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Top : **Lavanya** *et al.*, P. 67
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JOURNAL OF HORTICULTURAL SCIENCES

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Short Communications

In this issue…

The farmers involved in the production of horticultural produce have defied the challenges due to second wave of the ***COVID pandemic****. Especially, the information that India's horticulture production is expected to increase by 2.93 per cent to a record level of 329.86 million tonnes (MT) in 2020-21, according to the second advance estimate of horticulture production released by the Ministry of Agriculture is very much motivating. Though many hurdles are faced by the farmers due to the prevailing* ***Covid Pandemic*** *with respect to timely marketing, storage and other issues, the horticultural production has maintained the steady growth. Wish that in other countries also the horticulture production enhances to provide the nutritional security. Journal of Horticultural Sciences takes pride in sharing the recent research developments in different disciplines of Horticulture.*

Many native vegetable crops have nutraceutical values. One of them is Moringa or commonly called as drumstick. ***Jattan et al.*** *have reviewed the traditional values, requirement for crop cultivation, crop improvement, value addition, nutraceutical and pharmaceutical values with respect to this crop.*

Many under-utilized fruit crops are brought to cultivation again with scientific interventions. One such crop is mountain sweet corn (Flacourtia montana). The fruits have high potential for processing into jam, jelly, wine, etc. and this plant has good medicinal value. ***Tripathi et al.*** *evaluated and characterized the mountain sweet corn accessions from Western Ghats and identified a suitable line with higher yield, regular bearing nature and less thorniness.* ***Ravishankar et al.*** *isolated and characterized the microsatellite markers from the under-utilized tree species Garcinia indica. In their study, 3725 microsatellites were identified and primers were designed for 1374 microsatellites. The SSR developed will be useful in studying genetic diversity, mapping and fingerprinting of Garcinia indica and related species.*

Among the leafy vegetables, Amaranthus ranks first in production. ***Agadi et al.*** *estimated the nature and extent of genetic variability among twenty Amaranthus genotypes. They found that Arka Arunima, Chikmagalur local, IC-551486, IC-551494 and IC-551466 recorded high foliage yield per plot and could be utilized in further breeding programme.****Challam et al.*** *studied the morpho-physiological parameters associated with resistance to iron deficiency induced chlorosis in potato and their effect on yield attributed. They found that genotype CP-3443) was found tolerant to Fe deficiency induced chlorosis.* ***Ayub et al.*** *evaluated responses of different okra (Abelmoschus esculentus) cultivars to water deficit conditions in Pakistan. They concluded that drought caused significant variation on physical and biochemical attributes of okra whereas the cultivar 'Sabz Pari' showed resistance towards the water stress.*

Gamma ray is an effective mutagen which creates useful variability in crops where the natural variation is very meagre and creation of variability by conventional methods is cumbersome. ***Lavanya et al.*** *studied the induced variability in cluster bean due to gamma irradiation and found that the traits like*

plant height, pod length, pod width, pulp to seed ratio showed sufficient variability. ***Sankaran et al. also*** *employed the gamma irradiation to generate variability in pummelo. They found that 60 Gy gamma dose can effectively be used for raising the mutant populations to identify a desirable mutation in pummelo*

Soil microbiome plays important role in crop production. There is need to pay attention on the nutrition management practices. They should encourage the soil microbe population that will indirectly help the plant health. ***Al-Mosour and Kalaivanan*** *have demonstrated that integrated nutrient management can maximize soil microbial community dynamics which is considered as driving force behind regulating soil processes that support sustainable sweet basil cultivation.*

While attempting to evaluate the spectral manipulations on cultivation of cut foliage crops, ***Nair et al.*** *found that coloured shade nets did not influence vase life of the cut foliage in Philodendron and observed that cultivation of Philodendron 'Xanadu' under white shade resulted in maximum cut foliage yield and quality.*

Mango ginger is an underutilized rhizomatous species that has been valued in the tropical Asian countries as a source of vegetable, spice, salad, medicine and essential oil. Huge quantity of seed rhizomes is required to promote this crop in larger area. ***Waman et al.*** *developed an in vitro protocol for the multiplication of mango ginger.*

Jamun seed is a rich source of polyphenolic compounds with antioxidant potential and alpha - glucosidase inhibitory activity. ***Arivalagan et al.*** *have optimized the methodology for the extraction of such polyphenols from jamun seeds. This will be of much in nutraceutical industry. Similarly,* ***González et al.*** *studied the post-harvest quality and quantification of betalains, phenolic compounds and antioxidant activity in fruits of three cultivars of prickly pear in Mexico. They observed that there was high correlation between antioxidant activity and phenolic compounds. The methodologies developed by them will be useful tool for the quantification of bioactive compounds fruit tissues.*

Post-harvest disease management is an important aspect in delivering the harvested produce safely to the end-user. ***Bhandari et al.*** *have recorded that application of essential oils with wax improved shelf life of sweet oranges in Nepal and this treatment enhanced juice retention, firmness, titratable acidity, vitamin C and disease reduction.*

S. Sriram
Editor in Chief

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Review

Moringa (*Moringa oleifera* L.) : An underutilized and traditionally valued tree holding remarkable potential

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ABSTRACT

Moringa (*Moringa oleifera* L.) commonly known as "drumstick tree" belongs to the family *Moringaceae*. It is now grown worldwide but its native region is India. It is a fast-growing tree that responds to low inputs and has high regeneration potential after cutting. Its nutritional value and capacity to grow economically in different soils and environmental conditions makes it a wonder tree. It is highly nutritious and each part is being utilized in various forms. It is widely cultivated for its young pods, flowers and leaves for use as traditional herbal medicine and vegetable. It is also used by indigenous people in the tropics and sub-tropics as a source of remedies. The leaves are also used as a source of fodder in many countries of the world as it can sustain green fodder availability round the year without extra efforts. Various parts of this tree are good source of ascorbic acid, calcium, iron, protein and antioxidant compounds. Hence, its remarkable properties help to fight nutritional deficiency, human diseases and improve performance of livestock.

Keywords: Antioxidants, Fodder, Livestock, Malnutrition, Moringa, Nutrients and Pharmaceutical

INTRODUCTION

Moringa (*Moringa oleifera* L.) also known as ben oil tree, benzolive tree, drumstick tree or horseradish tree is the most versatile and comes under the category of underutilized perennial plant species. All parts of this plant *i.e.* leaves, roots, flowers, pods and seeds have remarkable range of properties due to the presence of essential nutrients like vitamins, minerals, phytochemicals etc. Therefore, this plant can serve as a highly nutritious food with medicinal properties for human beings as well as fodder for livestock (Nouman *et al.,* 2014 and Masih *et al.,* 2019). Essential nutrients present in *Moringa oleifera* such as vitamins, minerals, fatty acids and amino acids are helpful in activating enzymes, hormones and osmotic adjustment in the body. Hence, enhance growth, body functions and maintenance of life processes (Anjorin *et al.,* 2010). Also, a wide range of antioxidants available in leaves, make it a valuable source of natural antioxidants, nutraceuticals and functional components (Anwar *et al.,* 2007). Therefore, *Moringa oleifera* can serve as an effective remedy for malnutrition particularly in the developing countries of the world.

In the present scenario when the cultivatable area for fodder production is decreasing day by day due to the cultivation of cash crops; *Moringa oleifera* can serve as an important source of quality fodder. This plant can be used as livestock fodder, as a supplement to enhance the dry matter intake (DMI) and digestibility of fodder in livestock. It is helpful in weight gain and enhances milk production in livestock. Fodder quality can be improved by mixing *Moringa oleifera* leaves with other fodder crops which contributes towards better livestock performance. Hence, *Moringa oleifera* can be used as nutritional supplement for livestock due to its nutritional value and good biomass production (Sanchez *et al.,* 2006).

Due to several medicinal and therapeutic properties of *Moringa oleifera* traditionally, it has been used to treat problems such as anemia, anxiety, asthma, bronchitis, skin infections in different cultures of the world. It also possesses anti-diabetic, anti-hypertensive, anti-inflammatory, anti-spasmodic and anti-tumor activities (Sharma *et al.,* 2012). Therefore, various bioactive compounds isolated from *Moringa oleifera* are utilized in medicinal field at large scale (Abalaka *et al.,* 2009). *Moringa oleifera* being rich in phytochemicals and nutrients as compared to vegetables; helps to enhance immunity. Hence, its consumption should be encouraged to strengthen immunity and cope up with malnutrition.

The biochemical composition of different parts of *Moringa oleifera* varies from location to location (Anjorin *et al.,* 2010). These variations in biochemical composition may be due to cultivation methods, environment and genetic background (Brisibe *et al.,* 2009). There is very scanty scientific information on the potential of this species to alleviate nutritional problems. There is lack of *Moringa oleifera* varieties due to less information available on genetic improvement programs for this tree (Padulosi *et al.,* 2013). Therefore, to solve the malnutrition problems such as protein deficiency in the population of developing countries of the world; further studies are needed on breeding and genetic improvement of this species. This information will be helpful in utilizing the full potential of this nutrient rich and incredible tree in various arenas of life.

ORIGIN AND DISTRIBUTION

Moringa is a member of the family Moringaceae which is comprised of thirteen species from various geographical locations (Shahzad *et al.,* 2013). It is commercially cultivated in parts of Africa and India. Four species *i.e. Moringa drouhardii* (Madagascar), *M. ovalifolia* (Namibia and southwest Angola), *M. hilderandtii* (Madagascar) and *M. stenopetala* (Kenya and Ethiopia) are characterized by bloated water-storing trunks. Three species *i.e. M. peregrine, M. oleifera* and *M. concanesis* are known for their slender trees with juvenile stage being tuberous. The remaining six species (*M. arborea, M. borziana, M. longituba, M. pygmaea, M. rivae* and *M. peregrina*) are tuberous in nature. *M. oleifera* is chiefly cultivated species of *Moringa* genus. It is a softwood deciduous tree. It is indigenous to the Himalayan foothills. It is widely cultivated in tropical regions of South Asia from northern Pakistan to northern Bengal state in India and northeastern Bangladesh, Nepal, Afganistan, Sri Lanka, West Asia, the Caribbean and sub-Saharan Africa, Latin America and reported in Florida and the Pacific Islands (Sachan *et al.,* 2010).

TREE MORPHOLOGY

Moringa oleifera tree bears spreading, open crown of drooping and fragile branches. The compound tripinnate leaves alternately bear leaflets in opposite pairs and leaf length ranges from 45-90 cm. The leaflets are dark green with red tinged midveins, entire margins and rounded at apex (Rollof *et al.,* 2009). The soft stem wood is light weight and bark is whitish-grey, thick and corky. The deep tap root system and spreading tuberous lateral roots provide support to the tree. It may flower twice in a year or round the year. The flowers have pleasant smell and these are produced in 10-25 cm long loose axillary panicles. The flowers are white or ceamy-white in colour, zygomorphic and pentamerous (Sachan *et al.,* 2010). Calyx contains five sepals which are green, lobed and tubular. Corolla comprises five narrowly spathelate, veined and creamy-white petals. Androecium is comprised of five fertile yellow stamens with alternating five smaller sterile stamens (staminodes). Gynoecium is represented by a single, stalked superior ovary with slender style. Ovary is one celled with double rows of ovules (10-25). Moringa fruit is called as pod and looks like drumstick. Pods are large (10-60 cm long) and turn brown at maturity. The seeds are rounded, one cm in diameter, dark brown to black in color with three papery wings.

TRADITIONAL VALUE

Each part of *Moringa oleifera* tree has immense potential **(Fig 1)**. It was documented in Indian Vedic literature nearly 5000 years ago (Patwardhan, 2003). In the tropical and sub-tropical regions, Moringa is highly valued by local people because of its pharmaceutical value and consumed as infusions and decoctions. This tree has served as a remedy to cure more than 300 diseases and can be regarded as a panacea. The roots have a pungent odour resembling with horseradish tree (*Armoracia rusticana*). It is used as a flavoring agent and in cardiac & circulatory problems (Mishra *et al.,* 2011). Root-bark is used as antiviral, anti-inflammatory, analgesic. The leaves are

Fig. 1: Moringa (*Moringa olefera* L.) at different growth and development stages: (A) seed, (B) direct seeded sapling in field, (C) nursery raised sapling, (D) harvesting stage for fodder, (E) flowering stage, (F) fruiting stage

used as a remedial source for the treatment of various ailments like influenza, malaria, typhoid, arthritis, inflammations, skin problems, hypertension and diabetes. The flowers are generally consumed as cooked vegetable or salad. Stem-bark and flowers are hypoglycemic. Pods are antipyretic and anthelmintic. Seeds have 30-40% oil and it is called as Ben oil which is resistant to rancidity and used in cooking (Yu *et al.,* 2005). It is also used for the treatment of hysteria, scurvy, prostate problems and bladder troubles (Fahey, 2005). Indian Ayurveda claims that Moringa oil possesses antitumor, antiepileptic, anti-inflammatory, antiulcer, antibacterial, antifungal properties and it is utilized for the treatment of different ailments in the indigenous system of medicine.

GROWTH CONDITIONS AND PLANTATION

Moringa oleifera grows very fast and it can adapt to variable ecosystems and farming systems with a temperature range of 25-35 ^{0}C. It is tolerant to drought and can be grown in sandy or loamy soils. The soils which are waterlogged or poorly drained are not suitable because it may cause rotting. The soil with a slightly acidic to slightly alkaline pH is suitable for it. An annual rainfall of 250-3000 mm is essential for its proper growth. During germination phase, it can withstand up to 3 dS m^{-1} electrical conductivity (EC). However, its resistance to salinity increases during later stages of growth. The antioxidant system of *Moringa oleifera* is responsible to tolerate moderate salinity with a mild effect on its mineral composition (Nouman *et al.,* 2012). Therefore, it can be grown in diverse environments *i.e.* hot, humid and dry with well drained soils. Although this plant can grow in versatile environments, yet the nutrient content and strength of the plant is ensured by the soil condition. Soil fertility, application of different doses and combinations of fertilizers; cause variations in nutrient composition of plant/plant parts (Dania *et al.,* 2014).

Moringa plantation can be done from seeds, cuttings or nursery saplings. However, direct cultivation through seeds is utilized when seed is sufficient in quantity. This method has high seed germination rate and takes 5-12 days for germination (Leone *et al.,* 2015). It can be grown to full-sized trees to get leaves, flowers, pods and seeds as economic parts. It can also be grown as a bush and intensively planted to get leaves as the economic part (Fig. 2). The best sowing

is the beginning of the rainy season. It ensures enough water to the growing tree during its first growing season to become well-established. After that, much water is not required. Planting method depends on available space and which economic part is to be utilized. For good seed production, 2-3 seeds per hole are planted 2-3 cm deep with 2-5 meter spacing between trees depending of the annual or perennial types. For good leaf production, dense planting should be done *i.e.*, 30 cm x 10 cm spacing. When the trees reach about 1.5 m tall, prune them down to 15 cm. The pruned rows grow back with a greater number of branches and leaves. It gives a continuous supply of leaves. Plantation can be done from cuttings having diameter 4-5 cm and length 100 cm; by digging a hole, adding manure and mounding the soil (Peter, 1978). The sprouting takes variable time depending on various factors like genotype, length of cuttings and growth conditions. However, plants grown from cuttings are sensitive to winds and drought due to lack of strong root system. It can also be cultivated through nursery raised saplings. The saplings are grown in plastic bags having sandy or loamy soils. Saplings have tender roots and hence transplanted carefully when attain the height of about 30 cm.

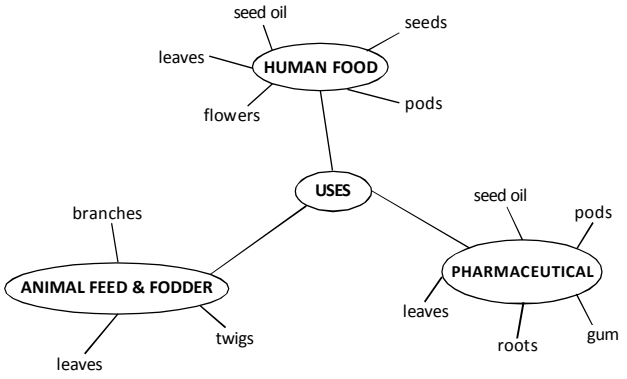

Fig. 2: Potential utilities of different parts of Moringa (*Moringa oleifera* L.)

AGRONOMIC MANAGEMENT

Field preparation for cultivation is done by adding farmyard manure (10 MT/ha) and chemical fertilizers. The application of chemical fertilizers depends on the soil condition. Generally, *Moringa oleifera* cultivation for fodder purpose needs 150 kg nitrogen, 60 kg phosphorus, 40 kg potash, 30 kg sulphur and 10 kg zinc sulphate for one hectare. Nitrogen is given in split doses; 30 kg at the time of filed preparation and later equal split doses *i.e.* after 45 days of sowing and after subsequent cuttings. Cultivation for the pod or seed purpose requires 20 kg farmyard manure per pit at the time of sowing and then application of chemical fertilizers *i.e.*, 100 g each of urea, super phosphate and muriate of potash per plant for getting higher yield (Kader and Shanmugavelu, 1982). The application of 7.5 kg farmyard manure and 0.37 kg ammonium sulfate per tree can increase yield up to three folds (Morton, 1991). For proper weed control weeding should be done at regular intervals whenever required. Irrigation requirement in Moringa depends on the rainfall. First irrigation should be given just after sowing. During early stages of development, irrigations should be managed properly for the establishment of plant. Then in later stages it should be given as per need. For the management of insect pests like hairy caterpillar; bio-pesticide like neem seed kernel extract (5 % solution) should be sprayed on the crop during infestation.

POST HARVEST MANAGEMENT AND VALUE ADDITION

Moringa plant parts can be used directly as a fresh harvest or these can be processed and value added through various methods for utilization in diverse forms. Leaves and flowers are generally shade dried and ground to a fine powder for utilization as tea or food additive. Moringa leaf dry matter in the form of powder or pellets is also utilized as a value-added product for livestock. The roots from young plants are dried after removing root bark and powdered for use as a hot seasoning base. In many countries, the use of Moringa as a food fortificant is increasing rapidly. Different parts of this tree are used in making soups (Babayeju *et al.*, 2014), weaning foods (Arise *et al.,* 2014), herbal biscuits (Alam *et al.,* 2014), bread (Chinma *et al.,* 2014), cake (Kolawole *et al.,* 2013) and yoghurt (Hekmat *et al.,* 2015). Hence, different parts of the tree put value to diversity of food items by enhancing their nutritional potential. Also, the Ben oil obtained from seeds after cold pressing or solvent extraction has been used in skin preparations and ointments since Egyptian times. The oil is used in making cosmetic products and perfumes. The oil cake is a byproduct of oil extraction and useful as an organic fertilizer. In this way, processing and value addition of Moringa leads to increase in its value and it can serve as a good source of income for small land holding farmers.

NUTRITIONAL POTENTIAL OF MORINGA

Moringa oleifera is used to fight malnutrition among infants and nursing mothers especially promising in the tropics because it can provide leaves as a source of food and fodder even in dry period when other sources are typically scanty (Folkard and Sutherland, 1996). Moringa has enormous potential as it is helpful in improving human health, weight gain and milk production in livestock.

