | 65.00 |
| Maximum | 108.00 | 98.00 | 93.00 | 11.00 | 2.52 | 234.00 | 32.00 | 98.00 |
| Standard deviation | 14.98 | 11.35 | 1.99 | 1.99 | 0.34 | 36.47 | 4.71 | 18.14 |
| Standard error | 1.50 | 1.14 | 1.10 | 0.23 | 0.04 | 4.27 | 0.87 | 3.37 |
| **Demon $F_1$** | | | | | | | | |
| Mean | 81.76 | 73.40 | 63.89 | 8.90 | 1.82 | 156.15 | 28.11 | 98.56 |
| Minimum | 65.00 | 60.00 | 57.00 | 4.00 | 1.44 | 61.00 | 17.00 | 90.00 |
| Maximum | 115.00 | 92.00 | 88.00 | 13.00 | 2.68 | 343.00 | 36.00 | 112.00 |
| Standard deviation | 9.52 | 9.47 | 9.67 | 1.85 | 0.31 | 50.28 | 6.29 | 7.02 |
| Standard error | 0.94 | 0.93 | 0.95 | 0.18 | 0.03 | 4.93 | 2.10 | 2.34 |

**Height of the lower most and higher most fruits bearing branches**

Though there is not much considerable variation in the mean plant heights among the three cultivars, but considerable variation was observed in fruits bearing canopy zone lengths. The fruits bearing canopy spread height was maximum (70.45cm) for Demon $F_1$ and the least 46.83cm for Mahyco Tejaswini. The average height of the lowermost chilli fruits bearing was observed 20.83 cm in Mahyco Tejaswini and highest value 28.11 cm in Demon $F_1$.

**Fruits geometrical and physical properties**

The size and shape of fruits play major role in separation of unwanted biomass and also immature harvested ones from the quality produce and otherwise more prone to storage disease in crop like chillies. The fruit shape description of chilli grown for dual purpose use in India is difficult, however in general it is triangular in shape with obtuse truncated shape pedicel attachment portion and blunt sunken at blossom end portion. Maturation is indicative of the fruit being ready for harvest and after full maturation, there will not be much change in fruit size and shape, since the edible part of the fruit or vegetable is fully developed. Dependence on colour parameter alone to harvest the matured vegetables at green colour stage may mislead in certain vegetables. Rather than decision taken based on fruit size, shape and colour may yield best results. Apart from that, fruits and vegetables geometrical parameters like length, width, thickness or diameter will give us an idea to design and develop sieve set to separate the discard able biomass from produce and graded marketable produce based on size.

In chilli the total fruit length and diameter at shoulder are two geometrical dimensions, based on which the separation and grading of produce equipment could be planned. The fruit length measured without pedicle for the selected chilli crops ranged from 2.60 to 14.70 cm and for the same the mean length values varied 4.97 to 10.44 cm. The maximum mean fruit length was observed in Arka Meghana (10.44 cm) and minimum value 4.97 cm in Demon $F_1$.

**Fruit diameter and 1000 fruits weight**

In certain fruits the shape can change during maturation and can be used as a characteristic to determine harvest maturity. As the fruit or vegetable matures on the plant the relationship between the shoulders of the fruit and the point at which the stalk is attached may change. The shoulders of immature ones slope away from the fruit stalk and on full maturity the shoulders become level with the point of attachment, and in certain cases the shoulders may be raised above this point also.

As per the forgone discussion, in chilli the size of fruits is maximum at shoulders, so the diameter was measured at this point. For the selected chillies, overall fruit diameter varied from 0.51 to 1.58 cm and the mean values were ranged from 0.80 to 1.25 cm (Table 3). The maximum values in all respects were observed in Arka Meghana and minimum in Demon $F_1$.

The weight of 1000 ripened chilli fruits widely ranged from minimum 1.24 kg to maximum 9.21 kg. The mean weight of 1000 ripped fruits was 1.96 to 6.97 kg. The maximum 1000 chilli fruits weight was recorded in Arka Meghana 6.97 kg and minimum value in case of Demon $F_1$ (1.96 kg).

**Table 3. Ripened chilli fruits geometrical and physical properties**

| **Cultivars** | **Arka Meghana** | | | **Mahyco Tejaswini** | | | **Demon $F_1$** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Properties** | **Fruit length (cm)** | **Fruit dia meter (cm)** | **1000 fruits weight (kg)** | **Fruit length (cm)** | **Fruit dia meter (cm)** | **1000 fruits weight (kg)** | **Fruit length (cm)** | **Fruit dia meter (cm)** | **1000 fruits weight (kg)** |
| Mean | 10.44 | 1.25 | 6.97 | 7.74 | 0.92 | 3.81 | 4.97 | 0.80 | 1.96 |
| Minimum | 6.20 | 0.81 | 4.13 | 4.30 | 0.69 | 2.40 | 2.60 | 0.51 | 1.24 |
| Maximum | 14.70 | 1.58 | 9.21 | 9.80 | 1.18 | 5.43 | 10.50 | 1.05 | 2.98 |
| Standard deviation | 2.00 | 0.16 | 1.02 | 1.30 | 0.09 | 0.64 | 1.06 | 0.11 | 0.30 |
| Standard error | 0.20 | 0.02 | 0.10 | 0.13 | 0.01 | 0.08 | 0.10 | 0.01 | 0.03 |

**Moisture content**

The moisture contents data of different plant parts at different ripeness stages was presented in Table 4. At full matured green stage of fruits, the moisture content of the leaves was about 72% (db) and fruits possessed considerably higher amount of moisture about 80%.As the fruits maturation changes from physiological mature green colour to full red and beyond, all the plant parts namely leave, stems and fruits moisture contents decreased. When compared to other parts, the per cent of moisture loss was more rapid in leaves followed by stems and minimum gradual reduction was observed in fruits. The moisture content trend is more or less same in all the three cultivars.

Moisture content is an influential factor in all the crop processing operations and greatly influences other physical and mechanical properties (Ilori *et al.,* 2010). In harvesting stage of crops, excessive loss of moisture may lead to the structural parts of the plant to become softer. The softer plant parts cling to the rotating or oscillating or jolting components which shake or vibrate or comb or push the plant branches reducing its effectiveness thus reducing harvesting efficiency of the fruits and vegetables. In certain species, reduced moisture contents in plant parts result in excessive detachment of leaves, twigs in considerable quantity thus increasing energy expenditure in cleaning and grading unit of harvesting machine.

**Table 4. Moisture content of plant parts at different maturity stages of different cultivars**

| Plant part | Green | Semi-red | Red | Semi-Dry | Dry |
|---|---|---|---|---|---|
| **Arka Meghana** | | | | | |
| Leaf | 71.33±2.08 | 54.04±0.47 | 40.78±0.64 | 33.63±0.64 | 20.53±0.87 |
| Stem | 68.90±0.87 | 66.54±0.76 | 49.15±0.31 | 46.15±0.75 | 39.71±1.27 |
| Fruit | 81.29±2.85 | 84.35±0.81 | 77.86±0.31 | 79.77±0.78 | 72.70±0.82 |
| **Mahyco Tejaswini** | | | | | |
| Leaf | 72.17±2.13 | 62.96±2.63 | 48.66±1.16 | 33.33±1.53 | 18.23±1.09 |
| Stem | 63.69±2.29 | 54.45±1.27 | 53.83±2.40 | 53.16±1.04 | 38.05±2.10 |
| Fruit | 77.86±0.31 | 76.72±0.46 | 74.85±0.17 | 74.76±1.57 | 72.17±1.02 |
| **Demon $F_1$** | | | | | |
| Leaf | 71.78±1.65 | 54.16±2.13 | 45.59±0.94 | 33.30±1.20 | 18.21±1.20 |
| Stem | 60.85±1.57 | 58.83±1.22 | 52.87±1.57 | 44.12±0.58 | 37.12±1.50 |
| Fruit | 77.58±0.59 | 76.96±0.93 | 74.79±1.40 | 72.49±0.69 | 71.04±1.72 |

**Detachment force of chilli fruits at different stages of ripeness**

The principles dictating at what stage of maturity the fruits or vegetable should be harvested are crucial to its subsequent drying /storage and marketable life and quality. Post-harvest physiologists distinguish different important stages in the life span of fruits and vegetables namely maturation (green), semi – ripeness, ripened, semi dried, dried and crop senescence (ageing) itself. All these stages have its own importance depending on how and where the produce being used and strategies being followed in collection, transportation, storing and marketing. Ripening follows or overlaps maturation, rendering the produce edible, as indicated by colour and taste in majority of fruits and vegetables. In certain crops plant senescence (ageing) also considered as indicative of crop harvest. Senescence is the last stage, characterized by natural degradation of the plants, as in loss of texture, colour, etc. In case of certain fruits and vegetables colour and moisture content are two majorly determining factors to harvest the produce that are to be dried to preserve for round the year use as it is or in size reduction form with or without pre-treatment.

Ripening stage has an important effect on the force required for removal or detachment of fruits or vegetables or nuts from the branches of plants or trees and on relative susceptibility to mechanical damage. Some researchers reported that the holding force of fruits and vegetables to pedicle decreased as the fruit

**Table 5. Force (N) required to detach chilli fruit at different growth stages**

| Different stages | Arka Meghana | | | | | Mahyco Tejaswini | | | | | Demon $F_1$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Green (N) | Semi red (N) | Red (N) | Semi Dry (N) | dry (N) | Green (N) | Semi red (N) | Red (N) | Semi dry (N) | Dry (N) | Green (N) | Semi red (N) | Red (N) | Semi Dry (N) | Dry (N) |
| Mean3.45 | 4.07 | 5.85 | 2.36 | 1.08 | 2.43 | 2.11 | 4.92 | 1.52 | 0.87 | 5.19 | 6.39 | 8.58 | 1.98 | 0.98 | |
| Minimum | 0.78. | 1.04 | 1.90 | 0.39 | 0.75 | 0.99 | 0.45 | 1.23 | 0.50 | 0.69 | 1.18 | 1.02 | 1.20 | 0.93 | 0.78 |
| Maximum | 9.86 | 11.30 | 17.49 | 6.21 | 1.43 | 8.79 | 5.80 | 13.18 | 4.47 | 1.13 | 13.82 | 12.21 | 15.65 | 5.95 | 1.32 |
| Standard deviation | 2.11 | 2.19 | 2.80 | 1.51 | 2.32 | 1.53 | 0.93 | 2.23 | 0.80 | 0.95 | 2.31 | 2.21 | 2.07 | 1.15 | 1.76 |
| Standard error | 0.19 | 0.22 | 0.28 | 0.15 | 0.19 | 0.15 | 0.09 | 0.22 | 0.08 | 0.14 | 0.21 | 0.22 | 0.21 | 0.11 | 0.34 |

matured, due to cork that is formed in the stem holding place. The detachment force required to pluck the fruits of selected cultivars of chillies at various stages of ripeness is presented in Table 5. The results indicates that, the detachment force increased as the fruits maturation increased from green to full red and there after it decreased. This may be due to the fact that up to full maturation of fruits, pedicle contains more fibre content compared to remaining stages and at dry stage pedicle contain less fibre content. It was observed that, the average force required to pluck the chillies at green stage for Arka Meghana, Mahyco Tejaswini and Demon $F_1$ was found to be 3.45± 2.11 N, 2.43±1.53 N and 5.19± 2.31 N, respectively. Similarly, in fully ripped red stage maximum plucking force noted, 5.85± 2.80N, 4.92± 2.23N and 8.58± 2.07N, respectively.

The data also indicates that, specifically the cultivars having pendent position fruits have recorded lower plucking force than erect position. These observations concur with the findings of Funk and Walker (2010), pendant fruit position with minimum fruit attachment force in green chilli genotypes aides for better mechanical harvesting. When mechanical harvesting components designs involving working principles such as rotating, oscillating, push-pull, combing and jolter actions are employed to harvest fruits and vegetables; fruit/ vegetable detachment force, size properties, mass and puncture property against mechanical damages must be known. Polat *et al.,* (2010) reported that for pistachio nut the pod detachment force decreased from 436 to 118 N within 100 days prior to harvesting to harvest date of partially dried nuts.

## CONCLUSIONS

Red chilli is an important commercial crop, besides its wide spread use in Indian food culture. However, the fruits harvesting still being carried manually at increased harvesting costs and in hot weather conditions. So, important morphological attributes, physical and mechanical properties of three popularly grown cultivars were studied to provide an idea about these for harvester developers, researchers. The results have revealed the importance of the difference among cultivars, while designing and manufacture of machines. These properties are highly useful in harvesting machine development and as well as post harvesting like cleaning and grading equipment.

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***(Received on 10.11.2021, Revised on 15.12.2021 and Accepted on 31.12.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 280-286, 2021*

***Original Research Paper***

# Assessment of soil and water quality status of rose growing areas of Rajasthan and Uttar Pradesh in India

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## ABSTRACT

**Rose is a commercial flower crop widely grown across India. It is highly sensitive to salinity and alkalinity. In the process of identification of salt and alkalinity resistant rootstocks of rose cultivars, a survey was conducted in the rose growing areas of Uttar Pradesh (UP) and Rajasthan. Total of 28 representative surface soil samples were collected from rose fields of these regions, processed and analyzed for the soil quality parameters. Similarly water samples (20 samples) from the bore wells of these fields were collected and analyzed. The results revealed that most of the soils of rose growing fields in UP were alkaline (pH >8.0) with normal salt content (electrical conductivity, EC < 0.5 dS $m^{-1}$). Many of these soils also had higher bicarbonates (> 3 meq 100 $g^{-1}$). In case of Rajasthan, few samples had higher pH, EC, chloride (>2 meq 100 $g^{-1}$) and bicarbonate contents. Exchangeable sodium percentage (ESP) of UP and Rajasthan samples ranged from 5.21-20.7% and 2.94-24.9%, respectively. In case of water parameters in these areas, pH was slightly in alkaline range, EC of some of the samples were high (>1 $dSm^{-1}$). Sodium content was slightly higher than other cations. Soluble sodium percentage (SSP) of water samples was also slightly higher than normal range (0-50%). Few samples had slightly higher chloride above the threshold limit. From the results, it is concluded that soil and water quality of the rose growing areas of UP and Rajasthan is marginal and proper management/reclamation measures need to be carried out for sustaining the production system.**

**Keywords**: Rajasthan, rose, Soil quality, Uttar Pradesh and water quality

## INTRODUCTION

Rose (*Rosa* spp.) is one of the most economically important ornamental crops in the world. Increasing demand for cut-flowers both in domestic and export markets encouraged many entrepreneurs to enter into the commercial cultivation of roses. Rose has been traditionally categorized as a salt-sensitive species with salt injury reported within a range of 0.5 to 3 dS $m^{-1}$ electrical conductivity (EC) depending on species, cultural medium, leaching fraction, and environmental conditions (Urban, 2003). Bernstein *et al*. (1972) classified roses as having very poor tolerance to salinity with a 25-50% decrease in shoot growth at electrical conductivity values in the saturation extract (ECe) between 2 and 3 dS $m^{-1}$, and experiencing lethal effects at $EC_e$ of 4 dS $m^{-1}$. In green houses electrical conductivity levels will increase significantly as roses are irrigated with water soluble fertilizers. High content of salts affect the plants by reducing water availability to the plants and by specific ion toxicity of Na, Cl, B, *etc*.