As a food sensere care

There should be sustainability and stability in quality food supply for raising any stable community. Cereals, pulses, vegetables, fruits, meat and milk are the sources of food which fulfill our nutritional requirements. However, many of these food items are not affordable by a large population, especially those living below the poverty line. In developing countries, the diet is devoid of proteins, vitamins and minerals. Therefore, for these people; plants that are particularly nutritious represent a valuable option to fulfill such needs. *Moringa oleifera* has the potential to fulfill many of such needs Due to the multitudes of properties which are harbored by this tree species. Moringa leaves have plentiful of nutritional components. The leaves can be taken fresh, cooked or in the form of dry powder. The dry powder retains its nutritional value for many months without refrigeration. The leaves and pods are generally used in common food items. Moringa leaves are rich in vitamin A, C and E (Hekmat *et al.,* 2015). Dry leaves contain ten times more vitamin A than carrot and seven times more vitamin C than orange. Minerals i.e., iron (25 times than spinach), potassium (15 times than bananas) and calcium (17 times than milk) are also in abundance in dry powder of Moringa (Rockwood *et al.,* 2013). Moringa leaves also contain large amount of magnesium, manganese, copper, zinc and iron (Hekmat *et al.,* 2015).

Moringa leaves are rich in protein and this is cited in various studies (Thurber and Fahey, 2009). The studies also reveal that average crude protein in animal milk is much less in comparison to fresh and dry moringa leaves (Stelwagen, 2003; Chandan, 2006). Moringa leaves possess higher amino acids content than those recommended by Food and Agriculture Organization (Mendieta-Araica et al., 2011). Moringa seed meal contains large number of amino acids, except for valine, lysine, and threonine (Oliveira *et al.,* 1999). Moringa dry leaves and fresh pods contain higher contents of arginine, valine and leucine. However, some amino acids (serine, glutamate, aspartate, proline, glycine and alanine) have not been identified in dry leaves and fresh pods (Fuglie, 2000).

The phytonutrients (carotenoids, tocopherols and ascorbic acid) have also been reported in Moringa leaves which help in free radical scavenging (Dan Malam *et al.,* 2001; Saini *et al.,* 2014a; Saini *et al.,* 2014b). Moringa flowers and pods also contain appreciable amounts of carotenoids. Therefore, moringa is the best food supplement for children and infants to combat malnutrition.

The whole seeds of moringa can be eaten as such, roasted or used in powdered form. Moringa seeds have sweet to bitter taste and are consumed mostly after frying (Makkar and Becker, 1996). Moringa seed oil (Ben oil) is used for cooking and salad dressing. Ben oil in seeds is about 30-40% which is rich in unsaturated fatty acids (82%) (Ferrao and Mendez, 1970). The fatty acids present in oil are; linolenic acid, linoleic acid, oleic acid, palmitic, stearic, and behenic acid. The seeds also contain fibre, minerals, proteins, vitamins and amino acids (Kasolo *et al.*, 2010).

As animal feed and fodder

Various tree species are utilized as a fodder for livestock or supplement low quality fodder especially in the dry period (Otsyina and Dzowela, 1995). *M. oleifera* has the potential to supplement low-quality livestock fodders thereby increasing dry matter intake (DMI) and digestibility. It serves as the best example of quality fodder for increasing milk and meat production along with its environment friendly nature and low input requirements. The green fodder as well as dry matter (DM) production depends on the fertilizer, genetic make-up, season and ecological conditions (Palada *et al.,* 2007). Macronutrients which are present in abundance in Moringa play important roles like tissue building, physiological, metabolic, and biochemical processes in livestock. Magnesium (Mg) and Potassium (K) are important for lactating animals. The lactating cows require 0.17–0.20% Mg in dry matter (NRC, 1996). Magnesium (Mg) deficiency during lactating period results in low blood Mg which causes low milk yield. Similarly, beef cows require 0.70% K in dry matter. Besides essential nutrients and multivitamins, Moringa leaves also possess crude protein (21.8%), acid

detergent fibre (22.8%), and neutral detergent fibre (30.8%). The crude fat (412.0 g kg^{-1}), carbohydrates (211.2 g kg^{-1}) and ash (44.3 g kg^{-1}) have also been reported in Moringa leaves (Oliveira *et al.,* 1999). All these compounds are important in increasing livestock production. Hence, Moringa leaves which are rich source of these nutrients; fulfill the nutritional requirements of livestock in the best possible way.

Different plant parts (leaves, twigs or branches etc.) of this tree have variable concentration of nutrients. Hence, the fodder mixtures containing different proportions of Moringa plant parts provide an array of nutrition. For example, mixing of Moringa leaves with soft twigs in fodder provides lower CP and higher NDF contents, whereas mixing Moringa leaves with seed cake in fodder provides higher CP contents (Murro *et al.,* 2003 and Fujihara *et al.,* 2005). Depending upon the nutritional requirement of an animal; specific combination of Moringa plant parts can be given to the animal so that it can utilize the fodder in a best way. As for an example, moringa leaves with twigs having low crude protein can be given to dry cows, requiring low nutrient fodders.

Animals like cattle, goat, sheep, rabbits as well as pigs easily eat green leaves and stems of Moringa (Mulugeta and Fekadu, 2014). It increases the animal weight gain and protein intake (Aharwal, 2018). Positive effects of different parts of Moringa have been reported in the form of increased milk yield in cows, growth rate in sheep and feeding behavior in goat. Livestock feed supplemented with Moringa leaves can increase up to 32% of daily weight gain in livestock. Mixing of fresh Moringa leaves (15 to 17 kg) in daily feed of livestock can boost milk production by 43%. The milk production can be increased further with the supplementation of dry matter feed *i.e.*, 58 and 65% increase by 2 kg and 3 kg dry matter feed, respectively (Foidl *et al.,* 2001). Moringa diet has the highest efficiency of protein & nitrogen utilization and nutrient digestibility (Sultana *et al.,* 2015). Moringa Leaf Meal (MLM) can also be included in poultry and fish meal. The MLM in poultry feed can be particularly used by the small farm holders as natural and healthy feed substitute to synthetic feed supplements (Hermogenes *et al.,* 2014). Moringa leaves which are rich in protein help in improving the microbial protein synthesis in rumen of animals. Hence, it is a suitable alternate of soybean and rapeseed meal and can be given to ruminants (Soliva *et al.,* 2005).

Anti-nutritional factors

Besides nutritional factors, antinutritional factors are also reported in many food and fodder trees and these exert negative effect on human and animal health by interfering with various physiological processes. Therefore, such trees are not selected as priority for food and fodder. Some trees belonging to genus *Acacia*, *Albizia*, *Sesbania* are utilized for fodder purpose and these possess antinutritional factors (Kumar, 1992). However, *Moringa oleifera* represents unique property of being rich in minerals and lower in antinutritional factors. Moringa leaves possess tannins that range from 13-20 g $kg^{-1}$of dry leaves and it is very less in comparison to *Sesbania sesban*, *Acacia angustissima*, and *Acacia cyanophylla* leaves (31, 66 and 38 g kg^{-1}, respectively). The concentration of phytates is also low in Moringa and it ranges from 25-31 g $kg^{-1}$of the dry leaves (Ahn *et al.,* 1989; Makkar and Becker, 1996; Salem *et al.,* 1999). Lectins, trypsin, and amylase inhibitors have not been reported in Moringa leaves (Ferreira *et al.,* 2008). The glucosinolates and isothiocyanates have been reported in Moringa leaves and roots (Bennett *et al.,* 2003; Newton *et al.,* 2010). However, their concentration depends upon the soil type, climatic conditions, cultivar and its growth stage. Moringa leaves possess saponins, which impart a bitter taste. Saponins and isothiocyanates do not always have harmful effects and hence can be consumed by both livestock and human beings (Foidl *et al.,* 2001; Price, 2000). Other antinutritional factors found in many fodder trees are oxalates. However, oxalates reported in Moringa leaves are mainly insoluble oxalates and hence do not cause harmful effect (Radek and Savage, 2008). Hence, *Moringa oleifera* is having better nutritional composition than other leafy vegetables or fodders and can be utilized for food as well as fodder purpose.

PHARMACEUTICAL POTENTIAL OF MORINGA

The various parts of *Moringa oleifera* possess antioxidative, anti-inflammatory, antitumor, antiulcer, antibacterial and antifungal properties. Many bioactive

compounds like glycosides, malonates and flavonoids have been isolated from *Moringa oleifera* (Bennett *et al.,* 2003; Miean and Mohamed, 2001). Isothiocyanates isolated from Moringa leaves possess the capability to fight against human tumor cells. The reason behind this activity may be its capability to induce reactive oxygen species in cancerous cells which is target specific (Tiloke *et al.*, 2013; Jung, 2014; Leelawat and Leelawat, 2014). Many of the plant glycosides can be used in treatment of cancer or chronic diseases (Chumark *et al.,* 2008; Ghasi *et al.,* 2000; Murakami *et al.*, 1998). Lipid peroxidation plays an important part in artherogenesis, thrombosis and cancer development. The flavonoids isolated from Moringa leaves could modulate this process of lipid peroxidation and hence can combat with these diseases. The flavonoids also help in scavenging of free radicals and inhibition of oxidative and hydrolytic enzymes which are involved in several chronic diseases (Rodrigo *et al.,* 2011; Siddhuraju and Becker, 2003). Quercetin, which is a powerful antioxidant, also helps in lowering blood pressure (Larson *et al.,* 2012). Moringa leaf powder also helps in reducing blood sugar levels due to the presence of isothiocyanates (Kushwaha *et al.,* 2014; Waterman *et al.,* 2015).

Moringa leaf, pod and seeds also possess anti-inflammatory properties (Cheenpracha *et al.,* 2010). However, these results are based on lab and animal studies and confirmation on human beings further needs to be verified. Pods are helpful in treating eye disorders, diarrhea, joint pain, liver and spleen problems. The immature pods are used against intestinal worms (Kasolo *et al.*, 2010). Moringa seeds help in treating many diseases like epilepsy, arthritis, viral diseases etc (Kasolo *et al.*, 2010 and Sutalangka *et al.*, 2013) . The flowers of Moringa can cure arthritis, cold, urinary and heart problems (Fuglie, 2005). The gum is used as an antiseptic (Rajangam *et al.,* 2001). The fresh root is used in case of intermittent fever. It can also be applied externally as a paste to treat inflammation, palsy, dropsy and animal bites. The root infusion helps in treating asthma and ascites. Root bark acts as an anti-ulcer, anti-inflammatory and cardiac stimulatory agent (Mishra *et al.,* 2011 and Fahey, 2005).

CROP IMPROVEMENT

Moringa oleifera is a cross-pollinated tree species having diploid chromosome number equal to 28. Therefore, high heterogeneity in morphological, physiological and quantitative traits is anticipated. This tree presents diversity of forms *i.e.,* annual to perennial types, deciduous to evergreen, semi-spreading to upright. Also, some trees flower in two seasons and others flower throughout the year (Raja *et al.*, 2013). Hence, the variability present in various characters can be a source for genetic improvement. However, lack of elite cultivars adapted to local environment and use of open pollinated seed for crop cultivation may be important factors that limit its productivity. Moreover, the number of germplasm accessions and active germplasm banks are incipient across the world. Despite India, some other research centres like AVDRC (Taiwan), Rural development initiative (Zambia) and Moringa Philippines foundation (Philippines); are also involved at the global level, in moringa improvement. Many ecotypes *viz.* Jaffna, Chavakacheri murungai, Chemmurungai, Kadumurungai and Kodikkal murungai have been reported in India (Kumar *et al.,* 2014). Despite huge variability in *Moringa oleifera* no institution has database for cultivated or natural germplasm accessions. There is great inconsistency between the inherent genetic variability in this species and reflected in available germplasm. Hence, priority must be focused on the fixation of poor variability in available germplasm in germplasm banks because it may hinder with the progress of crop improvement programs.

Any crop improvement program depends on the available genetic diversity. There is much less information available on genetic diversity of genus *Moringa*. Some of the studies show a wide genetic diversity present in Moringa (Ramachandran *et al.,* 1980; Shahzad *et al.,* 2013; Suthanthirapandian *et al.,* 1989). These studies also emphasize on the great potential for genetic improvement of this species to enhance the use of this under-utilized tree. There is one downside also i.e., some antinutritional factors in Moringa can reduce the absorption of minerals and protein (Richter *et al.,* 2003; Teixeira *et al.,* 2014). Hence, different strategies should be explored to harness the available genetic diversity. The true understanding of genetic diversity can be gained by the characterization of both cultivated and natural accessions present all over the world. Various

breeding programs, including plant introduction, evaluation, selection, hybridization and use of biotechnological methods have been employed for the development of varieties with dwarf stature, high biomass production, high seed yield and oil content, better quality, resistance to pest and diseases.

Introduction

The variety, Jaffna is presumed to have been introduced from Sri Lanka. It is cultivated in Southern India. Chavakacheri murungai is also an introduced variety from Sri Lanka.

Selection (Mass and Pure line)

The selection of plants starts with open pollination. The plants are selected based on production potential and tested under various conditions and sites. Then, it is followed by controlled pollination. The variety, PKM 1, has been developed through pure line selection. It is annual type and best suited for tropical plains.

Hybridization and selection

It involves hybridization between diverse genotypes followed by selection. The variety, PKM 2 is derived from a cross involving MP31 and MP28 as parents. It is annual type and gives 48% more yield over PKM1. It is suitable for cultivation in tropical plains of India.

Mutation breeding

It is also an important breeding method for creation of variability or novel traits. However, this method is not much utilized in Moringa.

Molecular breeding

Work on Moringa crop improvement based on biotechnology or molecular breeding is very limited and it is just at budding stage. It is obvious from the nucleotide database (NCBI) which possess very less sequence information (DNA and RNA). Recently, due importance has been given to micro propagation (Kantharajah and Dodd, 1991) and use of molecular markers for characterization of germplasm for crop improvement. Use of molecular markers can enhance the genetic improvement as it helps in identifying diverse genotypes. These diverse genotypes can be utilized in hybridization programs to produce recombinants with wide variability.

Biotechnological studies in Moringa started with the use of dominant markers. The amplified fragment length polymorphism (AFLP) marker technique was used to study diversity in natural populations from India and introduced populations in Malawi and Kenya (Muluvi *et al.*, 1999). In this study it was noticed that the population differentiation was significant and genotypes separated according to geographical origin. Further, randomly amplified polymorphic DNA (RAPD) marker technique was used by different researchers to study diversity among cultivated and non-cultivated population of Moringa (Mgendi *et al.,* 2010; Abubakar *et al.,* 2011; Da Silva *et al.,* 2012; Saini *et al.,* 2013 and Rufai *et al.,* 2013). In all these studies an appreciable level of genetic diversity was reported in natural populations with respect to cultivated ones. Hence, less genetic diversity in cultivated accessions indicates that there is an utmost requirement to widen the genetic base for crop improvement programs. Later, with the advent of co-dominant markers like simple sequence repeats (SSRs), the molecular genetic diversity studies got improved. Moringa accessions collected from different countries (Pakistan, India, Tanzania, Senegal, Mozambique, Zimbabwe, Florida, Mexico, Haiti and Belize) have been evaluated earlier and it has been reported that there was high genetic diversity in wild Pakistan accessions, as compared with other accessions (Shahzad *et al.,* 2013). Both morphological and molecular markers (SSRs) have been used for characterization of 300 genotypes of Moringa collected from different locations of India and large diversity have been observed (Ganesan *et al.,* 2014). Both these markers have been used later to screen 34 genotypes of Moringa collected from different regions of Tamil Nadu (Natarajan and Joshi, 2015). It has resulted in identification of superior genotypes which can be utilized in hybridization program to improve oil yield. In this way, morphological markers along with molecular markers can prove an authentic tool to enhance and strengthen breeding programs of Moringa.

CONCLUSIONS AND FUTURE PERSPECTIVES

Moringa oleifera has several strengths and potential utilities. The usage and merits of Moringa as food, livestock fodder and pharmaceutical have been documented widely. Although Moringa plant has

enormous potential, however it has some challenges. Many aspects of this tree e.g., the most successful utilization in agricultural practices, harmful effects of antinutritional factors on human beings and livestock, are still unanswered. Some antinutritional factors reported in this species may limit its utility in form of food and fodder. Therefore, more attention and research are warranted in this direction i.e., how it should be used in diets. Aside from this, agronomic practices requirements for high fodder biomass production have not yet been studied. Therefore, more emphasis should be given to these arenas of research on Moringa to highlight this underutilized tree in a better way.

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Original Research Paper

Characterization and evaluation of mountain sweet thorn (*Flacourtia montana* J. Grah) collections

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ABSTRACT

Mountain sweet thorn (*Flacourtia montana* J. Grah) is an indigenous underutilized fruit of Western Ghats and other regions of peninsular India. It is a close relative of Governor's plum. It is bushy shrub or small tree with spiny trunks and branches which may grow up to 2.5 m height. The fruits are bright dark red 1-2 cm in diameter, sweet edible and have potential for processing into jams and jellies. The presence of thorn is one of the major hurdles forcommercialization of this crop. Thus, seeds of the thorny plants of the Mountain sweet thorn were collected from different locations and seedlings were planted to identify suitable line .The plant height ranged from 425 cm (accession 0208) to 710 cm (accession 0202). The plant girth ranged from 34.5 cm to 82 cm. The growth data revealed that all the accessions are vigourous, fast growing and various levels of thorniness. All the accessions were found to be spreading type. Significant variability was recorded for leaf characteristics among the accessions studied. Three major clusters were observed in cluster analysis for morphological and fruiting characterisconsisting of 1, 8 and 9 accessions, respectively. The number of fruits ranged from 0 to 4008. Highest yield (9.46 kg/plant) was obtained in accession - 0106 followed by accession- 0201 (7.83 kg). The average fruit weight ranged from 1.51 g to 3.94 g. Highest fruit weight (3.94g) was also recorded in 0106 followed by 2.84 (0102).The total soluble solids ranged from 10 ^{0} Brix to 15.1 ^{0} Brix. Over all, accession 0201 was found better than others with respect to yield, regular bearing and less thorniness.

Key Words: Mountain sweet thorn, segregation, thornless and yield

INTRODUCTION

There are several underutilized and wild fruits are native to Western Ghats of India (Arora, 1998; Tripathi *et al*, 2015) of which mountain sweet thorn (*Flacourtia montana* J. Grah) one of the important indigenous underutilized fruits of tropical region. It belongs to the family flacourtiaceae. It is a close relative of governor's plum. The plants of several species of *Flacourtia* genus are found growing in wild in Western Ghats and other regions of peninsular India (Barwick, 2004). It is bushy shrub or small tree with spiny trunks and branches which may grow up to 2.5 meter height. Fruiting begins in the month of January to March. It bears dark red coloured fruits which are pleasantly acrid, eaten raw and used also for jelly preparation. The fruits have high potential for processing into jam, jelly, wine, *etc*. (Mundaragi *et al*., 2019). Ripe fruits are often dried and stored. Ripe fruits are delicious to taste and rich in sugars, calcium, magnesium, potassium, sodium, phenolics, flavonoids and ascorbic acid (Mundaragi and Thangadurai, 2015). It has also been reported that the fruits leaves and bark of this species have various pharmacological activities like antibacterial, antidiabetic, anti-inflammatory and hepatoprotective *etc*. (Joshy *et al*., 2016). The presence of thorn is one of the major hurdles in commercialization of this crop. Thus, some accessions of Mountain sweet thorn which are less thorn were selected and evaluated for various characteristics during 2016-19.

MATERIAL AND METHODS

The *Flacourtia montana* J. Grah accessions were collected from Kerala during 2008-09 through extensive surveys from Palode. The seedlings

were directly planted in the field at a distance of 5x5m at ICAR-IIHR, Bangalore which is located at 13.133999 ° N latitude and 77.47880 ° E at 855m above sea level. It has sub humid tropical climate with annual rainfall of 86cm. The soil is red. The plants started fruiting in third year and yieldgot stabilized after five harvesting seasons. Eighteen accessions were evaluated for two years for vegetative growth, flowering, fruiting and fruit characteristics. Twenty-five mature fruits were harvested randomly from each accession to record the physico-chemical parameters. The fruits were washed with distilled water and the surface water was removed using blotting paper. The cleaned air-dried samples were used. The fruit weight, fruit length, fruit diameter was recorded using electric balance and digital vernier caliper. The fruit shape and fruit colours were recorded as per the standard fruit shape (Gupta, 1972 and Berg, 2007) and colour charts (RHS colour chart,1966). The fruits were cut and pulp colour was recorded. The total soluble solids (TSS) were determined with Erma hand refractometer (0-32^{o}Brix) and titratable acidity (%) was estimated using procedures described by Ranganna (1986).

Data analysis

The data was analysed using R software(R-4.0.3). The distance matrix required for hierarchical clustering was computed using the 'dist' function which is based on Euclidean distances between the accessions. The hierarchical clustering of the accessions was done using 'hclust' function based on complete linkage method. Principal Component Analysis (PCA) was done using the 'pca' function. (Team, 2019).

RESULTS AND DISCUSSION

Morphological characterization

Growth characteristics

Morphological characterization of 18 accessions of mountain sweet thorn was done as per the standard descriptors. The plant height ranged from 425 cm to 710 cm with highest in accession0202. The plant girth ranged from 34.5 cm to 82 cm. The plant spread (E-W) ranged from315 cm to 625 cm where as N-S ranged from 315 cm to 625 cm. The growth data revealed that all the accessions were vigourous, fast growing and spreading type. The foliage was dense and leaves were dark green. The new leaf colour was coppery red in all accessions (Table 1). Leaf length ranged from 8.53 cm to 15.9 cm.The leaf width ranged from 4.23 cm to 6.89 cm. The petiole length ranged from 0.33 to 0.59 cm in different accessions. The leaf blade was pinned and all the accessions have petiolate leaves. The leaf margin was serrated while leaf apex was acute or acuminate type. The leafbase was elliptical in most of the collections but it was ovate in accessions- 0103,0106,0202,0306. The leaf base was acuminate or rounded (Table 2). These all accessions are fast growing but different levels of thorniness. Some collections werehighly thorny while some have moderate thorny.The number of thorns per 1m stem trunk were highest (63) in accession-0105 followed by 47 thorns in accessions-207 and 0208. The accessions-0103,0104, 0201 were found thornless. All the accessions started flowering in December and continued for almost one month. There was only 1-2 weeks difference in the flowering period of different accessions. The flowering time varied in both years which may be due to weather conditions (Table 3).

Cluster analysis

Clustering of the 18 accessions was done based on the morphological data. As the dataset consisted of mixed data types viz. numeric and attribute, the distance between the accessions was computed using Gower's distance measure. Hierarchical clustering of the accessions based on the complete agglomeration method was done for the above distance matrix and a dendogram was generated (Fig 1). Three major clusters were observed consisting of 1, 8 and 9 accessions, respectively. The first cluster consisted of the one accession i.e. O102. Cluster 2 consisted of 7 female plants and one male plant (O206) while cluster 3 consisted of 6 male plants and two female plants. The minimum distance was found between the accessions O201 and O205 (cluster III) while the maximum distance was observed between O102 (cluster I) and O103 (cluster III) (Fig 1).

Principal Component Analysis

The principal component analysis was done based on the numeric characters of morphological and leaf data. 68.7% of the total variance was explained by the first two components (39% by 1st component and 29.7% by 2nd component) (Fig 2 & Table 4).

Table 1 : Morphological characterization of *Flacourtia montana* accessions

Accession No.	Plant height (cm)	Plant girth (cm)	Plant spread (E-W) cm	Plants spread (N-S) cm	Habit	Growth pattern	New Leaf colour	Foliage density	Leaf Colour
O102	705	82	577.5	487.5	Small Tree	Spreading	Coppery red	Dense	Light Green
O103	615	49.5	502.5	450	Small Tree	Spreading	Coppery red	Dense	Light Green
O104	655	53	490	492.5	Small Tree	Spreading	Coppery red	Dense	Light Green
O105	633.5	71.5	442.5	570	Small Tree	Spreading	Coppery red	Dense	Light Green
O106	660	59.5	625	602.5	Small Tree	Spreading	Coppery red	Dense	Light Green
O201	592.5	45	535	495	Small Tree	Spreading	Coppery red	Dense	Light Green
O202	710	53.5	447.5	471	Small Tree	Spreading	Coppery red	Dense	Light Green
O203	560	62	470	485	Small Tree	Spreading	Coppery red	Dense	Light Green
O204	585	53.5	510	560	Small Tree	Spreading	Coppery red	Dense	Light Green
O205	600	54	450	480	Small Tree	Spreading	Coppery red	Dense	Light Green
O206	580	77.5	497.5	492.5	Small Tree	Spreading	Coppery red	Dense	Light Green
O208	425	34.5	315	320	Small Tree	Spreading	Coppery red	Dense	Light Green
O302	595	42	550	535	Small Tree	Spreading	Coppery red	Dense	Light Green
O303	655	47	385	505	Small Tree	Spreading	Coppery red	Dense	Light Green
O304	655	53.5	432.5	500	Small Tree	Spreading	Coppery red	Dense	Light Green
O305	622.5	56	440	482.5	Small Tree	Spreading	Coppery red	Dense	Light Green
O306	590	40	387.5	525	Small Tree	Spreading	Coppery red	Dense	Light Green
O307	655	71.5	557.5	505	Small Tree	Spreading	Coppery red	Dense	Light Green
Average	616.31	55.86	478.61	497.69	-	-	-	-	-
Min	425	34.5	315	320	-	-	-	-	-
Max	710	82	625	602.5	-	-	-	-	-
SD	63.67	12.96	76.07	58.30	-	-	-	-	-

Table 2 : Leaf characteristics of *Flacourtia montana* accessions

Accession No.	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leave type	Leaf Blade	Venation	Leaf Margin	Leaf Apex	Leaf Shape	Leaf Base
O102	8.53	4.23	0.33	Petiolate	Pinned	Pinnate	Serrate	Acuminate	Elliptical	Acuminate
O103	14.8	5.43	0.38	Petiolate	Pinned	Arcuate	Serrate	Acute	Ovate	Rounded
O104	13.04	5.4	0.34	Petiolate	Pinned	Arcuate	Serrate	Acute	Elliptical	Acuminate
O105	14.8	5.27	0.33	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O106	13.85	6.09	0.39	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Ovate	Rounded
O201	11.98	5.57	0.33	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O202	10.65	4.79	0.37	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Ovate	Rounded
O203	13.5	6.12	0.44	Petiolate	Pinned	Pinnate	Serrate	Acute	Elliptical	Acuminate
O204	12.63	5.56	0.41	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O205	12.17	5.46	0.33	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O206	13.7	6.89	0.48	Petiolate	Pinned	Arcuate	Serrate	Acute	Elliptical	Rounded
O208	11.52	5.23	0.45	Petiolate	Pinned	Arcuate	Serrate	Acute	Elliptical	Rounded
O302	13.93	5.68	0.44	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O303	14.6	6.18	0.55	Petiolate	Pinned	Pinnate	Serrate	Acute	Elliptical	Rounded
O304	12.24	6.23	0.53	Petiolate	Pinned	Arcuate	Serrate	Acute	Ovate	Acuminate
O305	12.09	5.20	0.59	Petiolate	Pinned	Arcuate	Serrate	Acute	Elliptical	Acuminate
O306	15.9	6.48	0.575	Petiolate	Pinned	Arcuate	Serrate	Acuminate	Elliptical	Acuminate
O307	13.58	5.76	0.475	Petiolate	Pinned	Arcuate	Serrate	Acute	Elliptical	Acuminate
Average	12.97	5.64	0.43	-	-	-	-	-	-	-
Min	8.53	4.23	0.33	-	-	-	-	-	-	-
Max	15.90	6.89	0.59	-	-	-	-	-	-	-
SD	1.73	0.63	0.09	-	-	-	-	-	-	-

Table 3: Flowering characteristics of *Flacourtia montana* accessions

Accession No.	Date of start of flowering		Date of end of flowering		Date of start of fruit maturity		Date of full fruit maturity	
	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
O102	Dec 21,2017	Dec 14,2018	Jan 20, 2018	Jan 21, 2019	Feb 14, 2018	Feb 14, 2019	March 26,2018	March 26,2019
O103	Dec 21,2017	Dec 14,2018	Jan 24, 2018	Jan 22, 2019	-	-	-	-
O104	Dec 18,2017	Dec 10,2018	Jan 24, 2018	Jan 21, 2019	Feb 14, 2018	Feb 14, 2019	March 26,2018	March 26,2019
O105	Dec 21,2017	Dec 14,2018	Jan 24, 2018	Jan 22, 2019	-	-		-
O106	Dec 15,2017	Dec 15,2018	Jan 18, 2018	Jan 21, 2019	Feb 14, 2018	Feb 14, 2019	March 26,2018	March 26,2019
O201	Dec 21,2017	Dec 14,2018	Jan 20, 2018	Jan 21, 2019	Feb 14, 2018	Feb 14, 2019	March 26,2018	March 26,2019
O202	Dec 21,2017	Dec 14,2018	Jan 24, 2018	Jan 22, 2019	-	-	-	-
O203	Dec 21,2017	Dec 15,2018	Jan 24, 2018	Jan 21, 2019	Feb 14, 2018	-	March 26,2018	-
O204	Dec 18,2017	Dec 142018	Jan 20, 2018	Jan 21, 2019	Feb 14, 2018	-	March 26,2018	-
O205	Dec 18,2017	Dec 142018	Jan 20, 2018	Jan 21, 2019	-	-	-	-
O206	Dec 18,2017	Dec 142018	Jan 20, 2018	Jan 21, 2019	-	-	-	-
O208	Dec 18,2017	Dec 24,2018	Jan 24, 2018	Jan 21, 2019	-	Feb 14, 2019	-	March 26,2019
O302	Dec 18,2017	Dec 14,2018	Jan 24, 2018	Jan 22, 2019	-	-	-	-
O303	Dec 15,2017	Dec 24,2018	Jan 20, 2018	Jan 21, 2019	Feb 14, 2018	Feb 14, 2019	March 26,2018	March 26,2019
O304	Dec 21,2017	Dec 24,2018	Jan 20, 2018	Jan 22, 2019	Feb 14, 2018	-	March 26,2018	-
O305	Dec 18,2017	Dec 24,2018	Jan 20, 2018	Jan 22, 2019	Feb 14, 2018	-	March 26,2018	-
O306	Dec 18,2017	Dec 24,2018	Jan 20, 2018	Jan 22, 2019	-	-	-	-
O307	Dec 21,2017	Dec 24,2018	Jan 20, 2018	Jan 22, 2019	Feb 4, 2018	-	March 26,2018	-

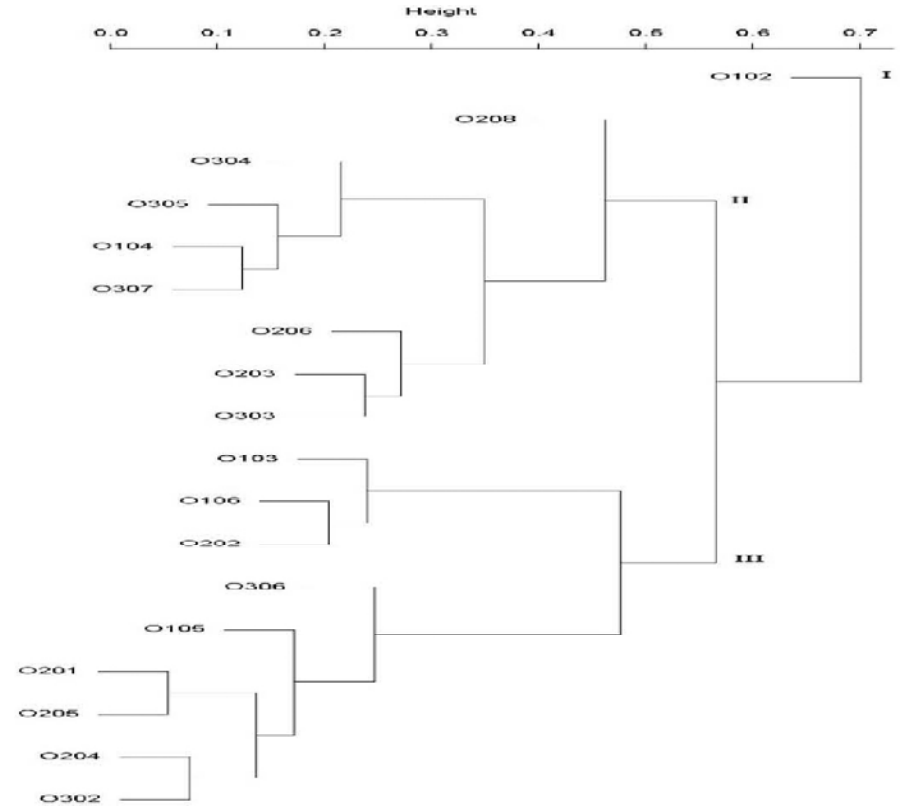

Fig. 1: Clustering of *Flacourtia* accessions Collections for morphological and leaf characters

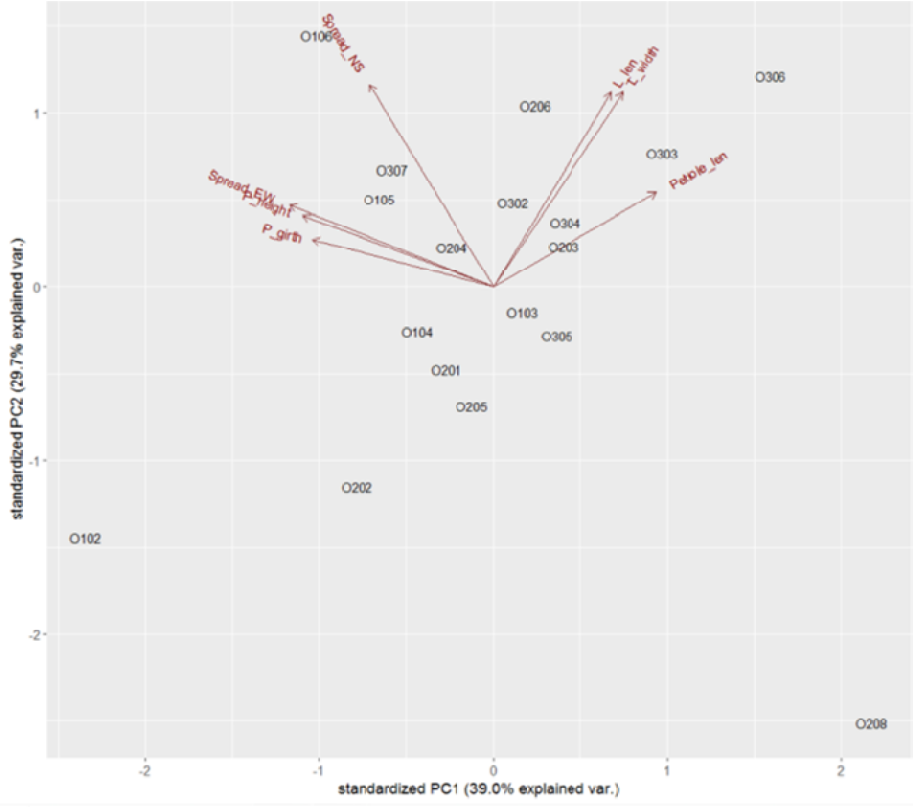

Fig. 2: Principal component analysis for morphological and leaf characters

Table 4: Fruit traits of *Flacourtia montana* collections

Accession No.	Pulp weight (g)	No.of Seed/ fruit	Seed wt. (g)/ Fruit	TSS (^{O}Brix)	Acidity (%)
O102	2.14	9.0	0.67	15.0	0.22
O104	2.62	11.0	0.66	15.0	0.51
O106	3.26	9.0	0.68	10.5	0.53
O201	1.50	10.0	0.74	15.0	0.25
O203	1.25	10.0	0.66	15.1	0.53
O204	1.32	10.0	0.67	15.0	0.47
O208	1.01	9.0	0.64	10.5	0.34
O303	2.13	10.0	0.70	15.1	0.43
O304	2.20	8.0	0.61	15.0	0.43
O305	0.85	8.0	0.63	15.1	0.39
O307	0.97	9.0	0.65	15.2	0.41
Average	1.75	9.36	0.67	14.23	0.41
Min	0.85	8.00	0.61	10.50	0.22
Max	3.26	11.00	0.74	15.20	0.53
SD	0.84	1.00	0.04	1.93	0.13

Characterisation for fruit traits and evaluation for yield

The fruit started maturing in the month of February and peak fruiting was in March. The number of fruits/tree ranged from 0 to 4008. There were no fruits in accessions 0103, 0105, 0202, 0205, 0206, 302 and 0306. It seems these were male plants. The accessions 0102, 0104, 0106, 0201, 0203, 0204, 0208, 0303, 0304, 0305 and 0307 produced fruits either in one year or both the year (Table 5). As it is well known that *Flacourtia* montana have unisexual flowers, Some trees produces only male flower while others produce only female flowers. (*http://www.flowersofindia.net)*. Among the remaining plants highest yield (9.46 kg) was obtained in accession- 0106. Higher yield was also obtained in accession- 0201 (7.83 kg).Average fruit yield ranged from 0 to 9.46 kg per plant. Most of the accessions are mid-season maturing and except accession 0307 which was early maturing. The fruits of all the accessions were spherical (Table 5). The productivity was low in most of the accessions. The low productivity may be associated with lack of pollination. The average fruit weight ranged from 1.51 g to 3.94 g. Highest fruit weight (3.94g) wasalso recorded in 0106 followed by 2.84 (0102).The fruit equatorial diameter ranged from 0.825cm to1.036 cm while fruit polar diameter ranged from 1.014cm to -1.062 cm. The fruit colour was dark red (Red 46b) .Fruit pulp was smooth and fibre content was low.There was no flavour.The average pulp weight ranged from 0.85 g to 3.26 g. Number of seeds/fruit ranged from 8 to 11. The highest numbers of seeds per fruit were found in accessions-0104 (11) while it was lowest in accessions-304,305(8). The total soluble solids ranged from 10 ^{0}Brix to 15.1 ^{0}Brix. Higher total soluble solids (>15 ^{0}Brix) were