As the availability of good quality water has become scarce, farmers are using poor quality water with high salt content and ground water from deep layers of borewells which contain high amounts of bicarbonates for rose cultivation. The poor quality water affects the pH and EC of the growing medium which inturn affects the nutrient availability to the plants. High bicarbonate content in soil affects soil pH and affects availability of micronutrients especially iron. This bicarbonate induced iron deficiency or iron chlorosis results in poor flower yield and quality. The high bicarbonate ($HCO_3$-) concentration and associated high pH of irrigation water is detrimental to plant growth, due to its adverse effects on availability and solubility of nutrients (Marschner, 1995). By application of phosphoric and sulfuric acids through fertigation, many polyhouse units try to control the pH. This is a costly, cumbersome and unsafe practice. Sustainable rose production will have to incorporate

economically feasible and environmentally sound solutions to problems associated with high levels of salts and $HCO_3$- in irrigation water. One of the ways to manage this problem is to use resistant varieties or rootstocks. Though there are good number of studies on rootstocks for high pH in other countries, the work on this aspect in India is scanty.

The area under salinity and alkalinity problems in Rajasthan is 1,95,571 ha and 1,79,371 ha, respectively. Similarly 21,989 ha of cultivated land is affected with salinity problems and 13,46,971 ha of land is affected with alkalinity problems in Uttar Pradesh (Mandal *et al.,* 2011). Rose is being cultivated in 1342 ha- in Rajasthan (Shekhawat, 2012) and 612 ha- in Uttar Pradesh (Sachan *et al.,* 2014). The present investigation was conducted to assess the soil and water quality status of rose growing areas of Rajasthan and Uttar Pradesh as a preliminary study for collection of rose germ-plasm for screening to tolerance of salinity and alkalinity problems of soil and water.

## MATERIALS AND METHODS

Investigative surveys were conducted in Udaipur, Haldighati, Sirohi, Pali and Jodhpur areas of Rajasthan during October, 2017 and in Lucknow, Kannauj, Etah, and Aligarh areas of U.P. during January, 2018. Representative soil and water samples were collected from rose fields to assess quality status with respect to rose cultivation. About 28 surface soil samples and 20 water samples from these regions have been collected and analyzed for quality parameters. Soil samples were analyzed for pH using glass electrode and EC using conductivity meter in 1:2.5 soil: water suspension (Richards, 1954). The exchangeable Na, K, Ca and Mg in the soils were analyzed using neutral normal ammonium acetate extraction method (Chapman 1965). Soluble bicarbonate and chloride content in the soil were analyzed by titration method (Richards, 1954). Exchangeable sodium percentage (ESP) of soil was calculated using the Equation 1 as given below (Richards, 1954).

$$ESP(\%) = \text{Exchangeable } \{(Na)/(Ca + Mg + K + Na)\} \times 100 \quad \text{.....Eq.(1)}$$

Similarly water samples have been analyzed for pH and EC using pH meter and conductivity meter (Richards, 1954). Na, K, Ca, Mg, $HCO_3$ and Cl were analyzed following standard analytical procedures (Richards, 1954). Sodium adsorption ratio (SAR) of water samples had been calculated by adopting the following equation (Richards, 1954).

$$SAR\ (me/L)^{1/2} = \{(Na) / [(Ca + Mg)/2]^{1/2}\} \quad \text{.....Eq.(2)}$$

Soluble sodium percentage was also calculated adopting equation 3(Richards, 1954).

$$SSP\ (\%) = \{(Na + K) / (Ca + Mg + K + Na)\} \times 100 \quad \text{.....Eq.(3)}$$

All the data were introduced to descriptive statistics for arithmetic mean and co-efficient of variation calculation.

## RESULTS AND DISCUSSION

### Soil quality parameters

Soil reaction in the study areas was found to slightly alkaline to highly alkaline range. The soil pH ranged from 7.83-9.34 in U.P with an average value of 8.55 (Table 1) and 7.18-8.42 in Rajasthan (average 7.91) (Table 2). The EC ranged from 0.12-0.76 dS $m^{-1}$ which was normal range in UP soils, whereas in Rajasthan soil it ranged from 0.14-4.59 dS $m^{-1}$, mostly under normal range but few samples had higher EC particularly in Haldigati and Pali areas. The exchangeable cations Na, K, Ca and Mg in the U.P soils ranged from 174-730 mg $kg^{-1}$, 48-228 mg $kg^{-1}$, 1109-2526 mg $kg^{-1}$ and 369-548 mg $kg^{-1}$, respectively. In Rajasthan, the corresponding values were 128-1575 mg $kg^{-1}$, 65-367 mg $kg^{-1}$,1289-2923 mg $kg^{-1}$ and 289-508 mg $kg^{-1}$, respectively. The results showed some soil samples had higher exchangeable sodium. The same had been reflected in the ESP of the respective soils. Soils of U.P had 5.21-20.7% ESP (mean 8.65%) and soils of Rajasthan had 2.94-24.9% ESP (mean 9.52%). This showed that many soils had ESP above the limit of 6% ESP, that reflect prevalence of alkalinity problems in the study area. The exchangeable sodium percentage (ESP) measures the proportion of cation exchange sites occupied by sodium. Soils are considered sodic when the ESP is greater than 6, and highly sodic when the ESP is greater than 15 (Tim *et al.,* 2019). This showed that many rose growing farms are having sodicity problems in Uttar Pradesh and some in Rajastan. Further bicarbonate content of soils were also high

**Table 1. Soil quality parameters of rose growing areas of Uttar Pradesh**

| S.No. | Location | pH | EC (dS $m^{-1}$) | $Na^+$ (mg $kg^{-1}$) | $K^+$ (mg $kg^{-1}$) | $Ca^{2+}$ (mg $kg^{-1}$) | $Mg^{2+}$ (mg $kg^{-1}$) | $HCO_3^-$ (meq 100 $g^{-1}$) | $Cl^-$ (meq 100 $g^{-1}$) | ESP (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lucknow-1 | 8.44 | 0.21 | 179 | 147 | 1800 | 462 | 3.1 | 0.4 | 5.56 |
| 2 | Lucknow-2 | 8.34 | 0.38 | 323 | 216 | 2526 | 539 | 2.5 | 1 | 7.36 |
| 3 | Basheerpur-1 | 8.61 | 0.12 | 175 | 48 | 1344 | 450 | 2.3 | 0.6 | 6.70 |
| 4 | Basheerpur-2 | 8.23 | 0.31 | 187 | 69.3 | 1109 | 369 | 3.2 | 1.2 | 8.46 |
| 5 | Narora-1 | 9.34 | 0.23 | 363 | 103 | 2006 | 473 | 2.0 | 3.6 | 9.98 |
| 6 | Narora-2 | 8.59 | 0.37 | 278 | 206 | 1694 | 436 | 0.3 | 4.0 | 8.73 |
| 7 | Sarkari-1 | 8.22 | 0.39 | 212 | 228 | 1797 | 548 | 0.5 | 4.0 | 6.12 |
| 8 | Sarkari-2 | 8.23 | 0.31 | 187 | 69.3 | 1109 | 369 | 0.3 | 2.4 | 8.46 |
| 9 | Jagdevpura-1 | 8.91 | 0.38 | 463 | 127 | 1417 | 479 | 3.5 | 10 | 15.0 |
| 10 | Jagdevpura-2 | 9.24 | 0.76 | 730 | 195 | 1577 | 452 | 3.9 | 2.4 | 20.7 |
| 11 | Safedpura-1 | 8.56 | 0.23 | 174 | 199 | 1862 | 472 | 3.6 | 3.6 | 5.21 |
| 12 | Sagedpura-2 | 8.40 | 0.25 | 215 | 110 | 1435 | 526 | 3.0 | 0.6 | 7.32 |
| 13 | Safedpura-3 | 8.45 | 0.20 | 174 | 199 | 1862 | 472 | 0.5 | 1.2 | 5.21 |
| 14 | Hapur-1 | 7.83 | 0.67 | 198 | 134 | 1475 | 431 | 3.2 | 1.4 | 7.07 |
| 15 | Hapur-2 | 8.83 | 0.27 | 250 | 193 | 1684 | 471 | 0.5 | 0.6 | 7.80 |
| Mean | | 8.55 | 0.34 | 274 | 150 | 1646 | 463 | 2.2 | 2.5 | 8.65 |
| CV (%) | | 4.67 | 50.9 | 55.3 | 40.1 | 22.1 | 11.1 | 63.1 | 100 | 47.7 |

in some soil samples (>2 meq 100 $g^{-1}$) and it ranged from 0.3-3.9 meq 100 $g^{-1}$ (mean 2.2 meq 100 $g^{-1}$) in UP and 0.3-10.1 meq 100 $g^{-1}$ (mean 2.83 meq 100 $g^{-1}$) in Rajasthan. The presence of higher sodium and bicarbonate in the soil could increase the soil alkalinity that is adverse to the plant growth. This is evident from the pH values of soil samples from the rose fields in both Rajastan and U.P. The chloride content of the soil varied from 0.4-4.0 meq 100 $g^{-1}$ (mean 2.5 meq 100 $g^{-1}$) in the UP region and 0.6-13.0 meq 100 $g^{-1}$ (mean 3.23 meq 100 $g^{-1}$) in Rajasthan samples. This indicated that chloride problem was more in Pali, Balarwa, and Haldigati regions of Rajasthan and in some pockets of Etah and Kannauj in UP.

Soil alkalinity will result in poor soil structure and surface crust formation. High pH is usually associated with high exchangeable sodium percentage. On the other hand, soil salinity and chloride toxicity could also be a serious problem that affects the germination, root growth and water availability of the plant (Munn and Tester, 2008). Excess $Na^+$ had been assumed to be largely responsible for reduction in crop growth and yield under salinity (Tsai *et al.,* 2004; Hong *et al.,* 2009). Though Cl- is an essential plant nutrient, it could be toxic to plants at high concentrations (Xu *et al.*, 2000; White and Broadley, 2001).

**Table 2. Soil characteristics of rose growing areas of Rajasthan**

| S.No. | Location | pH | EC (dS $m^{-1}$) | $Na^+$ (mg $kg^{-1}$) | $K^+$ (mg $kg^{-1}$) | $Ca^{2+}$ (mg $kg^{-1}$) | $Mg^{2+}$ (mg $kg^{-1}$) | $HCO_3^-$ (meq 100 $g^{-1}$) | $Cl^-$ (meq 100 $g^{-1}$) | ESP (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Chikada, Udaipur | 7.18 | 0.35 | 1223 | 68 | 2760 | 428 | 3.0 | 0.8 | 2.94 |
| 2 | Fatehnagar, Udaipur | 8.29 | 0.33 | 1341 | 123 | 2617 | 497 | 2.3 | 1.0 | 24.9 |
| 3 | Haldigati-1 | 7.67 | 0.35 | 194 | 367 | 2923 | 508 | 2.4 | 0.6 | 4.09 |
| 4 | Haldigati-2 | 7.93 | 0.31 | 207 | 103 | 2140 | 493 | 3.0 | 5.0 | 5.63 |
| 5 | Haldigati-3 | 8.08 | 0.18 | 168 | 187 | 2559 | 454 | 3.2 | 3.0 | 4.11 |
| 6 | Haldigati-4 | 7.55 | 4.59 | 1575 | 228 | 1633 | 483 | 2.2 | 2.0 | 34.9 |
| 7 | Arathwada | 8.42 | 0.15 | 195 | 65 | 1289 | 409 | 0.3 | 1.8 | 7.80 |
| 8 | Posalia | 8.02 | 0.15 | 138 | 119 | 1913 | 410 | 0.5 | 2.0 | 4.32 |
| 9 | Balarwa-1 | 7.95 | 0.14 | 128 | 107 | 1677 | 289 | 0.5 | 1.2 | 4.79 |
| 10 | Balarwa-2 | 8.05 | 0.14 | 207 | 180 | 1719 | 313 | 3.0 | 5.0 | 7.16 |
| 11 | Balarwa-3 | 7.87 | 0.24 | 138 | 174 | 1724 | 311 | 2.5 | 3.6 | 4.89 |
| 12 | Balarwa-4 | 7.83 | 0.37 | 186 | 172 | 1696 | 313 | 3.8 | 3.0 | 6.55 |
| 13 | KVK, Pali | 8.02 | 0.45 | 428 | 222 | 2045 | 404 | 10.1 | 13.0 | 11.6 |
| Mean | | 7.91 | 0.60 | 387 | 163 | 2053 | 409 | 2.83 | 3.23 | 9.52 |
| CV (%) | | 4.01 | 202 | 125 | 49.9 | 24.8 | 19.3 | 86.7 | 102 | 100 |

**Irrigation water quality parameters**

The irrigation water quality parameters of rose growing areas of UP (Table 3) and Rajasthan (Table 4) were analyzed and the results revealed that pH of the water samples were slightly alkaline in nature. Particularly water samples of UP had pH of 7.53-8.36, and water samples of Rajasthan had 7.23-7.70 pH range. It showed that irrigation waters of both the region had slightly higher pH (i.e.,) above the neutral pH (6.5-7.5). In case of EC, it ranged from 0.07-2.44 dS $m^{-1}$ in UP samples and 0.45-2.63 dS $m^{-1}$ in Rajasthan samples and few samples from Pali, Haldigati and Udaipur (Rajasthan), and Etah and Aligarh (UP) had higher EC (>1 dS $m^{-1}$). The cationic concentrations of the samples were within the safe range for K and Ca, but Na and Mg were higher than the FAO threshold levels in some samples (Ayers and Westcot, 1985). Further SAR of the water samples of UP region was 2.4-10.5 (4.5 meq $L^{-1}$) and Rajasthan region was 2.92-10.3 (5.41 meq $L^{-1}$). The SSP of the water samples were also very high that ranged from 33.5-82.5% in UP samples and 45.7-75.7% in Rajasthan samples. Most of the samples had higher SAR (more than 3) and SSP (>50%), which indicated presence of more Na than other cations. It was also reflected in higher pH of water samples. The SSP and the SAR were important factors for studying sodium hazards. The water samples with greater than 50% SSP and more than 3 (meq $L^{-1}$) SAR might result in accumulation of sodium in soil that cause the

**Table 3. Irrigation water quality of rose growing areas of Uttar Pradesh**

| S.No. | Location | pH | EC (dS $m^{-1}$) | $Na^+$ (mg $L^{-1}$) | $K^+$ (mg $L^{-1}$) | $Ca^{2+}$ (mg $L^{-1}$) | $Mg^{2+}$ (mg $L^{-1}$) | $HCO_3^-$ (meq $L^{-1}$) | $Cl^-$ (meq $L^{-1}$) | SAR (meq $L^{-1})^{1/2}$ | SSP (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lucknow | 7.59 | 0.65 | 5.28 | 0.02 | 2.98 | 0.51 | 5.0 | 0.6 | 4.0 | 60.3 |
| 2 | Bashirpur Khannoj | 7.66 | 0.48 | 6.26 | 0.03 | 0.75 | 0.58 | 5.2 | 1.2 | 7.7 | 82.5 |
| 3 | Bashirpur Khannoj | 8.32 | 0.07 | 16.9 | 0.02 | 3.5 | 1.67 | 1.0 | 0.6 | 10.5 | 76.6 |
| 4 | Narora, Etah | 7.55 | 2.44 | 3.87 | 0.08 | 2.81 | 2.6 | 7.0 | 5.0 | 2.4 | 42.2 |
| 5 | Narora, Etah | 7.54 | 0.76 | 8.83 | 0.08 | 3.38 | 3.87 | 5.1 | 0.6 | 4.6 | 55.1 |
| 6 | Sarkari Gram, Awaghad | 7.53 | 1.00 | 8.75 | 0.02 | 3.51 | 6.34 | 3.8 | 1.8 | 3.9 | 47.1 |
| 7 | Jagdevpura, Hasayan | 7.77 | 0.63 | 6.20 | 0.01 | 4.54 | 6.00 | 3.9 | 1.7 | 2.7 | 37.1 |
| 8 | Jagdevpura, Hasayan | 7.7 | 2.07 | 5.28 | 0.01 | 3.60 | 1.67 | 3.8 | 0.2 | 3.3 | 50.1 |
| 9 | Safed pura, Alighar | 7.63 | 0.60 | 6.26 | 0.04 | 1.56 | 6.26 | 4.1 | 1.2 | 3.2 | 44.6 |
| 10 | Hapur | 7.53 | 0.85 | 6.08 | 0.04 | 5.10 | 7.07 | 5.0 | 2.0 | 2.5 | 33.5 |
| Mean | | 7.68 | 0.96 | 7.37 | 0.04 | 3.17 | 3.66 | 4.39 | 1.5 | 4.5 | 52.9 |
| CV (%) | | 3.10 | 76.7 | 49.8 | 74.1 | 40.2 | 70.3 | 35.0 | 92.0 | 58.8 | 30.6 |
| FAO threshold (Ayers and Westcot,1985) | | 6.5-8.0 | 1.0 | 3.0 | 0.5 | 5.0 | 1.0 | 1.5 | 3.0 | 3.0 | 50.0 |

breakdown of physical properties and reduce permeability of soil, and stunted growth in plants (Joshi *et al.* 2009). The bicarbonate content was also higher than threshold value of 1.5 meq $L^{-1}$ in both the region, as per the FAO guidelines. The chloride concentration of the samples were within the safe limit (below 3 meq $L^{-1}$) in some samples and exceeded in some samples as in soil samples of Pali (17.5 meq $L^{-1}$) which was excessively high. Necessary precautionary measures could be taken while using the poor quality waters for irrigation over a longer period, because these lead to accumulations of salts and other hazards in the soil become harmful to production system.

## CONCLUSIONS

In comparison with other crop species, rose crop is highly sensitive to salinity and alkalinity. In the current study, it has been observed that most of the soil and water samples of the rose growing areas of Uttar Pradesh and Rajasthan are degraded due to alkalinity, sodium and bicarbonate hazards, and in some cases chloride hazards and salinity problems. Long term use of marginal quality water for irrigation can further aggravate the problems of soil salinity and alkalinity. Therefore, proper precautionary measures, reclamation and management of degraded soils and marginal quality waters is inevitable for sustaining the production system.

**Table 4. Irrigation water quality of rose growing areas of Rajasthan**

| S.No. | Location | pH | EC (dS $m^{-1}$) | $Na^+$ (mg $L^{-1}$) | $K^+$ (mg $L^{-1}$) | $Ca^{2+}$ (mg $L^{-1}$) | $Mg^{2+}$ (mg $L^{-1}$) | $HCO_3^-$ (meq $L^{-1}$) | $Cl^-$ (meq $L^{-1}$) | SAR (meq $L^{-1})^{1/2}$ | SSP (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Chapiri, Udaipur | 7.70 | 0.62 | 5.86 | 0.087 | 1.73 | 1.75 | 5.8 | 0.8 | 4.44 | 63.1 |
| 2 | Chikada, Udaipur | 7.59 | 1.05 | 8.74 | 0.032 | 1.01 | 1.80 | 7.1 | 3.0 | 7.37 | 75.7 |
| 3 | Fatehnagar, Udaipur | 7.51 | 1.57 | 15.90 | 0.043 | 3.50 | 2.08 | 10.0 | 5.0 | 9.52 | 74.1 |
| 4 | Haldigati-1 | 7.52 | 0.45 | 4.86 | 0.076 | 1.56 | 1.95 | 5.3 | 1.2 | 3.67 | 58.4 |
| 5 | Haldigati-2 | 7.32 | 1.76 | 7.74 | 0.076 | 4.54 | 2.24 | 10.9 | 3.6 | 4.20 | 53.5 |
| 6 | Posalia | 7.31 | 0.99 | 7.57 | 0.043 | 2.33 | 1.59 | 5.1 | 3.0 | 5.41 | 66.0 |
| 7 | Balarwa-1 | 7.50 | 0.53 | 5.18 | 0.022 | 3.81 | 1.82 | 3.0 | 2.0 | 3.09 | 48.0 |
| 8 | Balarwa-2 | 7.56 | 0.63 | 5.12 | 0.043 | 4.25 | 1.88 | 4.0 | 1.8 | 2.92 | 45.7 |
| 9 | Balarwa-3 | 7.58 | 0.62 | 5.27 | 0.011 | 3.60 | 1.82 | 3.8 | 2.0 | 3.20 | 49.4 |
| 10 | KVK, Pali | 7.23 | 2.63 | 20.8 | 0.151 | 6.01 | 2.09 | 3.9 | 17.5 | 10.3 | 72.1 |
| Mean | | 7.48 | 1.09 | 8.70 | 0.06 | 3.23 | 1.90 | 5.89 | 3.9 | 5.42 | 60.6 |
| CV (%) | | 1.98 | 64.7 | 61.8 | 69.9 | 48.1 | 10.0 | 45.5 | 123 | 50.3 | 18.6 |
| FAO threshold (Ayers and Westcot,1985) | | 6.5-8.0 | 1.0 | 3.0 | 0.5 | 5.0 | 1.0 | 1.5 | 3.0 | 3.0 | 50.0 |

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***(Received on 13.01.2021, Revised on 29.07.2021 and Accepted on 30.07.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 287-291, 2021*

***Original Research Paper***

# Qualitative and organoleptic evaluation of immature cashew kernels under storage

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## ABSTRACT

**Cashew cultivars, based on flowering behaviour, are categorized into three types, *viz*., early season, mid-season and late season. In late season type, the harvesting of cashew nuts coincides with the rainy season during which the quality of matured nuts are affected by increased pest and disease attack. This loss can be reduced if the nuts are harvested before it reaches its complete maturity. In this context, present study was conducted in immature cashew kernel to find out suitable storage treatments to enhance the shelf life. Immature cashew kernels were stored in different concentrations of brine solution (5%, 10% and 15%), sugar syrup (50°B, 60°B and 70°B) and by drying in hot air oven until the moisture content of kernel reaches 2-3 per cent. Storage period was for four months and the observations like tannin content, microbial content and organoleptic qualities of kernels stored in each treatment were analysed at the beginning and at the end of the storage. The treatment with 10% brine and 70°B sugar syrup for four months were found as best for storing immature cashew kernels.**

**Keywords**: Cashew, immature kernels, organoleptic qualities and storage

## INTRODUCTION

Cashew, an important horticultural crop of India, has a great socio-economic significance in this country. It is native of Brazil and the Lower Amazons. The demand for raw and processed cashew nut is high in both internal and export markets. According to the annual report of CEPCI (2019), India continued to be the largest producer of cashew nut in the year 2017-2018 and the state that contributed maximum towards production is Maharashtra (33%), followed by Andhra Pradesh (14%), Kerala (11%) and Karnataka (11%). At present cashew kernels are consumed directly or used for various food preparations. Raw cashews contain 5% water, 30% carbohydrates, 44% fat and 18% protein. In a 100 gram reference quantity, raw cashews provide 553 Calories, 67% of the Daily Value (DV) in total fats, 36% DV of protein, 13% DV of dietary fiber and 11% DV of carbohydrates (USDA, 2015). The research efforts are concentrated mostly on mature kernels, and immature kernels gained little attention. In Kerala, sprouted cashew nuts are eaten as raw and also after cooking. Considerable quantities of cashew nuts are produced during rainy season in Kerala, especially in the late season flowering varieties like Madakkathara-2 and Sulabha, which are inferior in quality and are being wasted. The occurrence of late season flowering is mostly observed in hilly regions of Kerala like Wayanad and Idukki districts. The quality of nut is affected mainly by the infestation of pests and diseases during this season. It is estimated that more than 50% of the crop is lost annually due to pests and diseases in cashew (Haribabu *et al*., 1983). If the nuts are harvested before it reaches complete maturity and if those can be economically utilized, the loss during rainy season can be reduced to a great extent. Therefore, in this experiment, the immature nuts were harvested in tender form, when the shells were not hardened and were green in colour. The shell is soft and can be cut with a knife and kernel can be extracted. The kernels can be put to use in a variety of ways like serving as a snack or it could be relished as salads by combining with mango and sweets like *tikka* and cashew cake can be prepared

(Anandkumar *et al.,* 2011). If we can store these immature kernels, it can be used round the year. In this context, the storage studies of immature cashew kernels were studied in College of Agriculture, Kerala Agricultural University, Thrissur, India.

## MATERIALS AND METHODS

The immature cashew nuts were collected from Cashew Research Station, Madakkathara, Thrissur, India. Inflorescences of cashew tree were tagged at the time of anthesis and the nuts were harvested after 55 days. This is the stage before the nuts turn from green to grey colour. The harvested tender nuts were cut into halves using sharp knife and the kernels were scooped out. The outer covering of kernel (i.e.,) testa, was removed and these kernels were washed thoroughly in water followed by steam blanching for two to three minutes.

Brine solutions of 5%, 10% and 15% concentrations were prepared and poured in sterilized glass bottles, to which the pre-treated kernels were added. Three replications were kept for each treatment. Similarly immature kernels were stored in sugar syrups of 50°B, 60°B and 70°B concentrations. Permissible quantity of preservative was added to every treatments. Another treatment under storage studies was by drying. The pre-treated kernels were dried in hot air oven for two days, until the moisture content of the kernels were reduced to 2-3% and then stored in glass containers after complete cooling. Observations taken were tannin content, microbial content and organoleptic evaluation of kernels at first and fourth months of storage.

**Tannin content**

Tannin content of the kernels were estimated using Folin-Denis method at first and fourth months of storage.

**Microbial Count**

In microbial analysis, total bacterial, fungal and yeast counts were evaluated for both stored kernels and keeping solution.

**Organoleptic Evaluation**

Organoleptic evaluation was carried out by a panel of judges consisting of 15 members. The evaluation was carried out at beginning and ending of the storage period. Different organoleptic parameters like appearance, colour, texture, flavour, taste, mouth feel and overall acceptability were evaluated.

## RESULT AND DISCUSSION

**Tannin content**

Tannin content could not be detected in any of the treated kernels at both first and fourth months of storage. It might be because of the primary processing steps like washing and steam blanching employed before storage treatments. Anand (1970) reported loss of tannins during pre-treatments like soaking, blanching and brining of fruits during preparation of aonla preserve. According to Afoakwa *et al*. (2007), blanching of Bambara groundnuts before canning reduced the tannin content. Thus, these pre-treatments and storage treatments were found to be very effective in removing tannin content of immature cashew kernels.

**Table 1. Microbial count of the keeping solution in different storage treatments**

| Treatments | Bacteria ($10^7$ cfu/ml) | | Fungi ($10^3$ cfu/ml) | | Yeast ($10^4$cfu/ml) | |
|---|---|---|---|---|---|---|
| | 1 MAS | 4 MAS | 1 MAS | 4 MAS | 1 MAS | 4 MAS |
| 5% brine | 7.67 (0.93) | 6.33 (0.86) | 1.67 (0.42) | 4.67 (0.75) | 1.33 (0.36) | 1.67 (0.42) |
| 10% brine | 0.00 | 0.00 | 0.00 | 0.33 (0.10) | 0.00 | 0.00 |
| 15% brine | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50°B syrup | 5.00 (0.77) | 1.67 (0.26) | 1.33 (0.36) | 2.00 (0.48) | 0.00 | 1.67 (0.42) |
| 60°B syrup | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 70°B syrup | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CD (5%) | 0.11 | 0.32 | 0.20 | 0.14 | 0.07 | 0.10 |

MAS - Months after storage (Values in the parenthesis are logarithmically transformed) Cfu/ml - Colony forming unit per ml

**Table 2. Microbial count of the kernels preserved in different storage treatments**

| Treatments | Bacteria ($10^7$ cfu/ml) | | Fungi ($10^3$ cfu/ml) | | Yeast ($10^4$cfu/ml) | |
|---|---|---|---|---|---|---|
| | 1 MAS | 4 MAS | 1 MAS | 4 MAS | 1 MAS | 4 MAS |
| 5% brine | 5.67 (0.82) | 4.33 (0.73) | 0.67 (0.20) | 1.67 (0.42) | 0.33 (0.10) | 1.00 (0.30) |
| 10% brine | 0.00 | 0.00 | 0.00 | 0.33 (0.10) | 0.00 | 0.00 |
| 15% brine | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50°B syrup | 3.33 (0.63) | 1.00 (0.30) | 3.33 (0.63) | 3.00 (0.60) | 0.33 (0.10) | 1.67 (0.42) |
| 60°B syrup | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 70°B syrup | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| drying | 0.00 | 0.00 | 0.00 | 0.33 (0.10) | 0.00 | 0.00 |
| CD (5%) | 0.04 | 0.03 | 0.12 | 0.17 | NS | 0.06 |

MAS - Months after storage (Values in the parenthesis are logarithmically transformed) Cfu/ml - Colony forming unit per ml
NS - Non-significant

**Microbial count**

The microbial count of both kernels and keeping solution is presented in table 1 and table 2. Among the seven treatments of storage, the bacterial count was beyond permissible limit both in the solution and kernels stored in 5% brine as well as in 50°B sugar syrup.

The fungal population was within the acceptable limit for all the treatments. The yeast population was also found above the permissible limit for 5% brine and 50°B sugar syrup. This might be due to the less concentration of salt and sugar content in both these treatments which might not be sufficient to control the microbes. Ranken *et al*. (1997) reported that placing vegetables in 8-11% of salt content inhibited the microorganisms that may cause spoilage of vegetables. Thus, the immature cashew kernels can be stored for four months without microbial attack in 10% brine, 15% brine, 60°B sugar syrup and 70°B sugar syrup. Storage after drying was also found to contain permissible limit of microbial population.

**Organoleptic evaluation**

Among the seven treatments of storage, kernels stored in sugar syrup were the most accepted ones, both at the first and last months of storage (Fig 1). According to Ponting (1973), sugar uptake by the product kept in sugar solution, through osmotic process, modified the composition and taste of the final product. In this experiment, the scores obtained for flavour (6.40-7.07) and taste (6.00-7.07) were higher for kernels in sugar syrup. The uptake of sugar in the kernel might have resulted in the increased taste and flavour leading to enhanced palatability and higher score. Kernels preserved in 70° B sugar syrup had the highest overall acceptability score (7.40) followed by kernels in 60° B (7.00) and 50° B (6.60) sugar syrup; higher sugar level might have resulted in more absorption.

Kernel stored in 15% brine was the least accepted treatment which might be due to its high salt content that made it unpalatable after four months of storage. Ross *et* al. (2002) reported that macadamia kernel pieces, which were immersed in salt solution, became unacceptable on extended storage. Kernels in 10% brine was found better than 5% and 15% brine solutions in sensory parameters like appearance, colour, texture, flavour and overall acceptability.

According to Hutton (2002), salt act as a preservative against microbial growth and also imparts characteristic flavour. All the quality parameters of organoleptic evaluation were found better for kernels stored in 10% brine after four months of storage compared to the first month. The preservative action of salt leading to enhanced storage life has been reported in many vegetables. Barwal *et al*. (2005) reported that blanched cauliflowers steeped in 10% and 15% salt solution were found acceptable up to 180 days.