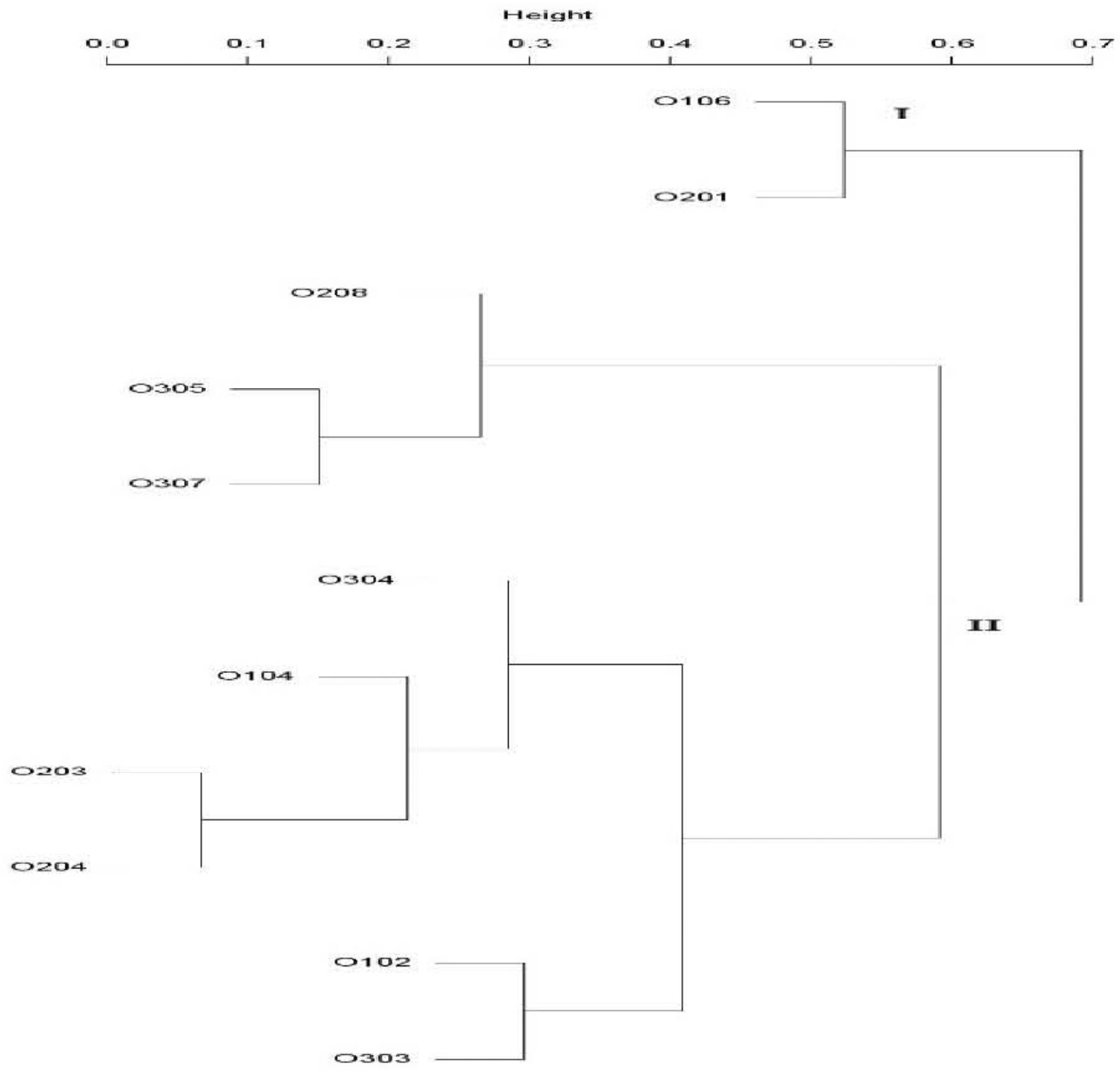

Fig. 3: Cluster analysis for fruit characters of *Flacouria montana* accassions

recorded in most of the accessions. The titrable acidity was ranged from 0.22 % to 0.53 %., it was height in accession- 0203 (Table 6).

Cluster analysis

Clustering of the 11 accessions was done based on the yield and yield contributing characteristics the dataset consisted of mixed data types viz. numeric and attribute, the distance between the accessions was computed using Gower's distance measure. Hierarchical clustering of the accessions based on the complete agglomeration method was done for the above distance matrix and a dendogram was generated (Fig 3). Minimum distance was observed between O305 and O204 while maximum distance was found between O104 and O102. Principal Component Analysis

The analysis of PCA for yield showed that 64.5% of the total variation was explained by the first two principal components (40% by PC1 and 24.5% by PC2) (Fig.4).

Table 5: Fruit yield and related traits in *Flacourtia montana* accessions

Accession No.	Branch thorniness	No. of thorns up to 1 m stem height	Type of plants	Immature fruit drop	Average No. of fruits/tree	Average Yield (kg /tree)	Maturity group	Fruit shape	Productivity status
O102	Less thorny	15.0	Female	High	1942	5.51	Mid	Spherical	Low
O103	Thornless	0.0	Male	-	0	0	-	-	Nil
O104	Thornless	0.0	Female	Low	441.5	1.43	Mid	Spherical	Low
O105	Highly thorny	63.0	Male	-	0	0	-	-	Nil
O106	Less thorny	3.0	Female	High	2400	9.46	Mid	Spherical	High
O201	Thornless	0.0	Female	High	4008.5	7.83	Mid	Spherical	High
O202	Less thorny	6.0	Male	-	0	0.00	-	-	Nil
O203	Moderate thorny	21.0	Female	Low	44.5	0.17	Mid	Spherical	Low
O204	Moderate thorny	31.0	Female	Low	14	0.02	Mid	Spherical	Low
O205	Moderate thorny	45.0	Male	-	0	0	-	-	Nil
O206	Moderate thorny	32.0	Male	-	0	0	-	-	Nil
O208	Moderate thorny	47.0	Female	Low	275	0.45	Mid	Spherical	
O302	Less thorny	18.0	Male	-	0	0	-	-	Nil
O303	Highly thorny	67.0	Female	High	1025	2.91	Mid	Spherical	Low
O304	Less thorny	6.0	Female	Low	175	0.98	Mid	Spherical	Low
O305	Moderate thorny	36.0	Female	Low	22.5	0.07	Mid	Spherical	Low
O306	Moderate thorny	33.0	Male	-	0	0.00	-	-	Nil
O307	Moderate thorny	47.0	Female	Low	69.5	0.23	Early	Spherical	Low
Average	-	26.11		-	578.19	1.61	-	-	-
Min	-	0.00		-	0.00	0.00	-	-	-
Max	-	67.00		-	4008.50	9.46	-	-	-
SD	-	21.79		-	1109.88	2.93	-	-	-

Table 6: Fruit traits of *Flacourtia montana* accessions

Accession No.	Fruit length (cm)	Fruit width (cm)	Fruit wt (g)	Fruit colour	Pulp colour	Pulp fibre content	Pulp taste	Pulp flavour
O102	1.019	1.048	2.84	Dark red	Dark red	Low	Sweet	No
O104	1.034	1.062	3.24	Dark red	Dark red	Low	Acid sweet	No
O106	1.036	1.055	3.94	Dark red	Dark red	Low	Acid sweet	No
O201	0.89	1.014	2.26	Dark red	Dark red	Low	medium sweet	No
O203	1.034	1.049	1.94	Dark red	Dark red	Low	Acid sweet	No
O204	0.942	1.055	2.0	Dark red	Dark red	Low	Acid sweet	No
O208	0.89	1.014	1.64	Dark red	Dark red	Low	Acid sweet	-
O303	1.037	1.054	2.84	Dark red	Dark red	Low	Acid sweet	No
O304	0.891	1.025	2.81	Dark red	Dark red	Low	Acid sweet	No
O305	0.936	1.025	1.51	Dark red	Dark red	Low	Acid sweet	No
O307	0.825	1.027	1.62	Dark red	Dark red	Low	Acid sweet	No
Average	0.96	1.04	2.42	-	-	-	-	-
Min	0.83	1.01	1.51	-	-	-	-	-
Max	1.04	1.06	3.94	-	-	-	-	-
SD	0.08	0.02	0.84	-	-	-	-	-

Correlation analysis between growth and yield characters

There was no significant correlation of growth characters with yield and plant height. The plant spread was positively corelated with yield and fruit weight. The petiole length was found positively correlated with thorniness (Table 7). The characterization and evaluation of mountain sweet thorn accessions revealed that most of them are thorny and about 45 percent are male.

Table 7: Correlation analysis between growth and yield characters

	No of thorns	No. of Fruits/tree	Yield	Fruit length	Fruit width	Fruit weight
Plant height	-0.27	0.20	0.33	0.30	0.46	0.55
Plant girth	-0.17	-0.01	0.11	0.17	0.33	0.13
Plant spread (E-W)	-0.54	0.48	0.59	0.15	0.34	0.43
Plants spread (N-S)	-0.34	0.24	0.41	0.33	0.57	0.53
Leaf length	0.27	-0.21	-0.12	0.10	0.26	0.11
Leaf width	0.07	-0.13	-0.06	0.00	0.09	0.17
Petiole length	0.63	-0.57	-0.53	-0.23	-0.26	-0.38

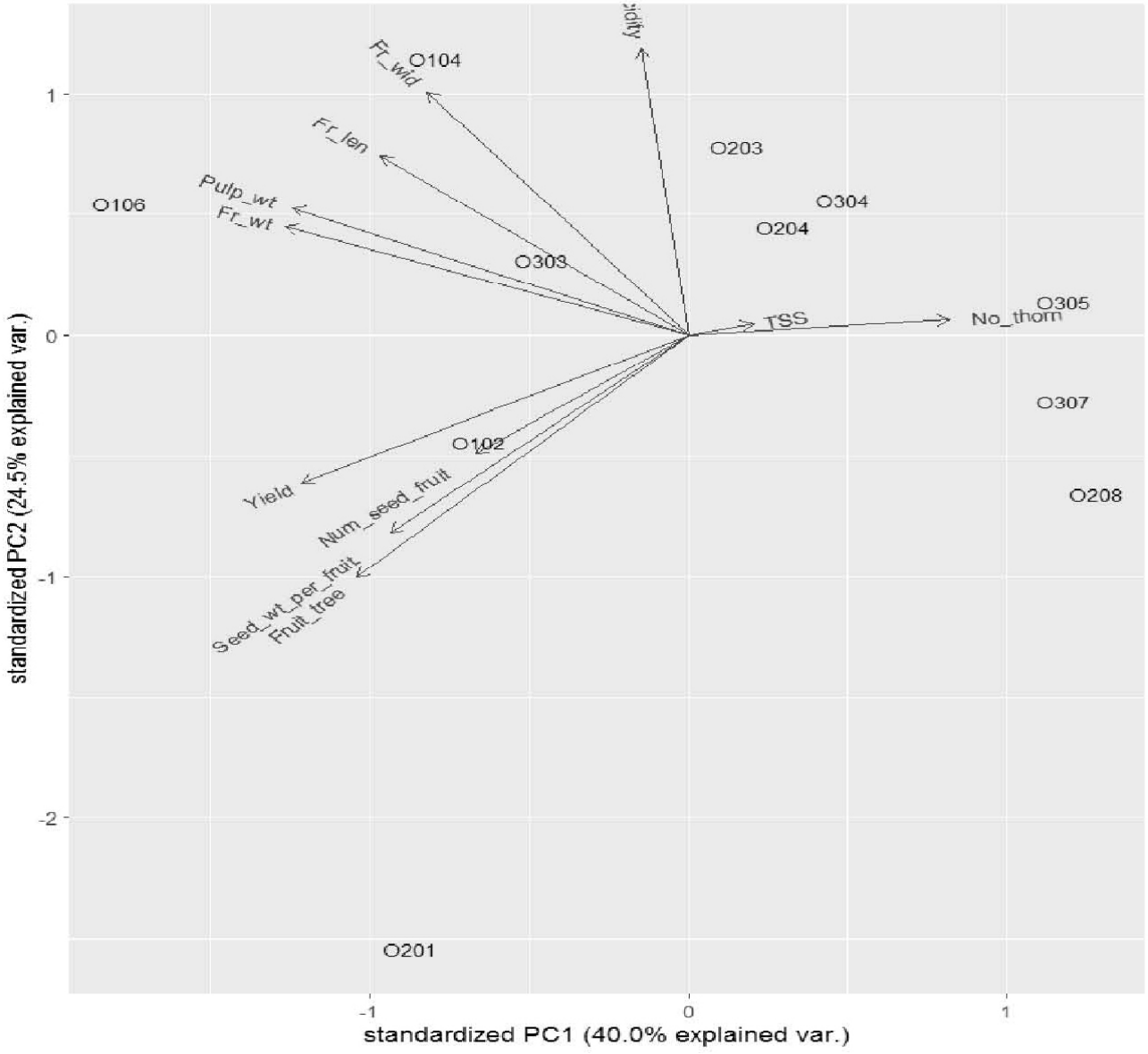

Fig. 4: Principal component analysis for fruit characters

All the accessions were dioecious as either they produce either only male flower or only female flowers. The fruits are bright and bearing habit is sparse to heavy. The fruits are dark red with weight ranging from 1 to 3 g. On the basis of the evaluation one collection (accession- 0201) was found thornless, heavy and regular bearer and average fruits weight (2.26 g).This high yielding, thornless accession is recommended for cultivation.

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Original Research Paper

Optimization of methodology for the extraction of polyphenolic compounds with antioxidant potential and α-glucosidase inhibitory activity from Jamun (*Syzygium cumini* L.) seeds

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ABSTRACT

Jamun (*Syzygium cumini* L.) seed is one of the rich sources of polyphenolic compounds with antioxidant potential and α-glucosidase inhibitory activity. A study was conducted to optimize the methodology for the extraction of polyphenolic compounds (total phenolic and flavonoid contents) with antioxidant potential and α-glucosidase inhibitory activity from jamun seed powder. The study showed that the nature of solvent and extraction conditions had a significant effect on total phenolic content (TPC), total flavonoid content (TFC), antioxidant potential, and α-glucosidase inhibitory activity. The TPC varied between 6.0 (mg/g jamun seed powder) for the acetone extract to 119.2 (mg/g) for 80% aqueous acetone extract, and TFC varied between 1.06 mg/g for the acetone to 10.81 mg/g for the 80% aqueous methanol. From the study, it was apparent that an aqueous form of acetone (acetone: water 80:20, v/v) is a better solvent system for extraction of polyphenolic compounds with high antioxidant potential and α-glucosidase inhibitory activity. Ultrasonication for 60 min increased the efficiency of phenolic extraction.

Keywords: á-glucosidase inhibitory activity, Antioxidant potential; Flavonoids, Jamun seed powder, Natural antioxidants and Polyphenols.

INTRODUCTION

Jamun (*Syzygium cumini* L.) is one of the most important indigenous fruits belongs to the family Myrtaceae. It is a treasure of wide range of secondary metabolites with numerous health benefits. The fruit pulp is sweet and the seeds are pungent and tangy. Both pulp and seeds are extensively used in traditional medicine against various ailments such as diabetes, diarrhea, and ringworm (Benherlal and Arumughan, 2007). While jamun fruits are a rich source of anthocyanins, their seeds are high in polyphenolic compounds. Oxalic acid, tannic acid, gallic acid, and some of the alkaloids are the major secondary metabolites responsible for the astringency taste of seed as well as pulp (Hameed *et al.,* 2020). Most of the secondary metabolites, especially polyphenolic compounds present in the jamun pulp and seeds are reported to possess free radical scavenging potential (Ayyanar and Subash-babu, 2012) as well as anti-inflammatory activities (De Bona *et al.,* 2016; Hossain *et al.,* 2016).

Earlier studies have suggested that some of the polyphenolic compounds present in jamun fruit pulp and seeds have the potential to inhibit the activities of the enzymes, alpha-amylase and intestinal alpha-glucosidase which are responsible for the digestion of dietary starch (Mahmood, 2016). These enzymes have been a target of many drugs developed for the treatment of type-II diabetes (Sim *et al.,* 2008). Since, diabetes is a major problem affecting the human population worldwide and synthetic drugs are risky due to associated side effects, plant-based secondary metabolites especially polyphenolic compounds are the ideal candidate to address the problems of hyperglycemia and diabetes in the human population. Investigation made earlier has reported that the presence of polyphenolic compounds in jamun seeds has appreciably good antioxidant potential and hypoglycemic effect (Aqil *et al.,* 2012). Thus, the extraction of polyphenolic compounds from jamun seeds and it's usage in the medication for regulation

of blood glucose level has attracted many researchers. Polyphenolic compounds possess diverse nature in terms of physical and chemical properties and some of them exist in the free as well as complex forms with carbohydrates and proteins. Due to this nature, the extent of the solubility of these polyphenolic compounds varies in different solvents (Khoddami *et al.,* 2013). Mostly water and other polar solvents such as ethanol, methanol, acetone, and diethyl ether have been extensively used for the extraction of polyphenolic compounds from plant sources (Arivalagan *et al.,* 2018). Since, the levels of active principle in the plant extract depends on the type of solvent used and the extraction methods being adopted (Sun and Ho, 2005; Turkmen *et al.,* 2006; Hayouni *et al.,* 2007), the present investigation was undertaken to optimize the suitable solvent system and extraction conditions for the extraction of polyphenolic compounds with antioxidant potential and alpha-glucosidase inhibitory activity from jamun seeds, as it is rich in polyphenolic compounds.

MATERIALS AND METHODS

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2- carboxylic acid), TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine), ABTS (2,2-azino-di-(3-ethylbenzothialozine-6-sulphonic acid) diammonium salt), potassium persulfate, and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma-Aldrich Co St. Louis, MO, United States of America. Analytical grade Ethanol, methanol, acetone, acetic acid (glacial), sodium acetate, hydrochloric acid (conc.), ferric chloride, ammonium acetate, copper (II) chloride, Folin–Ciocalteu's phenol reagent, aluminium chloride, sodium nitrite, and sodium carbonate were purchased from Merck KGaA, Darmstadt, Germany.

Sample preparation

The experiment was condcuted at ICAR- Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India during 2020. Matured and fully ripened fruit samples were randomly collected from jamun germplasm (IC-0621954, 8 years old tree with semi-spreading type with broad leaves) and brought to the laboratory in the icebox. After removal of pulp, the seeds were dried in a mechanical tray dryer (Make: M/s Servewell Instruments Pvt. Ltd, Karnataka, India) at 50°C until the constant dry weight was achieved. The seed coat was then removed and the kernels were powdered finely using a ball mill (Mixer Mill MMOL400, RETSCH GmbH, Germany) to get uniform particle size and stored in a vacuum desiccator for further analysis.

Solvent system and extraction procedure

About 100 mg jamun seed powder was mixed with 5 mL of selected solvent in 15 mL centrifuge tube and extracted. For extraction seven solvent systems *viz*. water, methanol, aqueous methanol (methanol: water 80:20 v/v), ethanol, aqueous ethanol (ethanol: water 80:20 v/v), acetone, and aqueous acetone (acetone: water 80:20 v/v) were used. The extraction procedure was carried out at two different conditions (room temperature [25±2°C] and ultra-sonic bath set at 50±2°C), each with 30 min and 60 min separately. Extracts were centrifuged (at 5°C for 10 min) and the supernatant was collected in amber reagent bottles. The residue was re-extracted twice using the same procedure as mentioned above. Filtrates collected from all three successive extractions were pooled and dried under a nitrogen atmosphere. The dried samples were dissolved in 5 mL of water, centrifuged, and then the clear supernatant collected was used for the analysis.