In dry storage, the dried kernels had an off taste after four months of storage which could be attributed to the rancidity of the kernels as experienced in nuts with

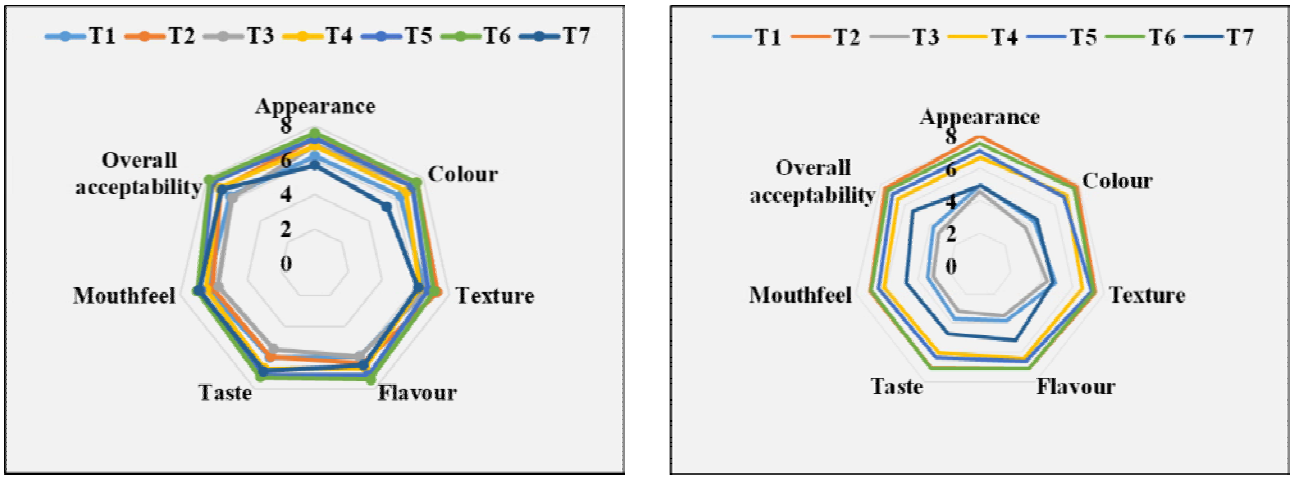


**Figure 1. Effect of different treatments on sensory attributes immature cashew kernel at (a) first month and (b) fourth month of storage**

$T_1$- Kernels preserved in 5% brine; $T_2$- Kernels preserved in 10% brine; $T_3$- Kernels preserved in 15% brine; $T_4$- Kernels preserved in 50° brix sugar syrup; $T_5$- Kernels preserved in 60° brix sugar syrup; $T_6$- Kernels preserved in 70° brix sugar syrup; $T_7$- Kernels preserved by drying

high fat content. According to Mexis and Kontominas (2009), the rancid taste of nuts during sensory evaluation occurred due to lipid oxidation. Young (2007) reported that rancidity was considered as the first sign of deterioration of nuts, since most edible nuts are rich in oil content. Hence the dried immature kernels cannot be used as such for consumption after a storage period of four months.

## CONCLUSION

According to the findings, tender cashew nuts can be stored safely for four months in preserve (70°Brix) and also in 10% brine added with allowed preservatives. This can be followed anywhere in the world, where the mature nuts are affected due to any biotic or abiotic problems.

## ACKNOWLEDGEMENT

The authors are very much grateful to Kerala Agricultural University, Thrissur, India for providing immense support and sufficient facilities for the conduct of this experiment.

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***(Received on 06.06.2021, Revised on 13.07.2021 and Accepted on 30.07.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 292-300, 2021*

***Original Research Paper***

# Physical quality of coffee bean (*Coffea arabica* L.) as affected by harvesting and drying methods

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## ABSTRACT

**Coffee is a stimulant crop with high socio-economic cultural value including economical significance in Ethiopia. This study was conducted in 2019-2020 to investigate the effect of harvesting methods and drying surfaces on the physical quality of the coffee beans. The experiment was carried out with two factors, harvesting methods and drying surfaces laid out in a two factorial completely randomized block design with three replications using a landrace coffee variety. The result showed that the interaction of harvesting methods and drying surfaces was highly significant (P<0.01) for coffee bean size and dried coffee berry weight. The highest beans retained above screen were recorded from the interaction of mesh wire (90%) and cemented drying (89%) surfaces with selective harvesting methods. The highest dried coffee berry weight (69.33 gm) were attained from the interaction of selective harvesting with mesh wire drying surfaces. The lowest dried coffee berry weight (63.79 gm) were attained from the interaction of strip harvesting with tin drying surfaces. Significant (P<0.05) variation for primary defects, length of drying period were recorded. Higher length of drying periods (41.67 days) was recorded from the interaction of mesh wire drying surfaces with selective harvesting method and the lowest (20.33 days) was recorded from the interaction of tin drying surfaces with strip harvesting method. The highest percentage of primary defected beans were recorded from the interaction of selective harvesting methods with mesh wire drying surfaces (15%) and the lowest number were recorded from strip harvesting method with drying on plastic (5%). Therefore, it can be concluded that using the interaction of selective harvesting and drying on mesh wire is better for optimum physical quality of coffee in the studied area**

**Keywords:** Coffee bean size, drying surface, ethiopia, export, harvesting methods and physical quality,

## INTRODUCTION

Ethiopia is naturally endowed with a suitable climate with a distinctive coffee profile and has the potential to produce large amounts of differentiated high-quality green coffee. But currently, Ethiopia's coffee qualities are quite average and need special attention to produce high-quality coffee to be competitive in today's world market (Asfaw, 2018). Coffee is the number one foreign exchange earning export commodity of Ethiopia. Almost 2% of the world's coffee comes from Ethiopia. Over 60% of the country's foreign exchange is obtained through the export of coffee. A quarter of the population is directly or indirectly engaged in the production, processing, and marketing of coffee (Chauhan *et al.*, 2015).

Coffee is grown by 6.3 million smallholder farmers in Ethiopia in an area of 758,523 ha with a production of 4.8 million qt and an average productivity of 6.36 qt/ha (CSA, 2020). Coffee is the most important commodity and there is huge potential to increase coffee production as the country is endowed with suitable agro-ecology, climatic, soil fertility, indigenous quality planting materials, and sufficient rainfall in the coffee-growing belts of the country. And, there is high national and international demand for the Ethiopian coffee product, increasing interest of private sector with high investment potential (Berhanu, 2017). Ethiopia produces a large volume of coffee beans

every year with 397,500 tons in 2014 alone, and ranking first in Africa and fifth in the world (ICO, 2015). However, coffee supplied and traded in the local market is usually has a lower quality. Coffee on the local market is mainly coffee destined for export through the Ethiopian Commodities Exchange (ECX) market but failed to meet ECX's quality standards (Asfaw, 2018) for export and got rejected. Quality is an important attribute of coffee and it is currently becoming even more important than in the past as coffee industry is generally going through a worldwide surplus production crisis (Petit *et al*., 2007).

Wollega is also a potential coffee growing area of Western Ethiopia (Stieger *et al.,* 2002). Though coffee quality is affected in several ways, the agronomic practices followed during harvesting, processing, and handling practices also influence its quality. According to Desse's (2008) report, poor harvesting practices such as stripping, collecting dropped fruits from the ground, improper postharvest handling practices such as bad processing and drying on the bare ground resulted in the low-quality green coffee bean. Among them, type of harvesting and drying methods used are important. However, there is little information on the effect of different practices such as harvesting methods and drying surface on coffee quality. Therefore, this study was initiated to investigate the influence of harvesting methods and drying surfaces on the physical quality attributes of coffee in Begi district West, Wollega of Ethiopia.

## MATERIALS AND METHODS

**Description of the study area**: The study site was in Begi district, West Wollega zone, Oromia Regional State of Ethiopia which is one of the major coffee-producing districts. The selected district represents the agro-ecological zones where coffee is produced. The agroecology of the area is semi-humid and the annual rainfall ranged between 1300-1500 mm per year and the mean annual temperature is 20-28$^0$C. Geographically it is located between latitude of 9$^o$26'North and longitude 34$^o$32'East at altitude range of 1768 meters above sea level.

**Treatments and experimental design:** The local land race of coffee (*Coffea arabica* L.) was used in the present study. The study consists of two factors *viz*., the harvesting method and drying surfaces. Two harvesting method *viz.,* selective and strip harvesting were tested. Under strip harvesting method, cherries were harvested when 75% of the cherries reached at full ripe stage whereas in selective picking the cherries were harvested as they attained full red ripe stage. Six drying surfaces *viz.*, bamboo mats, bare ground, cemented floor, mesh wire, plastic sheet and tin sheet were tested. The cherries harvested using both methods were spread out to dry in the sun on the six drying surfaces. They were stirred regularly to promote even drying, prevent fermentation and the development of mold in each treatment. Then each sample cherries were dried till their outer shell skin became dark brown and brittle. When the approximate moisture content of 11.5% was attained, dried coffee cherries were collected and de-hulled with mortar carefully and cleaned (Boot, 2006). Each of the drying surfaces had an area of 1m x 1m = 1m$^2$.

**Laboratory analysis:** Clean coffee bean sample of 500 g was taken from each treatment combination based on sampling procedure set by Ethiopian standard (ESBN 8.001), which is on the basis of drawing 3 kg per 10 tons. Representative samples were assigned an arbitrary code in order to secure an unbiased judgment and brought to coffee quality laboratory of the Jimma Agricultural Research Center where the green coffee beans were evaluated for different raw quality attributes. The moisture content of the sample was checked using Electronic Rapid Moisture Tester (HE 50, Germany) to make the uniform required moisture level of all samples.

**Data collection:** The data on length of drying period (days), weight of dried coffee berry (g), bean moisture content (%), dried bean weight (g), primary defect (count), secondary defect (weight), odor, coffee aroma and coffee flavor were collected according to their respective procedures.

**Data Analysis**: The various coffee quality data collected were subjected to analysis of variance using statistical procedures as described by Gomez and Gomez (1984) using SAS 9.3 version. The differences between and among treatment means were compared using the least significance difference test at 5% of significance when the ANOVA shows the presence of significant difference.

## RESULTS AND DISCUSSION

**Bean size screen (%):** The main effect of harvesting methods and drying surfaces as well as their interaction were highly significantly (P<0.01) (Table 1) influencing the bean screen size. Moreover, the interaction effect of harvesting methods and drying surfaces on the total percentage of bean size retained above screen size 14 ranged from 90% to 73%. The highest beans retained above screen were recorded with wire mesh drying surfaces with selective harvesting methods (90%). However, it was at par with cemented floor. The result indicated that coffee beans harvested in selective picking and treated with different drying surfaces met the export standards except when selective beans dried on tin surfaces (82.3%) (Table 2). The present finding is in agreement with Mekonnen (2009) who reported the highest percentage of beans retained above screen were recorded when different varieties of coffee beans were harvested. All the interaction of strip harvesting methods with respective drying surfaces ranged from 75.67% to 73% (Table 2) which failed under the category of rejected commercial coffee based on ECX (2010) standard. According to ECX (2010), any Ethiopian coffee export shall have a minimum of 85% of bean weight remaining on the top of screen 14 (Table 2). Similarly, Mohammedsani *et.al*. (2017) reported bean size was significantly influenced by harvesting methods and the interaction of harvesting and postharvest processing methods. Selective harvesting of red fruits produced a uniform bean size that is above the minimum required bean screen size. To improve quality coffee, traders practice some value-adding activities like removing the defect and undersized beans thorough cleaning and sorting (Anteneh, 2011), and Belete (2014) indicated coffee with larger beans usually get a good grade and fetch a higher price than smaller ones. The current study confirmed the report of Getachew *et.al* (2015) who indicated drying coffee on wire mesh and bamboo mats with a thin layer of thickness earned above screen size of beans (>85%).

**Dried bean weight** : The result showed a significant difference in 100 bean weight due to the main effect of harvesting methods but a non-significant result was obtained due to the main effect of drying surfaces and their interactions (Table 1). And, from this study, the highest 100-bean weight was recorded when coffee was harvested by selective methods (16.51 g) and the lowest recorded in strip harvesting methods (15.39 g) (Table 3). Similarly, Vaast *et.al*. (2006) indicated harvesting methods significantly influenced the bean weight of coffee due to the lower biochemical composition of the bean, hence reducing the cup quality. This study confirms also the finding of Mohammedsani *et.al., (*2017), the highest bean weight was obtained from selective harvesting compared to strip harvesting. This study showed the selective harvesting method was 7% more than strip harvesting (Table 3). Another report by Boot (2006) showed that the weight of ripe cherry was more by 20% than that of immature cherry. This might be due to the fact that on bamboo, cement, and mesh wire there was a gradual moisture loss and less burning effect, whereas on a tin bed, there was a burning effect on coffee berry which may decrease the weight of coffee seed. The result regarding drying surfaces was supported by Mohammedsani *et.al.,* (2017). And, report of Wintegens, (2004) and Yigzaw (2014) showed that Arabica coffee average bean weight with values ranging between 9.2 g and 18.2 g.

**Primary defects:** The analysis of variance revealed that the main effect of harvesting methods and drying surfaces were highly significant (P<0.01) on the primary defect. And, the interaction effect of harvesting methods and drying surfaces were also significant (P<0.05) for primary defect (Table 1). The highest percentage of many defected beans was recorded on selective harvesting methods and drying on a wire mesh (15) and the lowest number of a defected bean is recorded from strip harvesting with drying on plastic (5) (Table 2). This might be because unripe cherries lead to light-green beans, which when dried, become black and these beans are counted as defective in strip harvesting. This study is in agreement with the finding of Bee *et al.,(*2005).

**Table 1. Mean squares values of raw quality attributes of coffee as affected by harvesting methods and drying surfaces in Begi district, West Wollega Zone, Ethiopia**

| Raw quality attributes | Harvesting methods (HM) (1) | Drying surfaces (DM) (5) | HM* DM (5) | Residual (22) | CV (%) |
|---|---|---|---|---|---|
| Bean size | 1332.25** | 13.89** | 15.31 ** | 0.596 | 1 |
| SED (±) | 0.257 | 0.924 | 1.307 | | |
| Bean weight (gram) | 11.177* | 0.538ns | 1.92ns | 5.45 | 3.1 |
| SED (±) | 0.286 | 0.166 | 0.843 | | |
| Primary defect (%) | 306.25** | 14.65** | 1.45 * | 0.523 | 7.2 |
| SED (±) | 0.241 | 0.417 | 0.59 | | |
| Secondary defect (%) | 361** | 17.4** | 2ns | 1.364 | 11.7 |
| SED (±) | 0.389 | 0.674 | 0.953 | | |
| Length of drying period ( days) | 215.11** | 230.73** | 1.178* | 0.371 | 2.3 |
| SED (±) | 0.352 | 0.203 | 0.497 | | |
| Dried coffee berry weight (gram) | 29.16** | 6.508** | 2.67** | 0.16 | 0.6 |
| SED (±) | 0.23 | 0.134 | 0.327 | | |
| Odor (%) | 21.78** | 5.24** | 0.44ns | 0.78 | 10.3 |
| SED (±) | 0.294 | 0.509 | 0.72 | | |
| Acidity | 25.00** | 1.2ns | 1.6ns | 1.84 | 10.1 |
| SED (±) | 0.452 | 0.783 | 1.108 | | |
| Body | 2.2ns | 4.00ns | 1.00ns | 2.636 | 2.2 |
| SED (±) | 0.54 | 0.94 | 1.33 | | |
| Flavor | 20.25 | 0.85 | 1.65 | 2.159 | 10.8 |
| SED (±) | 0.49 | 0.85 | 1.2 | | |

* Significant at P<0.05, ** highly significant at P<0.01, ns= non-significant difference, Numbers in parenthesis indicates degree of freedom. CV (%) = coefficient of variation in percent, Sed (±) = Standard error of difference.

**Table 2. Bean size screen using ECX (2010) standard**

| Harvesting methods | Drying methods | Average value analysis screen beans size | | ECX (2010) standard |
|---|---|---|---|---|
| Selective harvesting | Bamboo Mats | 86.67 | | |
| | Plastic Sheet | 85.00 | | |
| | Cement | 89.00 | → | Export Standard |
| | Wire Mesh | 90.00 | | |
| | Bare Ground | 84.67 | | |
| | Tin | 82.00 | | |
| Strip harvesting | Bamboo | 75.67 | | |
| | Bare Ground | 75.67 | | |
| | Cement | 73.55 | → | Rejected for Export |
| | Wire Mesh | 73.67 | | |
| | Plastic Sheet | 73.00 | | |
| | Tin | 73.33 | | |
| | Mean | 80.19 | | |
| | LSD (5%) | 1.307 | | |
| | CV (%) | 1.00 | | |

ECX (2010) stated that Moisture and screen analysis are the two requisites before grading any coffee. The moisture content should be less than 11.5 percent, while the size of the bean should be above screen size 14 for 85 percent of the bean sample.

**Table 3. The main effect of harvesting method and drying surfaces on raw and physical quality attributes of coffee in Begi district, Ethiopia**

| Treatments | Bean weight | Secondary defect | Odor | Body | Flavor |
|---|---|---|---|---|---|
| **Harvesting method (HM)** | | | | **to the power** | |
| Selection | 16.51 | 13.17 | 9.33$^{ab}$ | 13.67 | 14.33$^{a}$ |
| Strip | 15.39 | 6.83 | 7.78$^{b}$ | 13 | 12.83$^{b}$ |
| LSD (5%) | 0.34 | 0.807 | 0.61 | NS | 1.016 |
| SED | | | 0.294 | 0.541 | 0.49 |
| **Drying surface (DS)** | | | | | |
| Bamboo | 16.34 | 11.00 | 9.667$^{a}$ | 13.5 | 13.5 |
| Bare Ground | 15.81 | 8.00 | 9.667$^{a}$ | 12.5 | 13 |
| Cement | 16.16 | 11.50 | 9$^{abc}$ | 14 | 14 |
| wire mesh | 16.13 | 12.00 | 9.33$^{ab}$ | 14 | 13.5 |
| Plastic sheet | 15.70 | 8.50 | 8.33$^{acb}$ | 13 | 13.5 |
| Tin | 15.58 | 9.00 | 7.667$^{bc}$ | 13 | 14 |
| LSD (5%) | ns | 1.39 | 1.056 | NS | NS |
| HM*DM | ns | ns | NS | NS | NS |
| CV (%) | 3.10 | 11.70 | 10.3 | 12.2 | 10.8 |

Means followed by the same letter(s) within rows and columns are not significantly different at P d" 0.05 level of significance, LSD= Least significant differences=Non-significant, CV (%) = coefficient of variation in percent

Similarly, with the report of Barel and Jacquet (1994), selective harvesting of coffee produced the best quality coffee by decreasing the percentage of defective coffee beans. Also, Berhanu *et al*., (2014) also indicated that inappropriate post-harvest management practices increased the number of defective coffee beans. Moreover, Tesfaye (2006) and Negussie *et.al*. (2009) stated that properly processed coffee is with very few defective beans.

**Secondary defects**

The result showed that there was a highly significant (Pd"0.01) variation of secondary defects due to the main effect of harvesting methods and drying surfaces. However, the interaction effect of harvesting methods and drying surfaces did not significantly affect secondary defects (Table 1). Selective harvesting had a high mean value of 13.17% indicating relatively pure coffee beans. However, the lower mean value (6.83%) was recorded from strip harvesting (Table 3), which indicated a high number of secondary defects due to improper harvesting. This showed that selective harvesting had more coffee beans free from secondary defects as compared to strip harvesting in dry-processed coffee. This is because selective harvesting involves only picking off the red, fully ripe, and normal cherries carefully from the tree while strip harvesting involves collecting of entire coffee bean just by one pass through cropping season. This result is in line with Hicks (2002) who described that although selective picking is more expensive, it can produce the best results of coffee by reducing the number of defects thereby increase the overall quality of coffee which is competent in the world market. And, Hicks (2002) reported that coffee that has been inappropriately dried would become brittle and produce too many broken beans that are considered as a secondary defect during hulling. Similarly, Olamcam (2008) result showed that the coffee well harvested and properly processed has no or very few broken beans and free of foreign matter.

**Length of drying periods:** The analysis of variance revealed that the length of drying periods was highly significantly (P<0.01) different due to the main effect of drying surfaces and harvesting methods and significant (P<0.05) difference due to the interaction effect of both factors (Table 1). Higher length of

drying periods (41.67 days)was recorded from the interaction of wire mesh drying surfaces with selective harvesting method and the lowest (20.33 days) was recorded from the interaction of tin drying surfaces with strip harvesting method but statically at par with the interaction of plastic drying surface with strip harvesting method (20.67) (Table 4). Harvesting red cherry would prolong the drying periods than harvesting in a strip. Besides, at the full maturity stage, there might be an increment of moisture and the development of luxurious mucilage. This result agrees with the findings of Berhanu *et.al.* (2014) that the shortest time drying periods were recorded when coffee was dried in bricks off the floor then raised bed. FAO (2006) and Martin *et al*. (2009) also reported coffee dried on a flat surface more quickly than that dried on raised-bed surfaces like mesh wire and bamboo mats.

**Table 4. Interaction effect of the harvesting method and drying surfaces on the primary defect, length of drying (in days), and dried coffee berry weight at Begi West Wollega Zone, Ethiopia.**

| Drying surfaces | Harvesting methods | | | | | |
|---|---|---|---|---|---|---|
| | primary defect | | length of drying (in days) | | Dried coffee berry weight | |
| | Selective | Strip | Selective | Strip | Selective | Strip |
| Bamboo | 12.00 | 8.00 | 28.00 | 21.67 | 65.67 | 65.30 |
| Bare ground | 12.00 | 6.00 | 27.33 | 23.33 | 65.73 | 63.80 |
| Cement | 15.00 | 9.00 | 26.67 | 22.67 | 67.53 | 64.90 |
| Wire mesh | 15.00 | 9.00 | 41.67 | 36.67 | 69.33 | 65.53 |
| Plastic | 12.00 | 5.00 | 25.33 | 20.67 | 65.53 | 65.20 |
| Tin | 12.00 | 6.00 | 25.67 | 20.33 | 65.50 | 63.76 |
| Mean | - | | 26.67 | | 65.65 | |
| LSD (5%) | 1.224 | | 1.03 | | 0.679 | |
| CV (%) | 7.20 | | 2.30 | | 0.60 | |

LSD= Least significant difference, CV= Coefficient of variation

**Dried coffee berry weight**

The analysis of variance revealed that the weight of dried coffee berry was highly significant (P<0.01) different due to the main effect of harvesting methods and drying surfaces. And, the interaction effect of harvesting methods and drying surfaces was also highly significant (P<0.01) on dried coffee berry weight (Table 1). The highest dried coffee berry weight (69.33) and lowest (63.76) was recorded as an interaction of Selective harvesting with mesh wire bed and strip harvest with tin drying, respectively (Table 4). This was because in selective harvesting the only red, matured and disease-free coffee berry was harvested.

The present finding supports Clifford (1985), who reported acceptable dry matter loss within the ranges between 35 and 14%. Mekonen (2009) also indicated that selectively harvested coffee of different drying surfaces showed significant variation in coffee weight by recording the highest percentage of beans retained above the screen. ITC (2011) also indicated that picking immature cherries with mature cherries could cause a reduction of the weight of the beans. Similarly, Boot (2006) reported that under almost all conditions, the specific weight of ripe cherry is greater than that of an immature cherry, it is heavier, weighing up to 20% more

**Odor:** The analysis of variance revealed there was a highly significant variation (Pd"0.01) for odor due to the main effect of coffee harvesting methods and drying surfaces (Table 1). However, their interaction effect showed non-significant variations for odor. For selective harvesting (9.33) the mean values of odor were higher than strip harvesting (7.78). For drying surfaces, the highest mean value of odor was recorded when beans dried on bamboo and wire mesh and the lowest was recorded in bare ground and tin (Table 3) showing that the odor was affected due to improper harvesting and drying surfaces. A similar finding was

reported by Olamcam (2008) indicating properly harvesting beans make free of unpleasant (bad) smells. Endale *et.al.,*(2008) reported that coffee with better management in each stage starting from harvesting until cupping turns out to have a better odor. Subedi (2010) reported coffee dried on bricks floor in contact with soil becomes dirty and blotchy resulting in a dull odor. Using incongruous drying surfaces and methods reduced raw and cup quality of coffee by producing off-flavor, abnormal color, and unpleasant odor, and finally cup cleanness (Mohammedsanni *et.al.,* 2017).

**Flavor:** The result showed that the flavor was highly significantly ($P<0.01$) different due to the main effect of harvesting methods. But, non-significant due to drying surfaces and interaction effect of drying surfaces with harvesting methods (Table 1). The highest percentage number of flavors is recorded in selectively harvested coffee (14.33) and the lowest in the number of flavors is recorded in the strip harvesting method (12.83) (Table 4). In strip harvesting, there might be a possibility of harvesting coffee with microorganisms that naturally present in the production environment which use sugars in the pulp and mucilage and excrete organic acids and other metabolites that may affect the final sensory characteristics of the beverage. This result conforms with Getu (2009) work that indicated flavor is identified as an all-round good cup quality attribute which embraces positive values of aromatic attributes, acidity, and body Similarly, Anteneh (2011) stated poor harvesting practices such as stripping and collecting dropped fruits reduced the quality attributes like flavor.

## CONCLUSIONS

The result revealed that the interaction of harvesting methods and drying surfaces were highly significant ($P<0.001$) difference for coffee bean size and dried coffee berry weight while significant ($P<0.05$) variation for primary defects, length of drying period. The main effect of harvesting methods and drying surfaces were highly significant on bean size, primary defect, secondary defect, length of the drying period, and dried coffee berry weight. Coffee beans harvested by selective harvesting and treated under different postharvest processing methods had 85%, except when coffee beans size dried on and above the minimum required bean size for export coffee as compared to strip harvesting beans in which all beans are recorded under rejected coffee due to many small beans (<76%).

The highest (16.51 gram) dried bean weight was verified in selective harvesting as well the lowest (13.59 gram) was in strip harvesting. Primary and secondary defects were highly significantly influenced by harvesting methods and drying surfaces. The highest length of drying period (41.67 days) was recorded from the interaction of wire mesh drying surfaces with selective harvesting method and the lowest (20.33 days) was recorded from the interaction of tin drying surfaces with strip harvesting method but statically at par with the interaction of plastic drying surfaces with strip harvesting method (20.67). The odor was significantly influenced due to the main effect of coffee harvesting methods and drying surfaces. The highest scale of the odor was recorded from selective harvesting and the lowest from strip harvesting. Acidity and flavor were affected by harvesting methods and selective harvesting produced a high raw quality of all attributes. The finding suggests that coffee physical quality could be better improved by the selective picking of red cherries. Moreover, drying coffee on bare ground highly reduced raw abnormal color and unpleasant odor.

## ACKNOWLEDGMENTS

The authors thank the Ethiopian Coffee and Tea Authority for their technical support in coffee quality analysis.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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***(Received on 13.10.2021, Revised on 02.12.2021 and Accepted on 10.01.2022)***

*J. Hortl. Sci.*
*Vol. 16(2) : 301-308, 2021*

***Original Research Paper***

# Vegetative vigour, yield and field tolerance to leaf rust in four $F_1$ hybrids of coffee (*Coffea arabica* L.) in India

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## ABSTRACT

**Four $F_1$ hybrids of arabica coffee (*Coffea arabica* L.) developed with the primary objective of pyramiding the $S_H3$ gene for leaf rust resistance in a commercial variety 'Chandragiri' for achieving the long-lasting resistance to leaf rust, have been evaluated in field. Two hybrids (S.5083 and S.5084) were derived from a donor heterozygous to $S_H3$, while the other two hybrids (S.5085 and S.5086) were developed from donor homozygous to $S_H3$. Among the hybrids, S.5086 recorded superior yield performance during individual years with a maximum yield of 1611 kg/ha during 2020-21 and the four year mean yield of 1313 kg/ha. The hybrid exhibited maximum heterosis over mid parent (29.10%) and better parent (17.12%) and S.5086 progeny also manifested high field tolerance to leaf rust pathogen as the entire plant population was free from the disease incidence throughout the study period. The findings of the present study established the efficiency of $F_1$ breeding strategy with simultaneous pyramiding of rust resistance genes for development of vigorous, high yielding and durable rust resistant $F_1$ hybrids in arabica. The $F_1$ hybrid, S.5086 with promising performance in terms of crop yield and high field tolerance to leaf rust has potential implications for commercial exploitation.**

**Key words**: Coffee, $F_1$ hybrids, durable resistance, pyramiding and $S_H3$ gene

## INTRODUCTION

Coffee, the most popular beverage crop is cultivated in over 50 countries across continents with high export potential. The foreign exchange revenues from coffee exports constitute substantial share in the national economies of the producing countries. The turnover of the world coffee industry is over $ US 40 billion annually and the industry provide direct employment as well as livelihood opportunities to approximately 20 million people in cultivation, on-farm processing, value addition and trade. India is the 7$^{th}$ largest producer of coffee with a cultivated area of 4.59 lakh ha and annual average production of 3.20 lakh MT, which accounts for about 3.5% of the world coffee production and 4.5% share in global exports. Commercial coffee comes from two species viz., *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner that are popularly referred as arabica and robusta coffees, respectively. Arabica coffee types manifest susceptibility to major diseases and pests while robusta is more tolerant to these diseases and pests but the bean and liquor quality of robusta is inferior to arabica. Among the various diseases that affect arabica, coffee leaf rust (CLR) caused by an obligate parasitic fungus, *Hemileia vastatrix* Berk et Br is the most prominent one that cause severe crop losses to an extent of 70% in susceptible cultivars, if proper control measures are not adopted (Anon., 2014). The wet weather with intermittent rainfall & sunshine, relative humidity over 80% and temperatures between 22$^0$ & 24$^0$C are the ideal conditions for CLR flare-ups.

Therefore, breeding for rust resistance has been pursued on highest priority in several arabica growing countries including in India, that resulted in development of several coffee varieties manifesting varying levels of resistance to CLR. However, because of the adaptive ability of the *H. vastatrix*, breakdown of resistance under field conditions has been experienced in the commercial varieties due to evolution of new virulent rust races from time to time. At present, as many as 45 different physiological races of rust with ability to infect different coffee genotypes are distributed in various coffee-growing countries (Rodrigues *et al*., 1993 and Prakash *et al*., 2005). The weather conditions that prevail in Indian coffee growing regions are highly favourable for leaf rust pathogen. As a result, high disease build up as well as race mutation leading to the evolution of new virulent races of rust with ability to overcome the resistance in varieties released for cultivation has been a common phenomenon. At present, over 35 different rust races are found distributed in coffee tracts of India. Therefore, development of varieties with long lasting resistance in field is the priority of arabica coffee breeding in India.