Determination of total phenolic and flavonoid content

Determination of total phenolic content (TPC) in the extract was done by Folin-Ciocalteu (FC) assay as described by Singleton *et al.* (1999) using gallic acid as a standard, and TPC was expressed as mg gallic acid equivalent (GAE), per g dry weight of jamun seed powder. The total flavonoid content (TFC) was determined according to Zhishen *et al.* (1999). Quercetin was used as a standard and the results were expressed as mg of quercetin equivalent (QE) per g jamun seed powder.

Determination of antioxidant potential

The DPPH and ABTS radical scavenging activities (IC_{50}) of the different solvent extracts of jamun seed powder were determined by the methods of Brand-Williams *et al.* (1995) and Arnao *et al.* (2001), respectively. IC_{50} value signifies the concentration of test samples to scavenge 50% of the DPPH and $ABTS^{\bullet+}$ radical and expressed as mM TE/g jamun

seed powder. The FRAP (Ferric Reducing Antioxidant Power) assay was done according to Benzie & Strain (1996) and CUPRAC (Cupric ion reducing antioxidant capacity) of the extract was determined according to the method of Apak *et al.,* (2004). In both methods, Trolox served as a positive control and results were expressed as mM TE/g dry jamun seed powder.

Determination of alpha-glucosidase inhibitory activity

Individual sample extracts of varying concentrations were made up to 1 mL with phosphate buffer (0.1M, pH 6.9) and 100 µL of alpha-glucosidase enzyme (25 µg of enzyme) was added. The contents were incubated at 25°C for 5 min. After pre-incubation, about 100 µL of p-nitrophenyl-α-D-glucopyranoside (250 µg/mL) added, contents mixed well and the reaction mixture incubated for 20 min at 25°C. The reaction was terminated by the addition of 1.0 mL of 1 N Na_2CO_3. After 10 min, the yellow color developed was measured at 405 nm and the α-glucosidase inhibition % (IC %) was calculated using the following equation:

IC% = [(Acontrol–Asample)/Acontrol]×100;

Where, Acontrol is the absorbance of the blank control (containing both enzyme and substrate except jamun seed extract): Asample is the absorbance of the test sample.

A graph was plotted with concentration along x axis and IC% along y axis, and IC_{50} values were calculated. IC_{50} value signifies the concentration of tested samples to inhibit 50% of the α-glucosidase enzyme.

Statistical analysis

All the experiments were conducted on triplicate samples. The effect of different extraction solvents, time, and conditions on TPC, TFC, antioxidant potential, and alpha-glucosidase inhibitory activity was evaluated by ANOVA using SAS (SAS, 2011). Pearson's linear correlation was performed to measure the correlation and strength of the relationship between TPC, TFC, antioxidant potential, and alpha-glucosidase inhibitory activity of different solvent extracts.

RESULTS AND DISCUSSION

Effect of solvent type with varying polarity, condition, and extraction time on the extraction of total phenolics and total flavonoids from jamun seeds

Total phenolics (TPC), flavonoids (TFC), antioxidant potential, and alpha-glucosidase inhibitory activity of different solvent extracts of jamun seeds are given in Table 1.

Total phenolic content (TPC)

The TPC varied between 6.0 (mg/g) jamun seed powder for acetone to 119.2 (mg/g) for 80% aqueous acetone. Significant variation was observed for the yield of total phenolic content with respect to solvent type, extraction condition, and time. The phenolic extraction ability was found significantly higher for aqueous solvents compared to their absolute forms. Absolute forms of methanol, ethanol, and acetone extracted less amount of TPC compared to their aqueous counterparts irrespective of extraction time and conditions. Among the solvents with absolute form, extractability of phenolics was found more in methanol (69.8 mg/g) followed by ethanol (42.3 mg), while acetone extracted significantly less TPC (19.3 mg). Water alone extracted a significantly high amount of TPC (78.1 mg) compared to solvents with absolute from. The addition of water to the solvents significantly increased the extraction ability of the solvents. Among the aqueous forms of solvents, aqueous acetone extracted higher amount of TPC (111.9 mg) followed by aqueous methanol (89.8 mg) and aqueous ethanol (83.6 mg). About 5 to 10-fold increase in TPC content was observed for aqueous acetone with different conditions and extraction time compared to its absolute form. From the results, it is clear that the solvents with varying polarity had a significant effect on extractability, and aqueous acetone was superior among the solvent systems studied.

Earlier studies also reported similar findings with high extractability of phenolics by aqueous acetone with varying proportions starting from 50 %, 70%, and 80% (Zhao *et al.,* 2006; Sulaiman *et al.,* 2011, Wijekoon *et al.,* 2011). In the present study, two extraction conditions were employed, one at room temperature [25±2°C] and the other using ultrasonication maintained at 50±2°C. Ultra sonication extracted significantly high amount of TPC compared to room temperature irrespective of solvent type. Similarly, in most cases, 60 min extraction time significantly increased the extraction ability of solvents compared to 30 min extraction time. From the results, it is clear that the aqueous form of acetone with ultrasonication for 60 min is the ideal condition for the extraction of more TPC from jamun seed powder.

Table 1: Total phenolics, flavonoids, antioxidant potential and alpha-glucosidase inhibitory activity of different solvent extracts of jamun seeds

Solvents	Condition	Time (min)	TPC	TFC	FRAP	CUPRAC	DPPH	ABTS	α-glucosidase inhibitory activity*
Water	RT	30	53.0	4.00	0.51	2.06	0.62	0.58	285.2
		60	53.6	3.93	0.52	1.92	0.62	0.92	206.0
	Sonication	30	67.7	5.65	0.61	2.54	0.86	0.86	222.2
		60	78.1	5.47	0.83	2.74	0.89	1.06	197.0
Methanol	RT	30	50.2	6.68	0.55	2.00	0.62	0.55	120.9
		60	57.1	6.82	0.54	2.09	0.61	0.77	57.7
	Sonication	30	68.8	8.41	0.65	2.24	0.76	0.93	194.2
		60	69.8	8.13	0.75	2.64	0.77	0.96	111.2
Aqueous methanol (80% v/v)	RT	30	74.7	9.20	0.89	3.09	0.90	0.98	58.4
		60	76.7	8.69	0.80	2.90	0.92	0.98	90.1
	Sonication	30	89.8	10.81	0.72	3.56	0.99	1.17	107.5
		60	72.4	9.09	0.62	2.38	0.70	1.19	86.6
Ethanol	RT	30	16.5	2.59	0.18	0.62	0.21	0.12	313.7
		60	17.8	3.20	0.16	0.50	0.23	0.22	206.8
	Sonication	30	31.2	3.86	0.34	1.13	0.35	0.34	215.7
		60	42.3	5.31	0.44	1.50	0.52	0.58	173.8
Aqueous ethanol (80% v/v)	RT	30	63.8	7.13	0.64	2.59	0.80	1.02	93.1
		60	65.9	6.61	0.66	2.53	0.83	0.90	83.8
	Sonication	30	79.0	8.48	0.78	3.05	0.94	1.06	120.6
		60	83.6	8.33	0.94	3.16	0.94	1.08	78.8
Acetone	RT	30	7.0	1.50	0.10	0.30	0.06	0.06	972.7
		60	6.0	1.06	0.07	0.16	0.10	0.28	771.0
	Sonication	30	14.0	1.94	0.16	0.53	0.12	0.28	536.9
		60	19.3	3.98	0.21	0.58	0.20	0.35	268.7
Aqueous acetone (80% v/v)	RT	30	83.7	8.60	0.93	3.54	1.08	1.24	81.6
		60	94.4	7.44	0.95	3.70	1.11	1.12	58.0
	Sonication	30	97.2	8.98	0.88	3.85	1.08	1.35	54.0
		60	111.19	9.01	1.08	4.23	1.17	1.42	47.0
Interaction effect with CD									
Solvent		2.29	0.48	0.03	0.09	0.05	0.08	51.7	
Condition		1.23	0.26	0.02	0.05	0.03	0.04	27.7	
Time		1.23	NS	0.02	NS	NS	0.04	27.7	
Solvent * Condition		3.24	NS	0.05	0.13	0.07	0.11	73.2	
Solvent * Time		1.73	0.36	0.02	0.07	0.04	0.06	NS	
Condition * Time		NS	NS	0.05	0.13	NS	NS	NS	
Solvent * Condition * Time		4.58	0.96	0.07	0.18	0.10	NS	103.5	

Three independent experiments were performed and data are presented as mean per gram of dried jamun seed powder;

RT- Room temperature [25±2°C]; TPC- Total phenolic content; TPC was expressed as GAE - Gallic acids equivalent (GAE); TFC- Total flavonoid content; TFC was expressed as QE - Quercetin equivalent.

DPPH and ABTS values are expressed as mM trolox equivalent/ g dry jamun seed powder, obtained from a trolox solution having a free radical scavenging activity (IC50) equivalent to that of sample.

FRAP and CUPRAC values are expressed as mM trolox equivalent / g dry jamun seed powder, obtained from a trolox solution having reducing power equivalent to that of sample. CD- Critical Difference at 5% level of significance;

*α-glucosidase inhibitory activity was expressed as the amount of sample (in µg) required to inhibit 50% of the enzyme alpha-glucosidase (25 µg) activity (IC50) in the presence of substrate p-nitro phenyl glucopyranoside (25 µg)

Total flavonoid content (TFC)

The TFC varied between 1.06 mg/g for acetone to 10.81 mg/g for 80% aqueous methanol. Significant variation was observed for the yield of TFC in terms of solvent type and extraction condition. Among the absolute solvents used, methanol was found to be a better solvent for TFC extraction (6.68-6.82 mg at room temperature and 8.13-8.41mg under ultra-sonication) followed by ethanol. Absolute acetone was found to be poor solvent that could extract only 1.06 to 1.50 mg of TFC at room temperature and 1.94-3.98 mg TFC under ultra-sonication. The addition of water to the absolute solvents significantly increased the TFC extraction ability. Among the aqueous forms, 80% methanol extracted the maximum amount of flavonoids (10.81 mg) followed by 80% acetone (9.01 mg). The increase in extraction ability was found high for acetone followed by ethanol and methanol. The extraction condition significantly increased the TFC, and ultra-sonication extracted a high amount of TFC compared to room temperature, and the effect was high for acetone. Extraction time did not vary significantly among the condition and solvent system used for extraction of flavonoids, except for acetone and methanol. In case of acetone, 60 min extraction time significantly increased the TFC content under ultra-sonication (1.94 mg to 3.98 mg), but in case of methanol, 60 min extraction time significantly reduced the TFC content from 9.20 to 8.69 mg and 10.8 to 9.09 mg for absolute methanol and 80% methanol, respectively). From the study, it was found the aqueous form of methanol with ultrasonication for 30 min is the ideal condition for extraction of more TFC from jamun seed powder.

Effect of solvent type with varying polarity, conditions and time on antioxidant potential and alpha-glucosidase inhibitory activity.

Antioxidant potential

Jamun seeds contain a wide array of polyphenolic compounds with different characteristics, thus employing a single method to evaluate the total antioxidant potential may not be appropriate to accurately measure the antioxidant potential. Thus, in the present study four complementary methods *viz*. DPPH and ABTS radical scavenging activity, FRAP, and CUPRAC reducing power – based on the single electron transfer mechanism were tested to evaluate the antioxidant activity due to their simplicity, stability, and accuracy. The results obtained in the study were expressed as mM TE per g jamun seed powder (Table1). Significant differences were observed between various solvent systems and extraction conditions for antioxidant potential measured by various methods. The antioxidant potential measured by FRAP and ABTS method showed significant variation with respect to extraction time. Among the antioxidant methods employed, CUPRAC method measured higher antioxidant potential, followed by ABTS and DPPH. Correlation studies clearly showed that the total phenolics and flavonoids contents have a significant positive correlation with antioxidant potential [0.987**, 0.991**, 0.987**, 0.967** for TPC with FRAP, CUPRAC, DPPH and ABTS; and 0.884**, 0.891**, 0.881**, 0.882**for TFC with FRAP, CUPRAC, DPPH and ABTS, respectively, at the 0.01 level ($P<0.01$)] (Fig. 1, 2). Since the antioxidant potential of the plant samples is directly related to the total polyphenolic content, the polarity of the solvents had a similar response to the total antioxidant potential. Among the studied solvents with different polarities, absolute methanol extract showed higher antioxidant potential measured by all methods followed by ethanol. Absolute acetone extract showed lesser antioxidant potential. Antioxidant potential due addition of water to the solvents had a similar effect observed for TPC. Aqueous solvent extracts had significantly higher amount of antioxidant potential compared to their counterpart with absolute forms.

Samples extracted using ultra-sonication had significantly higher amount of antioxidant potential compared to the samples extracted under room temperature. The time of extraction did not vary significantly for antioxidant potential measured by CUPRAC and DPPH, while FRAP and ABTS significantly varied with the time duration of extraction. In most cases, radical scavenging values obtained for ABTS radical scavenging

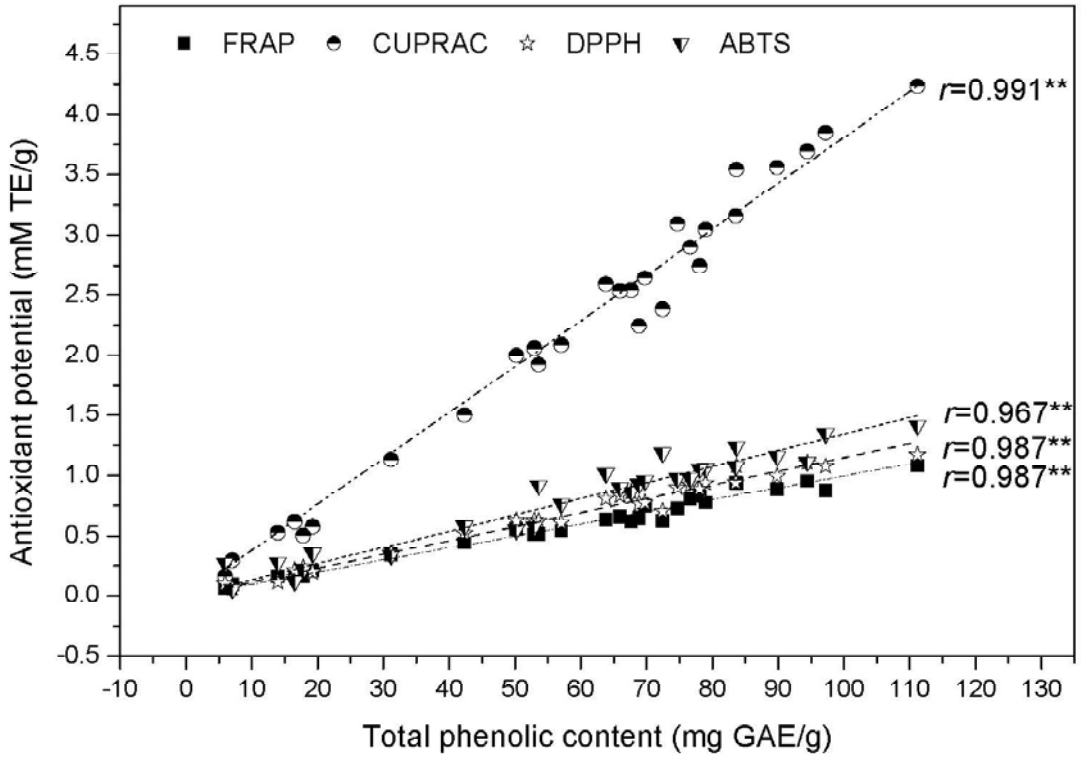

Fig. 1: Relationship between TPC and antioxidant potential.
**Correlation is significant at the 0.01 level (P<0.01).

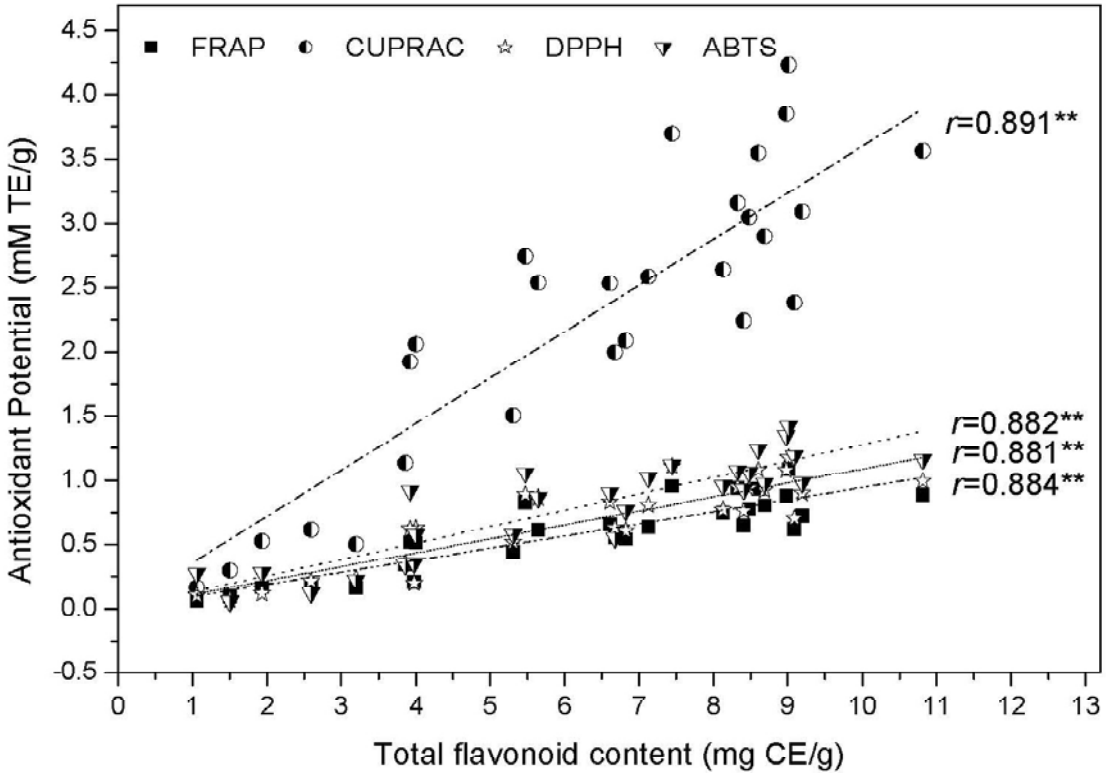

Fig. 2: Relationship between TFC and antioxidant potential.
**Correlation is significant at the 0.01 level (P<0.01).

activity were significantly higher than DPPH radical scavenging activity. This could be because of the ABTS radical's solubility in hydrophilic and lipophilic solvents, and its activity in a wide range of pH (Cano *et al.,* 1998). In case of reducing power, CUPRAC method resulted in higher reducing power compared to FRAP, and it is mainly due to the pH of the reaction medium. In FRAP method, the reaction is carried out in acidic pH (3.6) whereas CUPRAC assay is carried out in neutral pH (7.0). Acidic pH may cause a reduction in reducing power due to protonation of antioxidant compounds, while in neutral pH and basic conditions, antioxidant potential increases due to proton dissociation (Apak *et al.,* 2004).