The host resistance to coffee leaf rust is reported to be governed by nine resistance genes, designated as $S_H1$ to $S_H9$, distributed across the coffee gene pool. Among the commercially cultivated species, four resistance factors ($S_H1$, $S_H2$, $S_H4$, $S_H5$) were identified in *C. arabica* the only tetraploid species of the genus while four other factors ($S_H6$, $S_H7$, $S_H8$, $S_H9$), were reported from diploid species, *C. canephora* and $S_H3$ factor from another diploid species *C. liberica* [Wagner and Bettencourt, 1965 ; Vishveshwara, 1974 ; Bettencourt and Rodrigues, 1988]. It has been well established that the resistance genes identified in *C. arabica*, are less durable under field situations while the genes viz., $S_H6$, $S_H7$, $S_H8$, $S_H9$ introgressed from the diploid species either from *C. canephora* or from *C. liberica* ($S_H3$) are found to manifest durable resistance. Hence, the resistance breeding strategies have been mainly focused on pyramiding of resistance genes of diploid origin into selected arabica genotypes by using the natural inter-specific hybrids as donors. In order to expedite the pyramiding of resistance genes into outstanding cultivars by conventional breeding and to reduce the required time frame, $F_1$ breeding strategy is gaining significance. Further, because of the dominant nature, the resistance genes are expected to express in $F_1$s resulting in long lasting resistance. With this objective, development of $F_1$ hybrids has been pursued as a priority of Arabica coffee breeding in India since 2011. The present communication highlights the field performance of four such $F_1$ hybrids in respect to vegetative vigour, yield and field tolerance to leaf rust.

## MATERIALS AND METHODS

The plant material included in the present study comprised of four $F_1$ progenies, S.5083, S.5084, S.5085 and S.5086 generated from reciprocal crosses between two arabica genotypes, 'Chandragiri' and Sln.10 (Table 1). Two elite plants of Sln.10, characterized as homozygous and heterozygous for $S_H3$ gene for rust resistance were used in crossing programme, in order to validate the differential response of hybrids, if any. All the four progenies were planted in a compact plot at research farm of Central Coffee Research Institute, Balehonnur, Karnataka, India (13º 22' N, 75º 25' E at an elevation of 2787 MSL), during the year 2012. The weather data pertaining to the study period is furnished in Table 2. In all, 60 plants per progeny were planted in a conventional square design at a spacing of 5'x5' under a two-tier shade canopy, the top cover of evergreen natural forest trees and lower canopy comprising of fast-growing leguminous trees like *Erythrina lithosperma* Miq. (Dadap). The plants were trained on topped single stem system and standard agronomic practices recommended for semi-dwarf arabica genotypes were followed. The progenies have been evaluated for vegetative vigour, field tolerance to leaf rust and crop yield.

**Table 1. Details of parents and cross combination of $F_1$ hybrids**

| Accession No. | Parents/$F_1$ cross combination and their response to leaf rust pathogen | |
|---|---|---|
| S.3827 | Sln.10 (double cross hybrid) {Caturra x Cioccie} x {Caturra x S.795} | Susceptible to 5 races of rust; Selected two plants homozygous and heterozygous to $S_H3$ gene (donors for $S_H3$ gene introgressed from a diploid species *C. liberica* and also for good liquor quality attributes) |
| S.4202 | Chandragiri | Resistant Plant selected from the base population that remained tolerant to all prevailing races of rust in India. It's a *C. canephora* introgressed line. |
| S.5083 | Chandragiri | Sln.10 – Heterozygous to $S_H3$ |
| S.5084 | Sln.10 - Heterozygous to $S_H3$ | Chandragiri |
| S.5085 | Chandragiri | Sln.10 – Homozygous to $S_H3$ |
| S.5086 | Sln.10 - Homozygous to $S_H3$ | Chandragiri |

### Evaluation of agronomic characteristics

For agronomic evaluation in respect of vegetative vigour, data was collected from 20 plants for each progeny (five plants per replication) for three crop seasons till the bush canopy was totally covered. For assessment of vegetative vigour, data on the growth parameters such as stem girth, bush spread, number of primary branches, length of the longest primary, number of nodes per primary, inter nodal length and yield component characters such as number of bearing nodes per primary, number of fruits per node was recorded. The fruit yield per plant and progeny yield was recorded during harvest season i.e., Nov. - Dec. for four crop seasons from 2017 to 2020. Data was also collected from Chandragiri and Sln.10 the parents.

**Table 2. Weather data pertaining to the study period (2017-2020)**

| Months | 2017 | | | 2018 | | | 2019 | | | 2020 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tem. | Rainfall | RH% | Tem. | Rainfall | RH% | Tem. | Rainfall | RH% | Tem. | Rainfall | RH% |
| January | 22 | 0 (0) | 67 | 23 | 0 (0) | 81 | 21 | 0 (0) | 82 | 23 | 0(0) | 74 |
| February | 24 | 0 (0) | 65 | 24 | 0 (0) | 79 | 25 | 0 (0) | 62 | 24 | 0(0) | 61 |
| March | 26 | 13 (0) | 65 | 26 | 26 (4) | 82 | 25 | 24 (3) | 79 | 25 | 23(2) | 72 |
| April | 27 | 48 (4) | 74 | 26 | 108 (16) | 78 | 27 | 59 (5) | 77 | 26 | 77(9) | 80 |
| May | 25 | 195 (10) | 76 | 25 | 356 (17) | 82 | 26 | 39 (6) | 87 | 25 | 219(14) | 84 |
| June | 23 | 404 (23) | 85 | 23 | 692 (20) | 88 | 25 | 199 (20) | 87 | 23 | 270(20) | 89 |
| July | 22 | 556 (30) | 88 | 22 | 1179 (28) | 86 | 24 | 530 (28) | 89 | 22 | 440(27) | 88 |
| August | 23 | 560 (20) | 87 | 21 | 1169 (20) | 91 | 23 | 1204 (31) | 86 | 21 | 1057(20) | 87 |
| September | 24 | 261 (17) | 86 | 23 | 128 (10) | 88 | 23 | 538 (21) | 88 | 22 | 466(17) | 88 |
| October | 24 | 109 (6) | 87 | 23 | 150 (8) | 84 | 24 | 431(22) | 88 | 22 | 163(11) | 85 |
| November | 23 | 11 (2) | 82 | 23 | 28 (2) | 78 | 25 | 48(2) | 84 | 21 | 44(5) | 79 |
| December | 22 | 1 (1) | 69 | 22 | 25 (1) | 78 | 25 | 9(1) | 85 | 21 | 1(1) | 85 |

Tem.-Average temperature, Rainfall - rainy days in parenthesis, RH-Relative Humidity

**Data analysis and estimation of heterosis among $F_1$ hybrids**

Analysis of variance was carried out for each character as suggested by Gomez and Gomez (1984). Wherever the treatment differences were found significant, Critical Differences (CD) were worked out at five per cent probability level and values were furnished. The treatment differences that were not significant are indicated as 'NS'.

Relative heterosis and heterobeltiosis manifested by each $F_1$ hybrid was calculated over mid parent (MPH%) = [($F_1$-MP)/MP x 100] and better parent, (BPH%) = [($F_1$-BP/BP x 100], respectively.

**Evaluation of Coffee Leaf Rust (CLR) incidence:**

Observations on leaf rust incidence were recorded during peak periods of disease expression i.e., May-June months during pre-monsoon season and Sept.-Oct. months during post-monsoon season, for four successive years, from 2017 to 2020. Data on CLR incidence was recorded on individual plants and plants with less number of pustules were also treated as susceptible to assess the population susceptible/ resistant in each progeny. The disease build-up on susceptible plants was also scored using the 0-9 scale of Eskes and Toma-Braghini (1981 cf Eskes 1989), where 0 = plants are free from the symptoms; 1= presence of one diseased branch. Likewise, grades were assigned based on progression of disease and 9= maximum disease incidence. Finally, the plants were grouped into four categories viz., 1= tolerant (free from CLR incidence), 2 = moderately tolerant (mild infection without any defoliation); 3= susceptible (medium levels of incidence) and 4= highly susceptible (high disease build up coupled with defoliation).

## RESULTS AND DISCUSSION

**Agronomic Evaluation – Growth characters**

All the four $F_1$ hybrid genotypes evaluated in the present study exhibited vigorous vegetative growth with compact bush stature which is expected when both the parents are semi-dwarfs. The data in respect of vegetative, yield and yield component characters is presented in Table 3.

**Table 3. Character means and analysis of variance for different morphological and yield component characters among different $F_1$ hybrids**

| $F_1$ hybrid/ parental line | Stem girth (cm) | Bush spread (cm) | No. of primary branches | Length of longest primary (cm) | No. of nodes per primary | Internodal length (cm) | No. of bearing nodes/ branch | No. of fruits per node | Avg. fruit yield per plant (kg) |
|---|---|---|---|---|---|---|---|---|---|
| S.5083 | 38.5 | 349.5 | 19.1 | 98.9 | 20.0 | 5.2 | 9.9 | 14.7 | 1.95 |
| S.5084 | 39.5 | 366.1 | 19.1 | 98.5 | 20.2 | 4.9 | 8.8 | 14.9 | 1.78 |
| S.5085 | 37.0 | 333.7 | 19.8 | 94.3 | 19.5 | 4.7 | 9.1 | 14.0 | 1.89 |
| S.5086 | 36.1 | 341.2 | 19.8 | 94.3 | 19.2 | 4.6 | 8.9 | 13.7 | 1.98 |
| Chandragiri (Parent 1) | 36.2 | 322.2 | 20.0 | 87.4 | 19.1 | 4.9 | 8.6 | 12.3 | 1.69 |
| Sln.10 (Parent 2) | 37.3 | 341.6 | 21.3 | 92.3 | 19.0 | 5.0 | 8.7 | 12.9 | 1.37 |
| SeM± | 1.9 | 12 | 0.5 | 5.3 | 1.5 | 0.7 | 0.6 | 0.5 | 0.14 |
| CD @ 5% | NS | NS | NS | NS | NS | NS | NS | NS | NS |

From the data it is apparent that the hybrids exhibited uniform vegetative vigour with marginal differences in various characters. Among the $F_1$ progenies, S.5084 recorded superior growth in terms of stem girth (39.5 cm), number of nodes (20.2), bush spread (366.1); number of fruits per node (14.9). In contrast, S.5086 recorded more compact growth pattern as reflected in most of the growth parameters recorded, compared to other $F_1$ hybrid progenies (Table 3). Analysis of variance carried out for each character revealed that the character means among different hybrids are significant at $P<0.05$ with their parent populations except number of nodes per primary. These variations in terms of growth characters can be attributed to the growth pattern of the parents and their cross combination used for generation of hybrids. Prakash *et al* (2006) evaluated 17 elite hybrid progenies for growth parameters, field tolerance to rust, clean coffee

yield and bean characteristics in a specific agro-climate in India. Analysis of variance of five morphological characters revealed significant differences between the genotypes in respect of four characters (stem girth, bush diameter, length of longest primary and number of primaries per plant) establishing that the genotypes are moderately heterogeneous for plant architecture. Heritability was high (60%) for yield and 64% for field tolerance to leaf rust indicating low genotype x environment interaction for these traits.

**Heterosis for crop yield**

Data on year wise clean coffee yield among the $F_1$ progenies and the parents is presented in Table 4. In general, the production trend over the years from 2017-18 to 2020-21 has shown alternate bearing pattern which is common in Arabica coffee. Further, there has been an increase in quantum of yield both in parents and hybrids over the years. All the hybrids recorded higher yields over parents both during the on and off years. Statistical analysis of year wise yields showed significant differences among different genotypes and also the parents (Table 4). During the lean cropping years of 2017-18 and 2019-20, the mean crop yields (clean coffee) among the four hybrid progenies varied from 996 kg/ha (S.5083) to 1102 kg/ha (S.5086) and 1161 kg/ha (S.5085) to 1232 kg/ha (S.5086), respectively. During the high cropping years i.e., 2018-19 and 2020-21, the mean yields ranged from 1233 kg/ha (S.5084) to 1424 kg/ha (S.5083) and 1415 kg/ha (S.5084) to 1611 kg/ha (S.5086). Among the hybrids, S.5086 recorded superior and consistent performance during individual years with a maximum yield of 1611 kg/ha during 2020-21 and the four year mean yield of 1313 kg/ha.

Among the $F_1$ hybrids, the relative heterosis (MPH%) ranged from 15.83% (S.5084) to 29.10% (S.5086) while heterobeltiosis ranged from 5.08% (S.5084) to 17.12% (S.5086). Among the four $F_1$ hybrids, S.5086 recorded superior yield performance.

Among the two parental lines, the yield in 'Chandragiri' ranged from 887 kg/ha (2017-18) to 1303kg/ha (2020-21) while in Sln.10, the year wise yields ranged from 741 kg/ha to 1041 kg/ha. Analysis

**Table 4. Year wise clean coffee yields in $F_1$ hybrids and parental lines**

| $F_1$ hybrid progeny/Parents | Clean coffee yield (kg/ha) | | | |
|---|---|---|---|---|
| | 2017-18 | 2018-19 | 2019-20 | 2020-21 |
| S.5083 | 996 | 1424 | 1179 | 1557 |
| S.5084 | 978 | 1233 | 1086 | 1415 |
| S.5085 | 1069 | 1325 | 1161 | 1457 |
| S.5086 | 1102 | 1307 | 1232 | 1611 |
| Chandragiri | 887 | 1224 | 1070 | 1303 |
| Sln.10 | 741 | 987 | 883 | 1041 |
| SEm ± | 31.17 | 73.17 | 55.56 | 46.70 |
| CD at P = 5% | 93.96 | 220.56 | 167.48 | 140.77 |

of relative heterosis and heterobeltiosis revealed that all the hybrids exhibited maximum relative heterosis (MPH%) that ranged from 20.7% (S.5084) to 37.5% (S.5086) during the high cropping year, 2020-21 except in S.5085. As regards to heterobeltiosis, all the hybrids except S.5083 recorded high BPH% that ranged from 10.26% (S.5084) to 24.24% (S.5086) during the low cropping year, 2017-18. These differences in relative heterosis and heterobeltiosis, could be attributed to the yield of the parents during the respective years. Among the four $F_1$ hybrids, S.5086 recorded high BPH% in all the years that ranged from 15.14% (2019-20) to 23.64 (2017-18) except during 2018-19.

Bertrand *et al.* (2005) evaluated the performance of $F_1$ hybrid plants derived from *C. arabica* for production variables and reported that the $F_1$ hybrids produced between 22% (trial 1) and 47% (trial 2) more fresh berries than the parental lines in two separate trials. This difference was highly significant for trial

2 ($P = 0.00$). From the studies on genetic parameters of Timor hybrid derived Arabica genotypes at IAC, Brazil, Mistro *et al*. (2007) reported that the greatest yield gains were achieved when selection was performed based on plot means and years of high yields. It was reported that under normal climate conditions, coffee yields usually increase from the first until the fourth/fifth year. Thereafter, the biennial yield cycles begin, characterized by the alternate high and low yields. From the results of the present study, it is apparent that both the parental lines and the hybrids reflected the alternate bearing behaviour. However, the consistency in production has been recorded in the hybrids in corresponding on and off years of production, alternatively.

Dula (2019) reviewed the heterosis and combining ability studies for yield of *Coffea arabica* varieties in Ethiopia. From the studies conducted by Mesfin and Bayetta (1983), the extent of heterosis for yield was up to 60% over better parent. Out of nine $F_1$ hybrids, only one hybrid exhibited negative heterosis of -8%. The highest yielding hybrids Melko-CH2 and Ababuna showed 20% and 18% heterosis over the better parent respectively. Given the fact that the genetic distance and combing ability of the parental lines is critical for achieving the maximum extent of heterosis in $F_1$ hybrids, the heterobeltiosis to the extent of 23.64% over better parent and 37.5% over mid parent for yield in best performing $F_1$ hybrid (S.5086) in the present study is a significant point to consider for commercial exploitation.

**Coffee leaf rust incidence**

Data on coffee leaf rust (CLR) incidence among the four $F_1$ hybrids and parental lines is furnished in Table 5. Among the hybrids, the leaf rust incidence ranged from nil (S.5086) to 51% (S.5083) while in the two parental lines, the CLR incidence ranged from 2.8% to 30% in Chandragiri and 22% to 73% in Sln.10 during different years of study. From the data in respect of individual $F_1$ hybrids, it is apparent that the two hybrid progenies, S.5083 and S.5084 recorded relatively high susceptibility as 46.1% and 50.9% of population manifested susceptibility during 2019-20, the year that recorded high rust flare up due to favourable weather conditions. The parental lines also recorded maximum susceptibility (30% in Chandragiri and 73% in Sln.10) during the high rust year (2019-20). In contrast, the remaining two $F_1$ hybrid progenies (S.5085 and S.5086) maintained high levels of field tolerance as the entire population of these two hybrids are free from the rust incidence. The high rust incidence manifested during the 2019-20 could be attributed to the favourable weather conditions i.e., high rain fall coupled with maximum number of rainy days during July and August months as well as ideal temperatures ($19^0$C to $24^0$C). The quantum of rainfall though higher during July and August 2018, the number of rainy days were low thereby the rust incidence was relatively low compared to 2019. However, inspite of the favourable weather conditions during 2018 and 2019, the disease build up levels were recorded to be very low, in the hybrids S.5083, S.5084 and parent variety 'Chandragiri', the disease build up was characterized by the non-sporulating necrotic spots (group 2), indicating the high levels of tolerance. Further, the high levels of field tolerance to leaf rust in $F_1$ hybrid progenies (S.5085 and S.5086) could be attributed to the integration of the $S_H3$ gene in these hybrid populations as the Sln.10 parent used as $S_H3$ donor is homozygous to $S_H3$.

In fact, the very objective of the $F_1$ hybrid breeding programme was to pyramid the maximum number of $S_H$ genes for rust resistance in a proven commercial arabica genotype to ensure long lasting resistance to leaf rust. The resistance in Chandragiri is governed by the $S_H$ genes of tetraploid arabica origin ($S_H1,2,4,5$) and $S_H$ genes ($S_H6$, $S_H7$, $S_H8$, $S_H9$) introgressed to *C. arabica* from diploid species, *C. canephora* (robusta coffee). The $F_1$ hybrids evaluated in the present study were developed with the primary aim of pyramiding of $S_H3$ gene of *C. liberica* origin in order to improve durability of resistance in Chandragiri. Two plants of Sln10, homozygous and heterozygous to $S_H3$ gene were consciously selected and used as donor parents (paternal) in crossing programme. Apparently, the two hybrid progenies, S.5083 and S.5084 were derived from the heterozygous donor while the other two hybrid progenies, S.5085 and S.5086 were developed from the homozygous donor plant. Thus, the variability for field tolerance to leaf rust in these four $F_1$ hybrid progenies, could be attributed to the differences in pyramiding/ integration of $S_H3$ gene from heterozygous and homozygous donor plants. Analysis of the selected $F_1$ plants representing the susceptible and resistant plants in different hybrid progenies with SCAR marker linked to $S_H3$ gene confirmed the presence of the $S_H3$ gene (un-published

data). This inference lend credence from the findings of Shigueoka *et al*. (2014) evaluated nine arabica coffee progenies with an objective to select high-yielding coffee progenies with resistance to coffee leaf rust for the state of Parana in Brazil. It was reported that the plant population of several genotypes derived from 'Sarchimor' and 'Catucai' were susceptible to coffee leaf rust and complete resistance was broken in several coffee plants of 'Catucai' germplasm. However, the interesting observation reported was that the genotype '$F_6$ of Catuai x (Catuai x BA-10 coffee)' probably a carrier of $S_H3$ gene manifested complete resistance in more than 80% plants and inferred that the genotypes were heterozygous to $S_H3$ gene. Infact, there were several other earlier reports from Brazil that the coffee genotypes carrying $S_H3$ gene manifested complete resistance to leaf rust (Fazuoli *et al.*, 2005, Pereira *et al.,* 2005. Sera *et al*., 2007). The findings of the present study also in conformity with that of the earlier reports on high levels of field tolerance manifested by the two $F_1$ hybrids, S.5086 & S.5085 derived from crosses employing Sln10 plant homozygous to $S_H3$ gene.

**Table 5. Leaf rust incidence (% population) in different $F_1$ hybrids**

| Acc. No | 2107-18 | 2018-19 | 2019-20 | 2020-21 |
|---|---|---|---|---|
| S.5083 | 10.3 | 44.5 | 50.9 | 10 |
| S.5084 | 8.2 | 12.8 | 46.1 | 11 |
| S.5085 | 0.0 | 0.0 | 0.0 | 5.0 |
| S.5086 | 0.0 | 0.0 | 0.0 | 0.0 |
| Chandragiri | 2.8 | 20 | 30 | 30 |
| Sln.10 | 22.0 | 60 | 73 | 60 |

## CONCLUSION

The findings of the present study are of high applied value and clearly established the efficiency of $F_1$ breeding strategy for development of vigorous, high yielding and durable rust resistant $F_1$ hybrids in arabica. The extent of heterosis in arabica coffee is found to be dependent on the genetic distance and combining ability of the parental genomes. The two $F_1$ hybrids, S.5086 & S.5085 that recorded promising performance in terms of crop yield coupled with high field tolerance to leaf rust have potential implications for commercial exploitation.

## ACKNOWLEDGEMENT

The authors are grateful to the Director of Research, Central Coffee Research Institute, Coffee Board, for the support and encouragement received throughout the research study.

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***(Received on 23.05.2021, Revised on 24.07.2021 and Accepted on 27.07.2021)***

*J. Hortl. Sci.*
*Vol. 16(2) : 309-314, 2021*

***Original Research Paper***

# Limonene extraction from the zest of *Citrus sinensis, Citrus limon*, *Vitis vinifera* and evaluation of its antimicrobial activity

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## ABSTRACT

**Citrus rinds contain essential oils. One of the major constituents of the essential oils in the zest of different fruits like *Citrus sinensis, C. limon,* and *Vitis vinifera* is limonene. In this research, limonene was extracted by hydro-distillation method using Clevenger set up and its antimicrobial activity against certain bacterial and fungal strains was determined by using Kirby Bauer's disc diffusion method. The primary antimicrobial screening of limonene without dilution exhibited a zone of inhibition (mm) comparable to Ampicillin (20mg/ml) and Amphotericin B (20mg/ml). The effect of pure limonene against all strains used was high as compared to the isolated samples. The MIC values also showed an expected decrease in the zone of inhibition from 1:2 to 1:8 dilutions. Based on this study, the cost-effective isolation of limonene and other essential oils is quite possible.**

**Keywords:** Anti-microbial citrus, hydro-distillation and limonene

## INTRODUCTION

Citrus fruits are generally cultivated throughout the world. A substantial load of waste is produced after their processing. An estimated amount of Citrus mash (CM) generated in South Korea was reported to be more than sixty thousand tons. Not only the edible part but also the citrus peels are well known for their phytochemical, polyphenolic, vitamin, and essential oil contents (Mahato *et al*., 2017). One of the major components of citrus waste is the limonene. In citrus, the outer skin known as zest contains the majority of the Limonene, whereas, traces of limonene can be isolated from the inner white kernel. Limonene is a colourless cyclic monoterpene with chemical formula C10H16 having 1-methyl-4(prop-1-en-2-yl) cyclohex-1-ene as its IUPAC name and commonly used in food and pharmaceutical industries (Atti-Santos *et al*., 2005). It derives its name from the lemon as it can be derived from lemon peel. Limonene in its two optical forms is not only found in the citrus fruits but is known to exist in about 300 plant essential oils (Bacanli *et al*., 2015). This ten carbon compound is known to be volatile in nature and is also prone to oxidation. This oxidation generally occurs at the time of extraction and packaging. Limonene has found a wide range of applications as a flavoring and fragrance agent in many products, such as perfumes, beverages, detergents and soaps (Erasto and Viljoen, 2008). Besides this, limonene is well known for its insecticidal properties as it can penetrate the insect body through the respiratory system (fumigation), the cuticle (contact effect), or the digestive system (ingestion effect)(Prates *et al*., 1998) .

Limonene has a characteristic citrus odor and is colorless with a molar mass of 136.24g/mol. Since it has a chiral center with four functional groups around so, it exists in two isomeric forms I, e d-limonene and l- limonene. The d-limonene is common in lime, lemon, orange, tangerine, etc. It has a density of about 84kg/m3 which is lesser than the water I, e 997kg/m3 (Erasto and Viljoen, 2008).

The presence of this 10 carbon compound is also attributed to various plant genera like *Lippia* (frog fruit) and *Artemisia* (Mugwort, Sagewort). This optically active compound is generally found in its d-form. However, l-form is also found in *Pinus* and *Menthe* species. Limonene has been found to exhibit herbicide and antifeedant properties and acts as an

attractant for pollinators. This compound with its two isoprene units has tremendous antioxidant properties as it saturates the pulmonary membrane and gives protection against endogenous and exogenous oxidant agents like ozone (Erasto and Viljoen, 2008). Limonene can generally be extracted by various methods like steam distillation, cold press, solvent extraction, and hydro-distillation using the Clevenger system.

The emergence of drug resistant bacterial and fungal species poses a serious global public health concern. Hence, it is imperative to search for novel compounds to contain these pathogens. In this regard phytochemicals can be very useful. Hence, in this study antibacterial and antifungal properties of limonene extracted from *Citrus sinensis, C. limon,* and *Vitis vinifera* were evaluated.

## MATERIAL AND METHODS

### Sample collectiona and extraction:

The samples of the known variety of *C. sinensis, C. limon,* and *V. vinifera* were collected from the local street vendor in the commonly used polythene bag. The samples were washed initially with tap water and then with distilled water to make the outer surface sterilized (Shaw *et al*., 1997).

The fresh orange (1000g), Lemon (500g), and grape (500g) after surface sterilization were gently pressed against the grater to remove the zest without removing any part of the white flesh. The peel of *V. vinifera* was obtained manually. Then the peels of *V. vinifera* and zest of *C. sinensis* and *C. limon* were transferred to round bottom flasks (RBF). After that water was added into the RBF along with porcelain chips to prevent splashing of the zest and peels to the neck and allow smooth boiling (Gelb *et al*., 1995; Malko and Wróblewska, 2016). The distillation process was proceeded at a temperature of 70°C. The distillate was collected after one and a half hours and then continuing the process again. The distillate was left undisturbed for about 20 minutes and then removed carefully using a pipette.

### Limonene isolation and detection

The crude essential oil rich in limonene isolated from zest of *Citrus sinensis, Citrus limon,* and *Vitis vinifera* were detected by comparing the odour of the pure limonene with that of the isolated samples. Furthermore, the detection of crude limonene was performed by comparing the pH with that of pure limonene (Lopez-sanchez and Pagliaro, 2014). For the detection of the crude limonene from the zest of *C. sinensis* (OIL)*, C. limon* (LIL), and *V. vinifera* (GIL) biochemical assays such as Iodine test and Bromine test as described by Wang and Weller, 2006, and Asbahani *et al*., 2015, respectively.

### Culture revivaland antimicrobial activity determination

The strains which include gram-positive (*B.subtilis, M.luteus, and S. aureus*), gram-negative (*E.coli, K.pneumoniae, A.hydrophila, and P.pituda*), and fungal (*C.albicans, C.parapsilosis and S.cerevisiae*) were obtained from microbial type culture collection (MTCC) (Lucchesi *et al.*, 2004)**.** All the bacterial and fungal cultures were initially revived in the nutrient broth and yeast extract peptone dextrose (YEPD) broth, respectively and growth was checked as per the method described by Bhattacharya *et al*., (2014).

After the revival of microbes the antimicrobial activity of the crude essential oils rich in limonene was determined by using Kirby Bauer's disc diffusion method (Akhtar *et al*., 2017a; Akhtar *et al*., 2017b; Choudhury *et al*., 2017). In brief the microbes were grown overnight at 37°C and 28°C in Nutrient broth and YEPD broth for bacterial and fungal species respectively. Then 100 µl of the culture were spread on Nutrient agar and YEPD agar plates. Then, sterile filter discs containing different concentration of the essential oils rich in limonene extracted from zest of *C. sinensis, C. limon* and *V. vinifera* were placed on the plates. The plates were further incubated at 37°C for bacterial species and 28°C for fungal species. After the incubation, the zone of inhibition was measured. Ampicillin was used as control for bacterial species and Amphotericin B was used as control for fungal species.

## RESULT AND DISCUSSION

### Isolatoion and detection of limonene

Distillate obtained after Hydro-distillation is given in the table 1. The pH of the crude essential oils was almost the same as that of pure limonene. The color change during the biochemical assays such as Iodine test and Bromine water test also confirmed the presence of limonene in the crude essential oils (Figure 1 and Figure 2). The limonene content in orange zest, limonene zest, and grape peels were 2.0 mL, 2.7mL and 3.46mL for every 100 g of samples respectively.

**Table 1. Distillate obtained after Hydro-distillation**

| Fruit name | Zest weight (g) | Solvent added (ml) | Tempe-rature (°C) | Initial distillate (ml) | Limonene volume (ml) |
|---|---|---|---|---|---|
| Orange | 150 | 150 | 70°C | 13 | 3 |
| Lemon | 90 | 200 | 70°C | 10 | 2.5 |
| Grape | 130 | 150 | 70°C | 16 | 4.5 |

**Microbial susceptibility**

The maximum anti-microbial activity was shown by the pure limonene (97%) for all the tested strains. Among the ten strains, the maximum effect of pure limonene was found in the case of *B. subtilis* with a zone of inhibition (ZOI) measuring about 12mm. The minimum effect was revealed against *C. albicans* with ZOI measuring about 3mm (Karr, 1989). On comparing the results of three crude essential oil isolates the maximum effect was shown by the OIL and LIL against *K. pneumoniae* (7mm) and *S. aureus* (8mm) respectively. The crude essential oil rich limonene isolated from the grape peels showed good positive effects against *E.coli* (0.7mm) and *B. subtilis* (0.5mm) while it showed ZOI in the range of 1mm-3mm against all other strains (Figure 3, 4, 5) (Bhattacharya *et al*., 2014) . However, GOl, was found to be ineffective against all the fungal strains. So, pure limonene showed the maximum anti-microbial activity in comparison to the three isolates, whereas, the OIL and LIL showed maximum effect against gram positive bacteria than those of gram negative bacteria. The effects shown by the limonene are given below (Table 2):

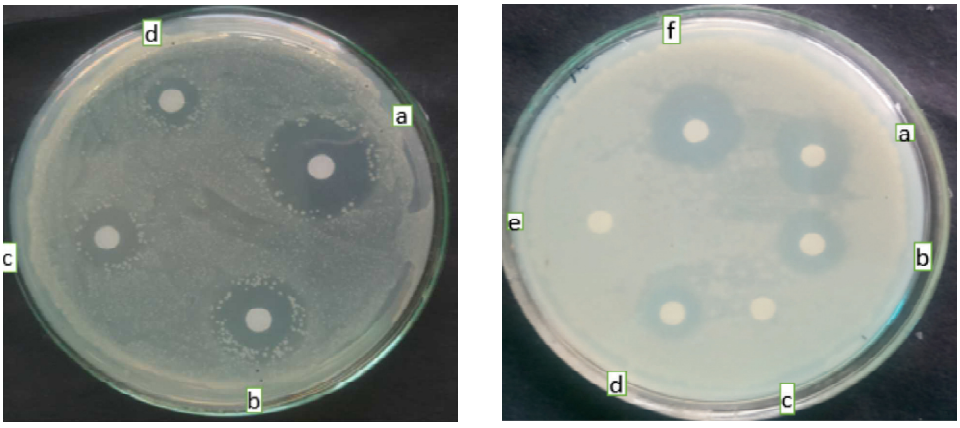


**Figure 1. The antimicrobial activity of (i) pure limonene (PL), (ii) crude essential oil isolated from C. limon (LIL), against *E. coli* at different concentration- (a). 1:2 (b). 1:4 (c). 1:6 (d). 1:8**