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory action of the different solvent extract of jamun seed powder was assessed and the results are expressed as the amount of sample (in µg) required to inhibit 50% of the enzyme alpha-glucosidase (25 µg) activity (IC50) in the presence of substrate p-nitrophenyl glucopyranoside (25 µg) (Table 1). Lesser the amount required to inhibit the α-glucosidase enzyme indicated the better inhibitory activity. As stated earlier, the α-glucosidase inhibitory activity is mainly due to the presence of polyphenolic compounds, the solvent system which extracted higher polyphenolic compounds showed better α-glucosidase inhibitory activity. Total phenolic content and flavonoid content negatively correlated [r=-0.754** and -0.784**, at the 0.01 level (P<0.01), respectively] with the amount of jamun seed powder required to inhibit the α-glucosidase enzyme (Fig. 3, 4). Solvent types, extraction condition, and time duration for extraction significantly affected the α-glucosidase inhibitory activity of the extract. Among the absolute solvents, the amount of jamun seed powder extract required to inhibit the enzyme is lesser for methanol followed by ethanol irrespective of extraction condition and time duration of extraction. Significantly very high amount of jamun seed powder is required to inhibit the enzyme when extracted with absolute acetone. Among the aqueous solvents, 80 % acetone showed better inhibitory activity compared to 80% methanol and 80% ethanol. Solvent with extraction condition and extraction condition with time duration did not vary significantly for α-glucosidase inhibitory activity, but compared to 30 min, 60 min extraction duration had better α-glucosidase inhibitory activity especially for 80 % acetone extract. From this assay, it was found the aqueous form of acetone is a better solvent for extraction of more α-glucosidase inhibitory compounds under ultra-sonication for 60 min. Presence of significant amount of antioxidant compounds such as phenolic acids, and flavonoids attributed to jamun seed's medicinal values such as anti-diabetic properties (Ayya *et al.,* 2015). Laboratory studies revealed that the antidiabetic effect of jamun seeds are mainly due to their inhibitory activity on the major enzymes *viz* alpha-amylase and alpha-glucosidase which involves in hydrolysis of carbohydrate (Omar *et al.,* 2012). Kim *et al.,* (2016) stated that the phenolics can impact carbohydrate metabolism and help maintain blood glucose homeostasis through multiple pathways. Results from the present study corroborated the earlier reports that the increased amount of phenolics compounds with antioxidant potential can inhibit more amount of α-glucosidase, which helps in maintaining blood glucose homeostasis.

Polyphenolic compounds derived from plant sources gaining more interest among researchers, food manufacturers, and consumers due to their numerous health-benefits. Jamun seed powder is rich in polyphenolic compounds with both antioxidant and α-glucosidase inhibitory activity. The present study showed that the nature of solvent and extraction condition had a significant effect on TPC, TFC, antioxidant, and α-glucosidase inhibitory activity. The aqueous form of acetone (acetone: water 80:20, v/v) is the better solvent system for extraction of polyphenolic compounds with antioxidant and α-glucosidase inhibitory activity. Ultrasonication for 60 min facilitated higher extraction of phenolics.

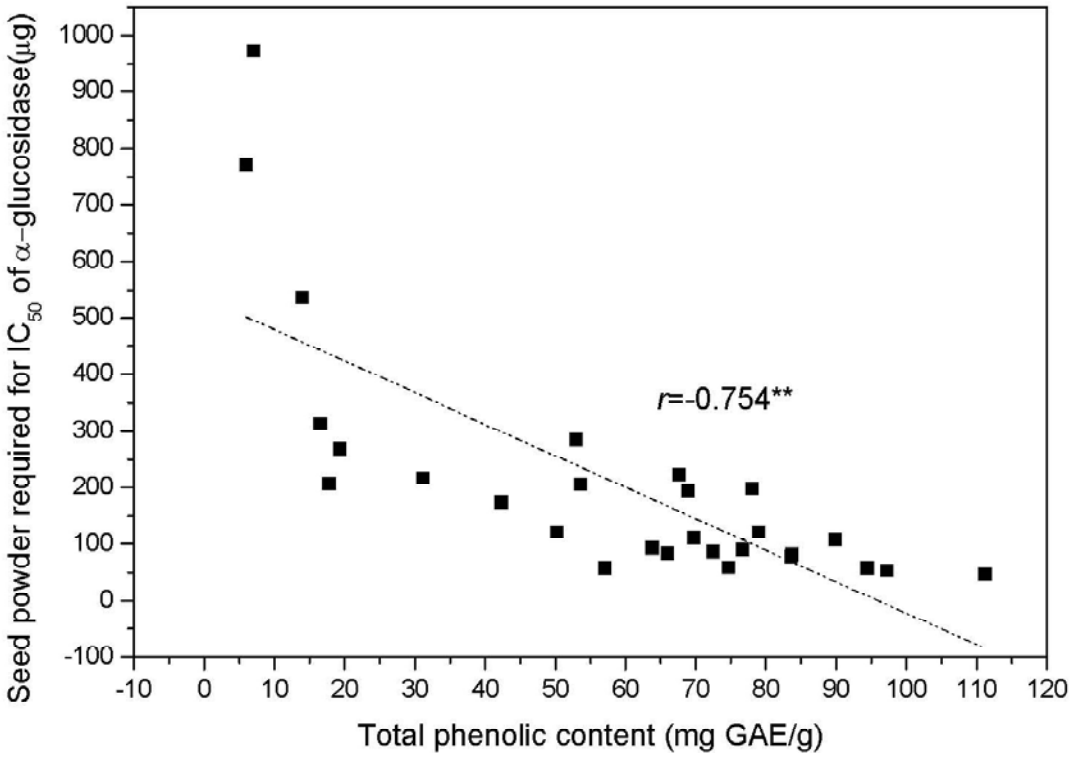

Fig. 3: Relationship between total phenolic content and α-glucosidase inhibitory activity.
**Correlation is significant at the 0.01 level (P<0.01).

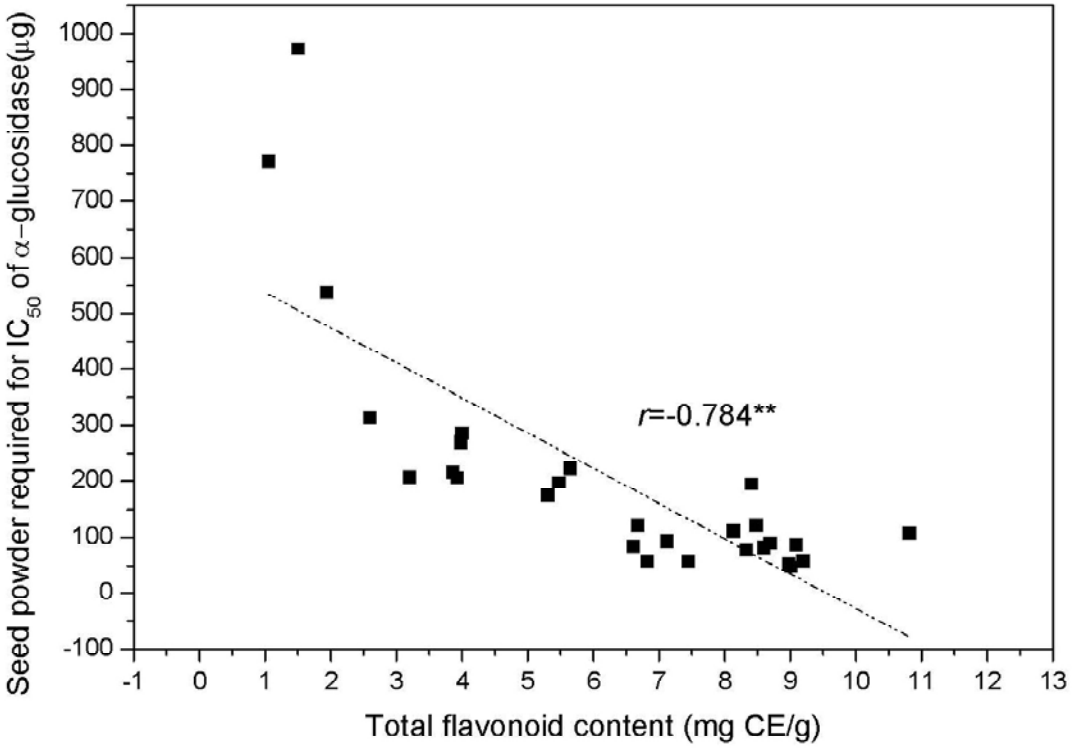

Fig. 4: Relationship between total flavonoid content and α-glucosidase inhibitory activity.
**Correlation is significant at the 0.01 level (P<0.01). 4. Conclusion

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Original Research Paper

Genetic variability studies in amaranthus (*Amaranthus* spp.)

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ABSTRACT

An investigation was carried out to estimate the nature and extent of genetic variability among twenty Amaranthus genotypes (*Amaranthus* spp.) under randomized block design during the year 2019-20. The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the traits. High genotypic coefficient of variation (GCV) and phenotypic coefficient variation (PCV) were observed for leaf area, leaf area index, leaf area duration, AGR, dry weight of leaf per plant, specific leaf weight, speed of germination, chlorophyll content and ascorbic acid (vitamin C). Moderate GCV and PCV were observed for leaf length, leaf width, petiole length, dry weight of stem per plant, leaf: stem ratio and foliage yield per plant. The high estimates of heritability coupled with higher values of genetic advance as per cent mean (GAM) were observed for the parameters like test weight, speed of germination, germination percentage, seedling dry matter, seedling vigour index-1, seedling vigour index-2, plant height, leaf length, leaf width, leaf area, leaf area index, leaf area duration, AGR, specific leaf weight, stem weight per plot, dry weight of leaf, dry weight of stem, leaf: stem ratio, foliage yield per plant, chlorophyll, ascorbic acid and beta- carotene content which indicates the predominance of additive gene action. Arka Arunima, Chikmagalur local, IC-551486, IC-551494 and IC-551466 recorded high foliage yield per plot and these can be utilized in further breeding programme.

Key words: Genotypic Coefficient of Variation, Genetic Advance as per cent Mean, Heritability and Phenotypic Coefficient Variation

INTRODUCTION

Amaranthus or chaulai (*Amaranthus* spp.) is a very popular widespread leafy vegetable throughout the tropics and the temperate zone of the world including India. It is grown throughout the year since it has very quick growth and is suited for crop rotation. Most of the cultivated species are monoecious and wind-pollinated, while the grain species with colourful inflorescence are occasionally visited by bees (Khoshoo and Pal, 1970). The genus *Amaranthus* includes 50-60 species, cultivated for leaf as well as for grains and few are wild species. Vegetable type of leaf amaranth *i.e.*, *A. tricolor* is originated in south East Asia, particularly in India (Rai and Yadav, 2005). Plant hybridization, mutation and polyploidy breeding are the ways and means to restore or create the variability in the existing germplasm. In order to achieve this, collection and characterisation of available genotypes could be the pre-requisite step which provide the necessary information to assess the variability. Studying the phenotypic expression of the plant characters and yield is controlled by the genetic makeup of the plant and environment which is composed of additive variance (heritable) and non-additive variance including dominance and epistasis (non-allelic) interaction. Therefore, it becomes necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as genotypic and phenotypic coefficient of variation, heritability and genetic advance. Further, genetic advance can be used to predict the efficiency of selection. With this background, the present investigation was carried out to assess the extent of genetic variability in *Amaranthus* genotypes for yield and its component traits.

MATERIALS AND METHODS

The experiment was laid out in randomized block design (RBD), with two replications during summer, 2019. The genetic factors *viz*., range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA) and genetic advance as per cent mean (GAM)were calculated. The details of genotypes used in the study are presented in the Table 1.

Table 1: Details of *Amaranthus* genotypes used in the study

S. No.	Germplasm	Source
1	Arka Arunima	IIHR, Bengaluru
2	IC-551486	NBPGR, New Delhi
3	IC-447684	NBPGR, New Delhi
4	IC-551482	NBPGR, New Delhi
5	IC-551483	NBPGR, New Delhi
6	IC-551462	NBPGR, New Delhi
7	IC-551492	NBPGR, New Delhi
8	IC-551466	NBPGR, New Delhi
9	IC-551477	NBPGR, New Delhi
10	IC-38174	NBPGR, New Delhi
11	IC-551506	NBPGR, New Delhi
12	IC-551494	NBPGR, New Delhi
13	IC-551459	NBPGR, New Delhi
14	IC551468	NBPGR, New Delhi
15	Dharwad local	Farmer's field
16	Kunigal local	Farmer's field
17	Arbhavi local	Farmer's field
18	Gokak local	Farmer's field
19	Mudigere local	Farmer's field
20	Chikmagalur local	Farmer's field

RESULT

The results of the present experiment carried out with twenty genotypes of amaranthus (*Amaranthus tricolor* L.) to assess the genetic variability among yield and its component traits are presented under the following headings.

Analysis of variance

The analysis of variance indicated that significantly higher amount of variability among the genotypes for growth and yield parameters *viz*., plant height, stem girth, leaf length, number of branches per plant, leaf width, stem girth, petiole length, leaf area, leaf area index, leaf area duration, absolute growth rate (AGR), dry weight of leaf per plant, dry weight of the stem per plant, specific leaf weight, stem weight of plant per plot, leaf :stem ratio, foliage yield per plant, foliage yield per ha, total yield per plant. Similarly, the quality traits *viz*., ascorbic, beta carotene, total chlorophyll were found to have higher variability Tejaswini *et al.* (2017). The results are presented in the Table. 2.

Genetic variability, heritability and genetic advance for different parameters of amaranthus genotypes

The results pertaining to genetic factors *viz*., range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA) and genetic advance as per cent mean (GAM) are presented in Table 3.

Morphological characteristics

***Plant height and stem girth*:** The variability for plant height and stem girth was high, as reflected by its wide range from 51.33 (IC-551477) and 11.54 (IC-551482) to 64.21 cm (Kunigal local) and 18.93 mm (Arka Arunima) with mean value of 57.08 cm and 15.35 mm respectively. The PCV and GCV values were low (5.79 and 6.15% respectively). Stem girth exhibited moderate PCV (11.85%), low GCV (8.96%). The magnitude of heritability estimates was high (88.80%) and moderate heritability (57.20%) with moderate genetic advance over per cent mean (11.26%) and (13.95%) respectively.

Table 2: Analysis of variance (mean sum of squares) for growth, yield and biochemical parameters in amaranthus (*Amaranthus tricolor* L.)

Source of variation/ characters	Replications	Genotypes	S. Em ±	CD @ 5%	CV
Degree of freedom	1	19			
Growth & yield parameters					
Plant height (cm)	0.0016	20.28 ***	0.75	2.30	1.92
Stem girth (mm)	1.14	5.20**	0.82	2.49	7.75
Number of branches per plant	0.021	2.38	0.80	2.43	9.10
Number of nodes per plant	0.006	2.55	0.78	2.39	8.14
Petiole length (cm)	0.05	1.007*	0.39	1.21	10.01
Leaf length (cm)	1.42	10.53***	0.59	1.82	8.98
Leaf width (cm)	0.36	1.93	0.34	1.05	9.22
Leaf area (cm^2)	0.17	7376.70 ***	1.98	6.01	2.01
Leaf area index	0.0007	0.01 ***	0.006	0.018	4.13
Leaf area duration	0.68	822.32 ***	1.79	5.46	3.70
AGR 0.001	0.005 ***	0.012	0.038	11.40	
CGR 0.09	0.63	0.49	1.51	0.89	
Dry weight of leaf/plant (g)	10.53	30.16 ***	1.18	3.58	10.10
Dry weight of stem/plant (g)	8.77	36.25 ***	1.17	3.57	7.04
Specific leaf weight	0.000002	0.003 ***	0.0004	0.0012	1.41
Stem weight of plant/plot (g)	0.20	8722.80 ***	0.86	2.62	0.22
Leaf : stem ratio	0.00036	0.009***	0.015	0.046	4.28
Foliage yield/plant (g)	8.36	489.47***	2.25	6.85	2.30
Foliage yield/plot (kg)	0.68	0.42	0.33	1.0148	10.61
Foliage yield/ha (t)	50.62	9.23	2.52	7.6702	0.88
Biochemical parameters					
Chlorophyll (mg/g)	0.046923	0.32***	0.08	0.26	7.00
Ascorbic acid (mg/g)	0.000023	6.38 ***	0.04	0.14	1.77
Beta carotene (mg/g)	0.001562	1.61 ***	0.02	0.06	0.38

Table 3: Estimates of mean, range, components of variance, heritability and genetic advance for growth, yield and biochemical parameters in amaranthus (*Amaranthus tricolor* L.)

Sl. No.	Character	Range	Mean	GCV%	PCV%	H^2 (%)	GA	GAM
Growth & yield parameters								
1	Plant height (cm)	52.3 - 64.21	57.26	5.39	5.72	88.00	5.99	10.46
2	Stem girth (mm)	11.54 - 18.93	15.35	8.96	11.85	57.00	2.14	13.95
3	Number of branches per plant	10.75 - 14.91	13.04	5.60	10.68	27.00	0.77	6.05
4	Number of nodes per plant	12.07 - 15.95	14.06	5.60	9.88	32.00	0.91	6.53
6	Petiole length (cm)	4.15 - 7.18	5.78	10.02	14.17	50.00	0.84	14.60
7	Leaf length (cm)	5.38 - 14.37	9.68	22.83	24.53	86.00	4.23	43.76
8	Leaf width (cm)	4.19 - 7.57	6.21	16.83	19.19	76.00	1.65	30.39
9	Leaf area (cm^2)	24.45 - 268.33	142.62	42.56	42.60	99.00	124.89	87.57
10	Leaf area index	0.07 - 0.38	0.21	42.06	42.26	99.00	0.18	86.22
11	Leaf area duration	38.39 - 114.16	69.83	28.92	29.15	98.00	41.25	59.07
12	AGR	0.09 - 0.26	0.16	30.15	32.23	87.00	0.09	58.08
13	CGR	1.84 - 3.66	2.56	9.36	29.68	9.00	0.15	6.07
14	Dry weight of leaf/plant (g)	12.39 - 25.02	16.96	21.76	23.98	82.00	6.89	40.64
15	Dry weight of stem/plant (g)	16.01 - 32.06	24.23	16.84	18.26	85.00	7.75	32.02
16	Specific leaf weight	0.01 - 0.19	0.041	95.01	95.02	100.00	0.08	195.69
17	Stem weight of plant per plot (g)	600.09 - 792.78	696.71	9.47	9.48	100.00	136.00	19.52
18	Leaf : stem ratio	0.41 - 0.67	0.51	13.25	13.93	90.50	0.13	25.97
19	Foliage yield/ plant (g)	113.71 - 169.40	142.18	10.88	11.12.	95.70	31.18	21.93
20	Foliage yield/ plot (kg)	3.89 - 5.43	4.56	6.75	12.58	28.80	0.34	7.47
21	Foliage yield/ ha (t)	19.4500 - 27.17	23.76	6.095	14.16	18.50	1.28	5.40
Biochemical parameters								
22	Chlorophyll (mg/g)	1.20 - 2.70	1.79	21.89	22.99	90.07	0.77	42.96
23	Ascorbic acid (mg/g)	1.32 - 7.00	3.84	46.47	46.51	99.90	3.67	95.67
24	Beta carotene (mg/g)	6.92 - 10.12	8.62	10.42	10.42	99.90	1.84	21.45

Number of branches per plant and petiole length: The number of branches per plant ranged between 10.50 (IC-551477) and4.15 (IC-551462)to 15.91 (Arka Arunima) and 7.18 cm (Arka Arunima)with mean value of 13.04 and 5.78 cm respectively. The moderate estimates of PCV (13.27) and (14.17), low GCV (3.98%) and moderate (10.02%) were noticed. Low heritability (27%) and low genetic advance as per cent mean (2.46) was observed for the trait.