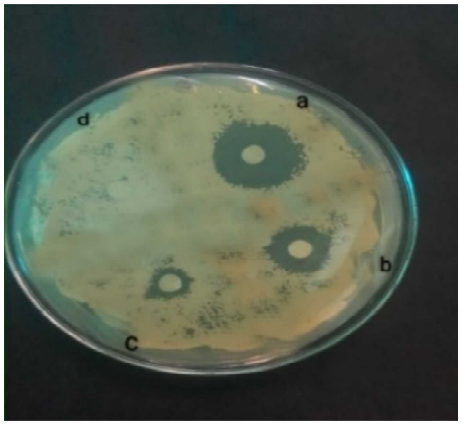


**Figure 2. Antifungal activity of essential oils against *C. parapsilosis* (a). PL (b). OIL (c). LIL (d) GIL**

**Table 2. Anti-microbial activity of essential oils against different bacteria and fungi**

| Organisms | Organism type | MTCC number | Incubation time (h) | Pure limonene | Zone of inhibition (mm) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Orange isolate | Lemon isolate | Grape isolate |
| *M. luteus* | GPB | 106 | 24 | 6 | 6 | 5 | 3 |
| *P. putida* | GNB | 102 | 27 | 5 | 6 | 4 | 3 |
| *A. hydrophila* | GNB | 1739 | 26 | 4 | 3 | 4 | 3 |
| *E.coli* | GNB | 739 | 25 | 6 | 2 | 8 | 7 |
| *S. aureus* | GPB | 3160 | 24 | 7 | 5 | 6 | 2 |
| *B. subtilis* | GPB | 121 | 24 | 10 | 4 | 2 | 5 |
| *K. pneumoniae* | GNB | 7028 | 27 | 7 | 6 | 5 | 3 |
| *C. albicans* | Fungi | 183 | 30 | 3 | 1 | 0.00 | 0.00 |
| *C. parapsilosis* | Fungi | 998 | 30 | 5 | 4 | 3 | 0.00 |
| *S. cerevisae* | Fungi | 36 | 30 | 5 | 4 | 2 | 1 |

**Microbiostatic/microbicidal effect of limonene with different dilutions:**

All those bacteria showing a zone of inhibition equal to 4mm or more were tested for determining the microbiostatic/microbicidal of a particular type of limonene. On the other hand, those with ZOI below 4mm were not tested (-). There was a continuous decrease in the ZOI of inhibition from less diluted sample to a more diluted sample (Figure 4 and 5). Besides the continuous decline in the ZOI, there were some unusual results shown by the *P.putida* for 1:2 and 1:4 LIL. Similar uncommon results were shown by *E. coli* and *B. subtilis* (Bold letters). Similar type of findings were reported in previous studies against *E. coli, P. putida,* and *S. aureus* (Akhtar *et al*., 2017a, 2017b; Bilal Ahmad *et al*., 2020). *C. parapsilosis* and *S. cerevisae* showing ZOI equal to 4mm or more for OIL and PL respectively were tested using different dilutions of these two samples. *C. albicans* was not tested because it did show susceptibility without dilution. There was a continuous decrease in the ZOI from less diluted sample to more diluted samples. The summary of microbiostatic/microbiocidal effect of crude essential oils rich in limonene with different dilutions is given in Table 3.

**Table 3. Microbio-static/Microbicidal effect of limonene with different dilutions**

| ZOI by ampicillin, PL, OIL, LIL and GIL ( mm) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Orga-nism MTCC | +ve Control 25 mg/ml | Pure limonene | | | | Orange isolated limonene | | | | Lemon isolated limonene | | | | Grape isolated limonene | | | |
| | | 1:2 | 1:4 | 1:6 | 1:8 | 1:2 | 1:4 | 1:6 | 1:8 | 1:2 | 1:4 | 1:6 | 1:8 | 1:2 | 1:4 | 1:6 | 1:8 |
| 106 | 7 | 4 | 2 | 0 | 0 | 4 | 3 | 0 | 0 | 3 | 2 | 0 | 0 | - | - | - | - |
| 102 | 5 | 4 | 3 | 1 | 0 | 3 | 2 | 1 | 0 | 3 | 0 | 2 | 0 | - | - | - | - |
| 1739 | 6 | 3 | 2 | 1 | 1 | - | - | - | - | 0 | 0 | 0 | 0 | - | - | - | - |
| 739 | 7 | 6 | 4 | 3 | 2 | - | - | - | - | 5 | 4 | 0 | 2 | 4 | 3 | 1 | 0 |
| 3160 | 7 | 3 | 2 | 1 | 1 | 4 | 3 | 2 | 1 | 3 | 1 | 1 | 0 | - | - | - | - |
| 121 | 5 | 3 | 4 | 2 | 1 | 4 | 3 | 2 | 1 | - | - | - | - | 4 | 2 | 1 | 1 |
| 7028 | 6 | 3 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | - | - | - | - |
| 183 | 1 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 998 | 5 | 0 | 0 | 0 | 0 | - | - | - | - | 0 | 0 | 0 | 0 | - | - | - | - |
| 36 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - |

## CONCLUSION

In the study crude essential oil rich in limonene were extracted from *Citrus sinensis, Citrus limon,* and *Vitis vinifera* by hydro-distillation with Clevenger set-up. The detection of limonene in these crude essential oils were confirmed by different biochemical assays. The isolated crude essential oil rich in limonene showed potential antimicrobial activity against different Gram-positive and Gram-negative bacteria as well as also three fungal species. This study shows that the limonene rich essential oils of *C. sinensis, C. limon* and *V. vinifera* has the potential to be used in drug and food industry to control various pathogens.

**Conflict of interest**: The authors declare no conflict of interest.

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***(Received on 16.01.2021, Revised on 06.07.2021 and Accepted on 05.10.2021)***

*Event Report*

# *National Horticultural Fair 2021 - A Success Story*

The ICAR-IIHR in its five decades of research on 54 horticultural crops, has released 302 varieties and licensed 104 technologies to 400 clients with 800 licenses, thus paving the way for entrepreneurship in the field of horticulture. The Institute has been popularizing the varieties by imparting training as well as by taking them to the farmers' field through conducting demonstration and field days, the effect of which can be seen in North-Eastern region of the country, wherein almost seven states have adopted the vegetable varieties of the Institute. However, to promote entrepreneurship in the field of horticulture it was decided to display the technologies developed in one place and through virtual and physical mode. Hence, 255 demonstration plots were set up. In addition to this provision was made for stalls so that other private and public institutions could display their technology. The main theme of this fair being 'Horticulture for Startup and standup India' and the main objectives were:

i. to showcase the new abiotic and biotic stress resistant technologies right from seed to input production to plant protection measures including biopesticides to post-harvest, value addition and machinery for horticulture to every stakeholder
ii. to make the stakeholders aware of the benefits of integrated farming systems, urban horticulture and the digitized seed supply system adopted by the Institute to reach them to the doorstep of the farmers,
iii. to create awareness with regard to the pollination and the need to conserve pollinators and also to highlight the importance of resistant varieties in organic farming,
iv. to make stakeholders aware of the opportunities in horticulture to become entrepreneurs through 'Atmanirbhar Krishi',
v. provide an opportunity to expose the farmers to the entrepreneurs who are successfully running their business.

The event was organized in collaboration with the Karnataka State Department of Horticulture, Karnataka State Department of Agriculture, Society for Promotion of Horticulture, Bengaluru, BESST-HORT a TBI of ICAR-IIHR, ICAR-ATARI (Zone 11) Bengaluru and The Sri Sri Institute of Agricultural Sciences & Technology Trust (AOL), Bengaluru. The modus operandi and the details of methodology followed in organizing NHF 2021 are discussed here.

## Modus operandi

The National Horticulture Fair (NHF 2021), which was held for five days from 8th to 12th February 2021 comprised of two approaches viz., Virtual mode and the Physical mode. The success of an event depends on how well it gets publicized. Publicity was carried out both at the national as well as the state level through social, print and electronic media. Forty (40) lakh messages were sent through mKisan to reach nook and corner of the country. A brochure depicting the highlights of the NHF 2021 was prepared in Kannada, Hindi and English and were widely circulated throughout the country especially among the line departments and educational institutions. Animated films depicting the technologies developed and the opportunities for entrepreneurship were widely circulated in Kannada, Malayalam, Tamil, Telugu and Hindi. Two press conferences were held with journalists representing leading dailies of various languages. Press conference was held with the leading electronic media representatives, the news on NHF 2021 was carried during prime time. Journalists were also taken for a guided tour of the demonstration plots with emphasis on the technologies showcased.

The fair was conducted on two modes viz., Virtual and Physical. To create awareness about the **Virtual mode** meeting was held with the DDG (Extension), ICAR and Directors of Agriculture Technology Application Research Institute (ATARI), which was followed up with two meetings with KVK heads, where in decision was taken to identify nodal officers from each zone. A virtual meeting with all the Directors under the Subject Matter Division of Horticultural Sciences with DDG (HS) and ADG's was conducted wherein decision was taken for cross posting the event links in their websites. Social media groups were created for KVK's and FPO's for each zone, which helped in the coordination of the activities.

Horticultural crops that are being cultivated in the 11 ATARI Zones, and the problems connected with these crops viz., seed, planting material to plant protection and post- harvest management were collected two months in advance. The problems thus collected were segregated and the compiled information was used for developing the technology videos, power point slides and for identifying experts connected with the crop, technology. Moderators for different languages were identified for the programme preparation. There were 11 moderators for 11 languages, because of which solution to the problems were clarified in local languages. The technical sessions in virtual mode were organized across 11 ICAR-ATARI Zones of the country covering 700 plus Krishi Vigyan Kendras and 600 plus FPO's, 160 Sri Sri Institute of farmers training centre's belonging to 16 states. In each login through a two-way link, around 50 to 100 farmers were mobilized at each Krishi Vigyan Kendras (KVK)/ Farmers' Producer Organization (FPO) for participation in the live technical session of the horticulture fair 2021. The setup of the virtual mode was carried out in the following way:

**Virtual platform for two-way and one-way telecast**


Pictorial representation of online logging in facilitated to attend the NHF-2021 virtually

Over 22 cameras were connected to live console, which were integrated with far end live feed. The master feed from PCR (Production Control Room) was broadcasted live on satellite as well as two-way feed to all the KVKs and FPO s in the country. The entire 5-day program was live casted on all social media platforms reaching around 18.7 lakh people. Six cameras were placed in the field to show the demonstration plots of various technologies. Cross posting of one-way link was also done through the websites of 9 Institutes. One-way participant entry was through the below platform, wherein anyone who wished to view the programme were able to do so by logging in through the registration using their mobile number.

In all, there were 11 technical sessions, which were developed after collecting the problems associated with different crops from KVK Heads. Subject matter

experts from ICAR Institutes and ICAR-ATARI's addressed the problems of the respective zones related to horticulture crops by showing the technology demonstration plots and also the videos of the farmers who have successfully grown the crop.

In different places (KVKs & FPOs) 7000 logins were observed, which accounted for 4.2 lakhs viewers, total primary source viewing being 18.7 lakhs. In all the reach from the secondary sources through the subscription to our YouTube channel (followers) was 38.2 lakhs. Hence, the total viewing was to the tune of 56.9 lakhs. The geographical reach apart from India, the programme was viewed in other countries viz., Philippines, United States, Sri Lanka, Bangladesh, Pakistan, Nepal, United Arab Emirates, Australia, Saudi Arabia, Algeria, and Kuwait.

The **Physical Mode** was carried out with the idea 'seeing is believing' and was made possible by the participation of various private and public Institutions who displayed their technologies through stalls numbering about 200. Apart from this the incubates who have taken the technology from the Institute also participated, which motivated the other farmers to become entrepreneurs. The live 255 demonstration plots having emphasis on resistant varieties and ecofriendly technologies were on display. The publicity given through the media helped in creating awareness about the NHF 2021 in the state of Karnataka and the neighboring states. The stakeholder mobilization was very effectively carried out by the State Departments. Electronic registration was also made to restrict the number of visitors.

The foot fall for the physical fair in all the five days was to the tune of 56.243. Six workshops / training programmes were conducted during the event days on; Terrace Gardening (Vegetable growing and medicinal plants for home remedies), Soilless Vegetable Cultivation, Home Scale Processing (Fruits: Lime, Orange, Vegetables: Tomato, Onion, Chilli, Flowers: Rose, Brine Preservation / Pickling), Safe Plant Health Management in Hobby Horticulture, Mushroom Products, Workshop on Hydroponics for Terrace & Peri urban horticulture, and Modern irrigation Technologies in Horticultural Crops. A total of 691 participants attended these workshops. Eleven Farmers from six different states of the country who have adopted ICAR IIHR technologies and one extension worker from Manipur who was instrumental in popularizing the ICAR IIHR technologies were felicitated during the fair.


Inauguration function of NHF-2021


Technical Session in progress

**Technology commercialization** - a goal achieved

The ICAR-IIHR during the past five decades has commercialized its technologies to more than 400 clients. The Institute developed varieties in fruits, vegetables and flower crops are being produced through 'Seed Village concept' and through private nurseries. The theme of NHF 2021 being 'Horticulture for Startup and Standup India', eight entrepreneurs took 15 technologies with an MOU.

The virtual mode was attended by the officials of ICAR Institutes, Horticulture and Agriculture Universities, Directors of State Horticulture Departments, Heads of KVKs and representatives from private industry, press personnel, farmers and students. The event had 255 demonstration plots of various varieties and technologies developed by the Institute. The problems related with the growing of horticultural crops in various regions of the country

were addressed by the experts through live video interaction with the help of the demonstration plots. A total viewing of 56.9 lakhs was recorded during the five days event, which included one way communication viewing through social media network (14.5 lakhs), video conference viewing through 7000 logins in various places of KVK's, FPO's and Sri Sri Institute of Agricultural Sciences & Technology Trust accounted for a total primary source viewing of 4.2 lakhs. The reach from the secondary sources through the subscription to our YouTube channel (followers) was 38.2 lakhs. Viewership was also noticed from 11 different countries. The footfall for the physical fair was nearly 56,000. Publicity preceding the fair by way of animated videos, press and media played a greater role in making the fair successful as commercialization of 12 technologies also took place. Various private and public institutions as well as entrepreneurs who have purchased the technology from the Institute got an opportunity to display their products.

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***(Received 07.07.2021; Accepted 31.07.2021)***

# AUTHOR INDEX - VOL. 16 ( 1&2) 2021

# SUBJECT INDEX - VOL. 16 (1&2) 2021

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(Form IV)

| | | |
|---|---|---|
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| Periodicity of publication | : | Half-yearly |
| Printer's Name | : | Mr. Ravikumar, B.A. |
| Nationality | : | Indian |
| Address | : | Resolution Print Media<br>#131, 6th Main, Meenakshinagar<br>Kamakshipalya, Bengaluru - 560 079. |
| Publisher's Name | : | Society for Promotion of Horticulture |
| Address | : | ICAR-Indian Institute of Horticultural Research<br>Hessaraghatta Lake P.O.<br>Bengaluru - 560 089 |
| Editor-in-Chief | : | Dr. S. Sriram |
| Nationality | : | Indian |
| Address | : | ICAR-Indian Institute of Horticultural Research<br>Hessaraghatta Lake P.O.<br>Bengaluru - 560 089. |
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