Petiole length (cm): Petiole length ranged from 4.15 (IC-551462) to 7.18 cm (Arka Arunima). The average value for the trait was 5.78 cm. PCV (14.17%) and GCV (10.02%) values were moderate along with moderate heritability (50%) and moderate genetic advance as per cent mean (14.60%).

Leaf characteristics

Leaf length and width: It ranged from 5.38 (IC-5515067) to 12.83 cm (IC-38174) with a mean of 9.68 cm. The high PCV (21.36%) and moderate GCV (18.95%) were observed. High heritability (78.80%) coupled with high genetic advance over per cent mean (34.66%) was noticed.

***Leaf width (cm)*:** The variability observed for Leaf width was high, as reflected by its wide range from 4.59 (IC-551468) to 8.58 cm (IC-551486) with an average value of 6.21 cm. High estimates of PCV (20.96%) and moderate GCV (16.29%) were observed. The estimates of heritability was high (60.40%) along with high genetic advance as per cent mean (26.08%).

Leaf area and Leaf area index (LAI): The values for leaf area ranged from 24.45 (IC-551486) to 268.33 cm^2 (Arka Arunima) with mean value of 142.62 cm^2. High estimates of GCV (42.56%) and PCV (42.60%) were found. High heritability (99%) coupled with high genetic advance as per cent mean (87.57%) was observed for the trait.

The values for leaf area index ranged from 0.07 (IC-551486) to 0.38 (Arka Arunima) with mean value of 0.21. High estimates of GCV (42.06%) and PCV (42.26%) were found. High heritability (99%) coupled with high genetic advance as per cent mean (86.22%) was observed for the trait.

Leaf area duration (days): The mean value for leaf area duration was 69.83 days with a range of 38.39 (IC-551486) to 114.16 days (Arka Arunima). The estimates of PCV and GCV were high (29.16% and 28.92%, respectively). High heritability (98%) coupled with high genetic advance as per cent mean (59.07%) was noticed.

Physiological characteristics

Absolute growth rate (AGR): The values for AGR ranged from 0.09 (IC-551494) to 0.26 (Arka Arunima) with mean value of 0.16. the estimates of GCV and PCV observed were high with values 30.15 and 32.23 per cent respectively, high heritability (87%) coupled with high genetic advance (58.08) was observed for the trait.

Crop growth rate (CGR): The values for CGR ranged from 1.84 (IC-551483) to 3.67 (Arka Arunima) with mean value of 2.56. The moderate estimates of GCV (9.36%) and high PCV (29.68%) coupled with low heritability (09.00%) and low genetic advance as per cent of mean (6.07) was observed.

Specific leaf weight: The values for specific leaf weight ranged from 0.01 (IC-551466) to 0.19 (IC-551486) with mean value of 0.04. The high estimates of GCV (95.01%) and PCV (95.02%) was observed. The high estimates of heritability (100%) coupled with high genetic advance as per cent of mean (195.69%) was observed for the trait.

Yield and its component traits

Foliage yield per plant (g): The mean value for foliage yield per plant was 142.18 g which range from 114.60 (IC-551468) to 169.40 g (Arka Arunima). Moderate estimates of GCV (10.88%) and PCV (11.12%) was observed. The high heritability (95.70%) coupled with high genetic advance as per cent of mean (21.93%) was noticed for the trait.

***Foliage yield per plot (kg)*:** The estimated value for foliage yield per plot ranged from 3.89 (IC-551468) to 5.43 kg (Arka Arunima) and mean value for this trait was 4.56 kg. Low GCV (6.75%) and moderate PCV (12.58%) values were recorded. The heritability estimated was low (28.80%) along with low genetic advance as per cent mean (7.47%) was noticed for the trait.

Total yield per plant (g): The mean value for total yield per plant was 175.03 g which ranged from 150.69 (IC-551468) to 207.59 (Arka Arunima). Low estimates of GCV (6.12%) and moderate PCV (11.34%) was observed. The low heritability (29.10%) with low genetic advance as per cent of mean (6.80) was noticed for the trait.

Total yield per plot (kg): The mean value for total yield per plant was 5.26 g which ranged from 4.52 (IC-551468) to 6.22 (Arka Arunima). The low estimates of GCV (6.50%) and moderate PCV (11.29%) was observed. The low heritability (33.50%) with low genetic advance as per cent of mean (7.80) was noticed for the trait

Biochemical parameters

Chlorophyll content (mg/g): Chlorophyll content was ranged from 1.20 (Arbhavi local) to 2.70 mg (Arka Arunima) with a grand mean of 1.79 mg. The estimates of GCV and PCV were high (21.90% and 22.99% respectively). High heritability (90.70%) was observed along with high genetic advance as per cent mean (42.96%) for the trait.

Ascorbic acid (mg/g): Ascorbic acid ranged from 1.32 (IC-447684) to 7.00 mg (Arka Arunima) with a grand mean of 3.84 mg. The estimates of GCV and PCV were high (46.47% and 46.51% respectively). High heritability (99.90%) was observed along with high genetic advance as per cent mean (95.68 %) for the trait.

Beta-carotene (mg/g): Beta-carotene content ranged from 6.92 (IC-551483) to 10.12 mg (Mudigere local) with a grand mean of 8.62 mg. Moderate estimates of GCV (10.42%) and PCV (10.42%) were observed. High heritability (99.90%) was observed along with high genetic advance as per cent mean (21.45%) for the trait.

DISCUSSION

For any crop improvement programme, it is important and a pre–requisite to maintain the genetic variability that allows identification of promising genotypes in the germplasm collections that can be used in the breeding programme to develop promising cultivars.

Variability, heritability, genetic advance and genetic advance over mean

Existence of genetic variability among the genotypes for the characters to be improved is the most basic requirement for successful selection. In the present investigation, variance due to treatments (genotypes) was significant for all the twenty for growth, earliness, yield and leaf quality parameters. It indicated that sufficient variability existed for most of the characters and considerable improvement could be achieved in these characters by selection. However, the analysis of variance by itself is not enough and conclusive to explain all the inherent genotypic variance in the collections. The phenotype of crop is influenced by additive gene effect (heritable), dominance (non-heritable) and epistatic (non-allelic) interaction. Hence, it becomes necessary to split the observed variability into phenotypic variation and genotypic variation which indicates the extent of variability existing for various traits. The estimation of heritability has a greater role to play in determining the effectiveness of selection for a character provided it is considered in conjunction with the predicted genetic advance as suggested by Panse and Sukhatme (1962) and Johnson *et al*. (1955) as the heritability is influenced by bio-metrical method, generation of hybrid, sample size of experimental material and environment. With these points in view, the results of the variability observed in the twenty amaranthus genotypes evaluated in the present investigation are discussed hereunder.

Phenotypic and genotypic coefficient of variability

The extent of variability present in the germplasm provides scope for the crop improvement programme and also depends on the extent of heritability for a trait. Variation can be created artificially but the examination of naturally existing variability in the germplasm of the species is also an important task for the breeder.

In the present study, totally twenty genotypes were evaluated to know the extent of variability for growth, yield and quality parameters. The analysis of variance indicated highly significant differences among genotypes for all the characters *viz*., plant height, stem girth, number of branches per plant, leaf length, leaf area, leaf area index, petiole length, leaf area duration, absolute growth rate, specific leaf weight, foliage yield per plant, total yield per plant, chlorophyll content, ascorbic acid content and beta-carotene content. Thus, it is implied that there was reasonably sufficient variability in the material used for the study, which provides ample scope for selecting superior and desired genotypes by the plant breeders for further crop improvement.

The results from analysis of variance revealed highly significant variation among the genotypes for growth, yield and quality parameters in amaranthus (Table 4) and considerable improvement could be achieved. However, the analysis of variance by

itself is not enough and conclusive to explain all the inherent genotypic variances in the genotypes. One of the ways, by which variability in the characters could be assessed is through simple approach of examining the range of variations. Range of variation observed for all the traits indicated the presence of sufficient amount of variation among the genotypes for all the characters studied.

Means of genotypes varied greatly for the traits *viz.*, stem girth, leaf area, leaf area duration, dry weight of leaf per plant, dry weight of stem per plant, foliage yield per plant, foliage yield per hectare indicated the higher magnitude of variability. In the present study, wide range of variability was observed for all the characters.

In the present experiment, high estimates of GCV and PCV values were observed for leaf area, leaf area index, leaf area duration, specific leaf weight. The similar results were also observed by Rani and Veeragavatham (2003) for specific leaf weight; Kusuma *et al*. (2007) for dry weight of leaf per plant; Anuja and Mohideen (2007), Ramesh *et al.* (2013) and Panda *et al*.(2017) for leaf area; Tejaswini *et al*. (2017) for leaf area index and leaf area duration; Yadav *et al*. (2014) and Tejaswini *et al.* (2017) for leaf length.

High GCV and PCV values with narrow difference is an indication of existence of broad genetic base among the population, suggesting stable expression of genotypes for these traits which provides scope for the improvement of these characters through simple selection.

Moderate GCV and PCV were observed for leaf width, petiole length and foliage yield per plant which indicated presence of moderate amount of variability for these traits and little influence of environment on these traits. The similar results are also observed by Ahammed *et al*. (2012), Hasan *et al*.(2013), Patial *et al*.(2014), Buhroy *et al*. (2017) and Diwan *et al*. (2017).

It appears that phenotypic variability may be a good measure of genotypic variability for almost all growth characters such as leaf length, leaf width, petiole length and foliage yield per plant as the estimates of GCV and PCV were closer and parallel indicating that they are least affected by environment.

Heritability and genetic advance

The effectiveness of selection for any character depends not only on the amount of phenotypic and genotypic variability but also on estimates of broad sense heritability.

High heritability was observed for plant height, leaf length, leaf width, leaf area, leaf area index, leaf area duration, AGR, specific leaf weight, foliage yield per plant, chlorophyll, ascorbic acid and beta-carotene content which indicates the prevalence of additive gene actions and lesser influence of environment in the expression of these traits, hence these characters are amenable for selection. The similar results were also observed by Pan *et al*., (2008), Chattopadhyay *et al*., (2013),Yadav *et al*., (2014), Venkatesh (2014), Mobina and Jagatpati (2015), Sarker *et al*., (2016), Buhroy *et al*., (2017), Lokeshkumar and Murthy (2017), Panda *et al*., (2017) and Yogendra *et al.,* (2018) for plant height, specific leaf weight, Yogendra *et al*. (2018) for leaf length. Kusuma *et al*., (2007) and Ramesh *et al*., (2013) for dry weight of leaf per plant, dry weight of stem per plant, Tejaswini *et al.,* (2017) for leaf length, leaf width, leaf area, leaf area duration, foliage yield per plant, chlorophyll content, ascorbic acid and beta carotene content.

Heritability in broad sense is not the true indicator of inheritance of traits. Since, only additive component of genetic variance is efficiently transferred from generation to generation. Therefore, heritability in broad sense may mislead in judging the effectiveness of selection for the trait. Considering heritability in broad sense along with genetic advance may reveal the prevalence of specific components (additive or non-additive) of genetic variance and thus, helps in judging the effectiveness of selection for the trait more accurately. High heritability accompanied with high genetic advance indicates the prevalence of additive gene effects and hence, selection would be effective for such traits. From the present investigation characters like leaf length, leaf area, leaf area index, leaf area duration, AGR, specific leaf weight, foliage yield per plant, chlorophyll content, ascorbic acid and beta-carotene content recorded high heritability with high genetic advance over mean. This indicates the predominance of

additive gene action and amenable for phenotypic selection in early generations, results are in accordance with the findings of Pan *et al.,* (2008), Chattopadhyay *et al*., (2013), Venkatesh (2014), Yadav *et al*., (2014), Mobina and Jagatpati (2015) Sarker *et al.,* (2015), Buhroy *et al*., (2017), Lokeshkumar and Murthy (2017), Panda *et al*., (2017) and Yogendra *et al*., (2018) for plant height, specific leaf weight, foliage yield per plot Yogendra *et al*., (2018) and Kehinde *et al*., (2013) for leaf length. Kusuma *et al*., (2007), Ramesh *et al*., (2013) and Tejaswini *et al.,* (2017) for leaf length, leaf width, leaf area, leaf area duration, foliage yield per plot, chlorophyll content, ascorbic acid and beta carotene content in *Amaranthus*.

Prevalence of high degree of additive components of genetic variance, high heritability coupled with high genetic advance as per cent mean, presence of high GCV and PCV for the characters viz., leaf length, leaf area, leaf area index, leaf area duration, AGR, specific leaf weight, which indicated lesser influence of environment in expression of these traits and prevalence of additive gene action in their inheritance. Hence, genetic improvements of these traits are amenable for selection.

CONCLUSION

In the present investigation, high GCV and PCV were observed for parameters like, leaf area, leaf area index, leaf area duration, AGR, dry weight of leaf per plant, specific leaf weight, speed of germination, chlorophyll content and ascorbic acid (vitamin C). It indicated existence of broad genetic base, which would be useful for further selection. The parameters like stem girth, number of branches per plant, leaf length, stem weight per plot, leaf: stem ratio, petiole length and foliage yield per plant are important characters to be accounted for gaining improvement in total yield per plant. Since, these characters had high significant and positive direct effects on foliage yield per plot.

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Original Research Paper

Morpho-physiological parameters associated with chlorosis resistance to iron deficiency and their effect on yield and related attributes in potato (*Solanum tuberosum* L.)

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ABSTRACT

The aim of the study was to assess genotypical differences over different stages for morpho-physiological parameters associated with iron (Fe) deficiency and their effect on yield. The factorial pot experiment was comprised of two major factors, i) soil-Fe status of natural vertisol [Fe-sufficient and Fe-deficient soils], and ii) genotypes [CP-3443, CP- 4105, CP-3486 and CP-4069] with differential iron-induced deficiency chlorosis (IDC) response. Data were recorded and associations between different traits were estimated. Under Fe-deficient soil, tolerant genotype (CP-3443) recorded significantly higher chlorophyll content, peroxidase activity in leaves and better yield compared to susceptible genotypes which verified usefulness as IDC tolerant potato genotypes characteristics.

Key words: Correlation, Iron-deficiency chlorosis, Morpho-physiological parameters; Potato and Yield

INDRODUCTION

Iron (Fe), one of the essential micronutrients for the growth and development of all living organisms, its deficiency is a serious problem in both agriculture and human nutrition. Iron is considerably less soluble in soils with a pH value of 8 than Zn or Mn; thus, inorganic Fe makes relatively little contribution to the Fe nutrition of plants in calcareous soils (Singh *et al.*, 2004). Fe level varies with the type and depth of the soil, ranging from 0.2% to 55% (20,000 to 550,000 mg kg1), and the highest concentration is found at 2–15 cm (Mahender *et al.*, 2019). For instance, the total Fe content in Indian soil ranges from 0.4% to 27.3% (40,000 to 273, 000 mg kg1), but that accessible to the plant is extremely variable, from 0.36 to 174 mg, which depends on the soil, plant, and environmental factors (Mahender *et al.,* 2019). Calcareous soils may contain high total Fe levels but are not accessible to the plants, because iron forms water-insoluble hydroxides and oxides, and/or Fe carbonates-bicarbonates. Thus, high bicarbonate ion concentration leads to iron-induced deficiency chlorosis (IDC) by suppressing iron uptake and/or translocation in plants (Li-Xuan *et al.*, 2005).

Potato (*Solanum tuberosum* L.) is a multipurpose crop and is the 4th most important crop contributing to the world's food requirement next to rice, wheat and maize with a global production of 388 MMT during 2017 (FAOSTAT, 2019). Potato crop is sensitive to non-optimal iron content in the substrate and marked decreases in tuber quality and yield were reported with reduced biomass, chlorophyll concentrations and alterations in enzyme activities (peroxidase, catalase and acid phosphatase) under Fe-deficient stress (Chatterjee *et al.*, 2006). Susceptibility to Fe chlorosis is based on a plant's response to Fe deficiency stress and this is genetically regulated (Simko *et al.*, 2008). IDC response is usually assessed by chlorophyll content in potato (Chatterjee *et al.,* 2006) and also other legumes like soybean, dry bean etc (Samdur *et al.,* 1999; Samdur *et al.,* 2000). Higher chlorophyll content indicates a lower occurrence of chlorosis in leaves. Iron deficiency was also found to decrease the production of oxidative stress-related enzymes such as peroxidase in several plant species and is due to less Fe concentrations in Fe-deficient leaves (M'sehli *et al.*, 2014).

Developing micronutrient-efficient genotypes can be a quick, fast and successful way to resolve soil micronutrient disorders and improve human health. In previous studies, we have screened genotypes at seedling stage with the intent to identify IDC tolerant potato genotypes. IDC tolerant genotypes had registered a significantly lower reduction in plant height, SPAD value, chlorophyll content, and peroxidase level under Fe-deficient condition. However, the association of morpho-physiological parameters under iron deficiency and their effect on yield in potato genotypes has not been examined. Given that genetic variability for IDC responses exist among potato genotypes. The objective of the study was to access genotypic differences for different morpho-physiological and biochemical parameters associated with IDC tolerance across growth stages and their yield under natural vertisol (normal and calcareous) soils using pot studies.

MATERIALS AND METHODS

Based on our earlier study (Challam *et al.,* 2021), 15 potato genotypes were evaluated for responses to Fe deficiency stress under controlled aeroponic condition. Potato genotypes with differential IDC response (tolerant: CP-3443; moderately tolerant: CP- 4105; moderately susceptible: CP-3486 and susceptible: CP-4069) were further selected for the pot experiment under net house condition at ICAR-Central Potato Research Institute, Regional Station, Shillong. Two soil type viz. Fe- sufficient and Fe-deficient were collected from two different sites Upper Shillong (25.54° N, 91.85° E) and Nongrah (25.57° N, 91.93° E), respectively. Soil samples were collected from identified sites by using hand auger at the soil depths of 0 - 30 cm by adopting random sampling procedure as described by Kalra, 1988. *In vitro* propagated potato plantlets of the genotypes were planted in twenty-five-centimetre plastic pots were filled with vertisol soils (pH: 4.3) having available Fe 7.25 mg/ Kg (DTPA extractable) for Fe sufficient plants whereas Fe deficient plants were filled with calcareous vertisol soil (pH: 8.6) having available Fe 4.53 mg/ Kg (DTPA extractable) (Table 1). All the major nutrients (N, P, K) were supplied in the form of urea, diammonium phosphate, and muriate of potash fertilizers according to the prescribed dosage. Micronutrients such as Zn, Mn and Mg were added in the form of sulphates to avoid the difficulty of concurrent symptoms of Fe deficiency. One-month old *in vitro* plantlets were transplanted into pots and were irrigated with de-ionized water as and when required. The experimental design was conducted with completely randomized design with four replication per treatment. Throughout the duration of the experiment, all the necessary management practices including pest and diseases control were carried out to ensure good growth and development. Plant growth parameters associated with Fe deficiency were assessed as described below. All the parameters were recorded at different intervals 30, 60, 90 and 120 days (harvest) after planting. The morpho-physiological and biochemical parameters such as plant height, number of main stems, number of leaves, chlorophyll content and peroxidase were recorded across the growth stages. Number and weight of tubers along with root: shoot ratio and were estimated after harvest stage.

All the parameters were recorded at different intervals 30, 60, 90 and 120 days after planting. The growth parameters such as plant height (round level to the tip of the last opened leaf), total number of stem and number of leaves were recorded. Physio-biochemical parameters viz. SPAD values (Boodi *et al.*, 2016), chlorophyll and peroxidise (Chatterjee and Chatterjee, 2003) were measured across the growth stages. To obtain tuber yield plants were maintained till maturity in each treatment. Number and weight of tubers along with root: shoot ratio were estimated after harvest stage.

Table 1: Properties of the initial soil used for pot experiment

Soil properties	Fe-sufficient soil	Fe-deficient soil
Soil pH	5.73	8.13
Electrical conductivity (dS m-1)	0.31	0.23
Organic carbon (%)	1.26	1.67
Free CaCO3 (%)	5.70	10.6
DTPA extractable-Fe (mg kg-1)	17.5	4.53

Mean squares for IDC tolerant associated traits, yield and it related traits were estimated with the aid of AGRES statistical package. Comparison between the treatments was made by using common least significant difference at (P = 0.05). Pearson's correlation coefficient between IDC tolerance associated traits across four stages, yield and it related traits were estimated for Fe-deficient soils and significance was tested (P = 0.05 and 0.01).

RESULTS AND DISCUSSION

Morpho-physiological parameters

Growth and development in plants are a consequence of excellent coordination of several processes operating at different growing phases of plant. Plant height, total number of stem and number of leaves are important morphological character representing vigour of the plant during the growth and development. These growth indicators were measured periodically during the crop period. Being an essential micronutrient, Fe promoted the growth of all potato genotypes when grown at Fe-sufficient soil. Significant difference was observed for plant height, total main stem and number of leaves between the genotypes under different soil condition at all the phenological stages viz., 30, 60, 90 and at harvest stage. The comparison between Fe-sufficient and Fe-deficient soil showed significant reduction in plant height and number of stems under Fe-deficient soil (Table 2). This reduction may be attributed to reduced cell division, meristematic activity in apical tissue, expansion of cell and formation of new cell wall under Fe starvations (Boodi *et al.*, 2016). The reduction of plant height during stress can serve an advantageous purpose for plants to reduce transportation distances, which can help to efficiently distribute water, nutrients and assimilates in plants (Aliche *et al.,* 2020). On the contrary, number of leaves was found to increase as many side shoots were observed as a result of Fe-deficiency in Fe-deficient soil. Tolerant genotype CP-3443 showed least reduction in number of leaves i.e., a greater number of leaves compared to other genotypes although lesser in plant height. Boamponsem *et al.,* (2017) made a similar observation under Fe-deficient conditions, Fe-efficient potatoes have a greater number of leaves due to shorter internodal distance and stem height. This may be influenced by genetic characteristics of genotype related to its ability to grow better and give the higher yield.

Chlorophylls, the plant pigments responsible for harvesting solar energy and converting into required chemical energy, exhibit a differential pattern in their accumulation in crop plants. Gradual increase in chlorophyll was observed from 30 to 90 days, but decline there onwards until harvest was evident for all the genotypes in Fe-deficient soils. The higher chlorophyll content in plants under Fe-deficient soil for initial growth periods could be attributed to the possible acquisition of appreciable Fe stores during prior growth of *in vitro* plants on sufficient Fe medium and available Fe in soil. Hence, plants were capable of sustaining chlorophyll biosynthesis during early stages on exposure to low Fe supplies. After 90 days, however, the plants could not acquire sufficient Fe from deficient soil led to inhibition of chlorophyll synthesis. The interaction effect was found significant across the growth stage in chlorophyll (a, b and total) (Table 2). Chlorophyll (a, b, and total) were significantly higher among tolerant followed by moderately tolerant genotype, but susceptible ones showed significantly lesser content during all the four crop growth stages under Fe-deficient soils (Table 2). The ability to absorb Fe from the soil under Fe-deficient soil as evident in tolerant and moderately tolerant genotypes helps to produce more chlorophyll pigments, since most of the leaf iron is found in chloroplast, primarily in photosynthetic electron transport chain complexes that comprise about 60 % of the total content of the leaf iron (Terry and Abadia, 1986).

Peroxidase (POD) content underwent a significant increase from 30, 60, 90 days but decline there onwards until 120 days was evident for all the genotypes under both soil conditions. However, Fe-deficient induced a noticeable decrease in the values of peroxidase in comparison to the Fe-sufficient condition (Table 2). The POD is a heme-containing enzyme and therefore its activity and/or synthesis are probably affected by iron deficiency. In the current study, decreased POD activity under limited Fe supply

Table 2 : Mean performance of potato genotypes for morpho-physiological parameters in Fe-sufficient and Fe-deficient soils across growth stages

Time interval		30 days		60 days		90 days		120 days (Harvest stage)		Mean		Change (%)
Trait	Genotype	C	T	C	T	C	T	C	T	C	T	
Plant height (cm)	CP-3486	26.3	21.8	35.6	29.3	48.9	45.9	58.8	50.9	42.4	36.97	13.4
	CP-4069	30.3	28.3	44.6	41.2	55.8	51.8	63.9	58.8	48.65	45.02	8.0
	CP-3443	28.5	23.3	40.9	38.9	52.9	48.4	60.6	54.6	45.72	41.30	9.9
	CP-4105	25.5	20.4	33.4	28.4	50.4	45.2	56.5	48.7	41.45	35.67	13.8
Mean		25.55		36.53		49.91		56.6				
LSD#		0.53**	0.37**	0.94**	0.66**	1.15**	0.81**	1.49**	1.06**			
C× T$		0.748*		NS		NS		NS				
Total main stem	CP-3486	1.3	1.1	2.2	2.0	2.9	2.2	3.3	2.5	2.42	1.95	24.2
	CP-4069	1.0	1.0	2.0	2.0	2.5	2.4	3.0	2.4	2.12	1.95	20.0
	CP-3443	1.2	1.0	2.3	2.0	2.4	2.3	2.4	2.3	2.07	1.90	4.2
	CP-4105	1.2	1.1	2.1	1.8	2.8	2.3	3.2	2.2	2.32	1.85	31.3
Mean		1.11		2.05		2.47		2.66				
LSD#		0.02**	0.01**	0.04**	0.03**	0.05**	0.03**	0.04**	0.04**			
C× T		0.022**		0.058**		0.068**		0.074**				
Number of leaves	CP-3486	15.2	15.0	30.4	36.4	39.2	50.2	56.0	67.9	35.2	42.37	21.3
	CP-4069	15.7	15.9	25.7	29.3	36.1	45.8	51.7	59.7	32.3	37.67	15.5
	CP-3443	15.0	14.8	24.5	28.5	33.1	47.1	50.6	63.0	30.8	38.35	24.5
	CP-4105	16.1	15.7	32.2	38.6	40.6	57.6	58.7	71.5	36.9	45.85	21.8
Mean		15.42		30.69		43.71		59.88				
LSD#		0.31*	NS	0.65**	0.46**	0.88**	0.62**	1.67**	1.61**			
C× T$		NS		0.921*		1.691**		NS				
Chl-a (mg/g FW)	CP-3486	0.8	0.7	0.9	0.6	1.0	0.7	0.9	0.6	0.9	0.65	33.3
	CP-4069	0.9	0.6	1.0	0.6	0.9	0.8	0.7	0.5	0.87	0.62	28.6
	CP-3443	0.9	0.8	1.1	1.0	1.2	1.0	1.1	1.0	1.07	0.95	9.10
	CP-4105	0.9	0.8	1.0	0.8	1.1	0.8	1.0	0.8	1.0	0.80	20.0
Mean		0.799		0.875		0.937		0.825				
LSD#		0.02**	0.01**	0.02**	0.01**	0.02**	0.02**	0.01**	0.01**			
C× T$		0.025**		0.024**		0.032**		0.019**				
Chl-b (mg/g FW)	CP-3486	0.4	0.3	0.5	0.4	0.5	0.3	0.4	0.3	0.45	0.32	25.0
	CP-4069	0.3	0.1	0.4	0.2	0.5	0.2	0.4	0.2	0.4	0.17	50.0
	CP-3443	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.4	0.32	25.0
	CP-4105	0.4	0.4	0.5	0.4	0.4	0.2	0.4	0.3	0.42	0.32	25.0
Mean		0.325		0.388		0.362		0.337				
LSD#		0.006*	0.005*	0.007*	0.005*	0.006*	0.005*	0.007*	0.005*			
C× T$		0.009*		0.011*		0.009*		0.011*				
Total Chl (mg/g FW)	CP-3486	1.2	1.0	1.4	1.0	1.5	1.0	1.3	0.9	1.35	0.97	30.8
	CP-4069	1.2	0.7	1.4	0.8	1.4	1.0	1.1	0.7	1.27	0.80	36.4
	CP-3443	1.3	1.1	1.5	1.3	1.6	1.4	1.5	1.3	1.47	1.27	13.3
	CP-4105	1.3	1.2	1.5	1.2	1.5	1.0	1.4	1.1	1.42	1.12	21.4
Mean		1.122		1.263		1.3		1.162				
LSD#		0.025*	0.018*	0.019*	0.013*	0.027*	0.019*	0.026*	0.018*			
C× T$		0.036*		0.027*		0.038*		0.037*				
POD (OD/ min/mg)	CP-3486	1.2	0.8	1.5	1.2	1.8	1.4	1.3	1.0	1.45	1.1	23.1
	CP-4069	1.7	1.0	2.0	1.5	2.3	1.7	1.7	1.5	1.92	1.42	11.8
	CP-3443	1.4	0.9	1.7	1.4	2.0	1.8	1.4	1.3	1.62	1.35	7.10
	CP-4105	1.1	0.7	1.4	1.1	1.7	1.3	1.3	1.1	1.37	1.05	15.4
Mean		1.1		1.475		1.749		1.325				
LSD#		0.025*	0.018*	0.031*	0.022*	0.034*	0.024*	0.027*	0.019*			
C× T$		0.036*		0.044*		0.048*		0.038*				

- least significant difference (P = 0.05) for Fe-sufficient and Fe-deficient soils individually, $ = common least significant difference for both Fe-sufficient and Fe-deficient soils for treatment comparisons, C – Fe-sufficient soil, T – Fe-deficient soil, LSD – Least significant difference (P=0.05), ** - highly significant, * - significant, Change (%) - % change for mean across four stages between Fe-sufficient and Fe-deficient soils.

may have been due to low activation and/or a reduced production of the POD enzyme. In comparison between genotypes, maximum peroxidase activity was noted in CP-3443 followed by CP-4105, CP-3486 and CP-4069 under Fe-deficient soils (Table 2). This implies during redox homeostasis, the antioxidant system in tolerant genotypes maintains a balance between production and scavenging of reactive oxygen species (ROS); such a balance is critical for the protection of the system against oxidative burst (Balakrishnan, 2000).

Yield components

Under Fe-deficient soil, reduced yield was observed in all genotypes compared to plants grown under Fe-sufficient soil. However, IDC tolerant genotype were found to have better yield and its related traits compared to susceptible genotypes. The decrease in yield under Fe-deficient soil being up to 16% in CP-3443, followed by CP-4105 (20%), CP-3486 (27%) and CP-4069 (28%) of its potential yield under Fe-sufficient (Fig. 1a). Similarly, reduced in number of tubers per plant (13-19%) and average tuber weight (3-12%) were recorded in all four genotypes (Fig. 1b & c). Under Fe-deficient soil, tolerant genotypes have better photosynthetic activity as shown by higher number of leaves and chlorophyll content while registered minimum reduction in number of tubers, average tuber weight and overall yield of plants as compared to susceptible genotypes.

Root architecture plays an important role in Fe uptake. Previous studies (Zou *et al.*, 2013; Li *et al.*, 2015) have shown that most species allocate more biomass to roots at reduced availability of Fe and allocate root biomass in shallow soil horizons, as well as increase root length and grow more and more root hairs and lateral roots; some also produce cluster roots, thereby promoting Fe uptake. Root-shoot ratio is the quantity of plant tissue that supports the quantity of those with growth functions. In case of root to shoot ratio an increase (15%) was observed in tolerant genotypes (CP-3443). A slight reduction of 3% was noted in medium tolerant genotype (CP-4105), whereas a reduction of 8% and 17% was observed in CP-3486 and CP-4069, respectively under Fe-deficient soil (Fig. 1d). Dry matter partitioning to different plant parts was significantly influenced both Fe-sufficient and Fe-deficient conditions. In the study, tolerant genotypes have more root biomass compared to susceptible genotypes. This may be due to better and deeper root system in the genotypes which help in absorption of nutrient from the deeper and surrounding layer of the soils. Root to shoot ratio clearly exhibit significant difference amongst potato genotypes in both the conditions. Genotypes with a higher root biomass actually compete more effectively for soil nutrients with the ability to produce higher yields, particularly under stress conditions. This is supported by the significant relationship of root to shoot ratio with yield (Table 3).

The available nutrient status of the soil is another important property which support the crop growth while, the crop growth and dry matter accumulation depends upon the ability of the soil to supply nutrients, the nutrients release from the soil in turn depends upon the demand from growing plants. Micronutrients, in particular Fe and Zn, either function as metal components of different enzymes or as functional structural or regulatory cofactors and are therefore connected to photosynthesis and protein synthesis. The concentration of Fe and Zn were estimated in the tubers to know their accumulation. A decrease in concentration of Fe ranges from 7.17 to 12.33% in tubers was observed in all the genotypes under Fe-deficient soil (Fig. 1e). This may be attributed to low expression of genes resulting in low xylem mobility and poor translocation capacity under Fe-deficient soil condition. A similar trend was observed in Zn, a stimulator under Fe-deficient conditions (6.54-10.05%; Fig. 1f)

Associations

A significant positive correlation was observed between morpho-physiological, biochemical and yield parameters in the study (Table 3). Number of leaves showed significant ($P < 0.05$) and positive correlation with parameters like total chlorophyll (0.981) and yield (0.970). While, yield per plant was also highly significant with number of tubers (0.957) and root to shoot ratio (0.973), respectively (Table 3). Under Fe-deficient condition, the genotype CP-3443 was able to produce more leaves which participated directly or indirectly in the manufacturing of more chlorophyll (Boamponsem *et al.,* 2017). Further, their leaves function as efficient photosynthesis structures and

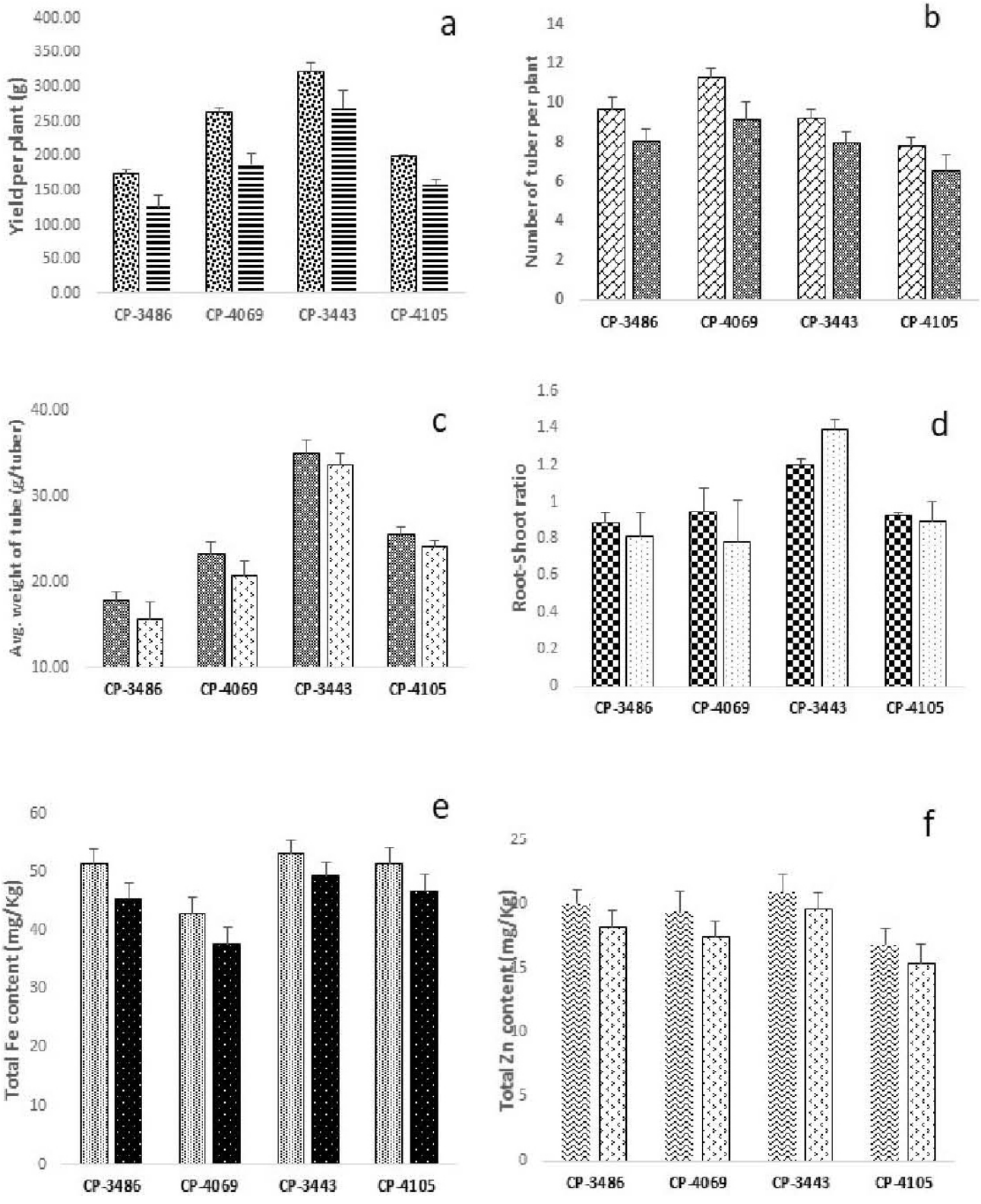

Fig. 1: Difference among IDC resistant and susceptible potato genotypes under normal and calcareous Fe soils for yield and its component traits a) Yield, b) Number of tubers, c) Avg. weight of tuber, d) Root-Shoot ratio, e) Total Fe content, f) Total Zn content. Mean of tolerant and susceptible genotypes considered for comparison; Standard error bar is common for both resistant and susceptible genotypes.

Table 3: Association between mean of morpho-physiological and biochemical parameters across all four stages and yield –related traits

	PH	TMS	NL	TC	POD	Yield	No.Tub	Wt.Tub	R:S
PH	1								
TMS	0.913	1							
NL	-0.542	-0.156	1						
TC	-0.461	-0.059	.981*	1					
POD	-0.027	0.366	0.765	0.867	1				
Yield	-0.352	0.06	.970*	.991**	0.895	1			
No.Tub	-0.358	0.039	0.9	.967*	0.943	.957*	1		
Wt.Tub	-0.914	-0.807	0.497	0.494	0.208	0.376	0.494	1	
R:S	-0.136	0.28	0.888	0.942	.962*	.973*	0.947	0.21	1

PH-plant height, TMS-total main stems, NL-number of leaves, TC-total chlorophyll, POD-peroxidase, No.Tub-number of tubers, Wt.Tub-weight of tubers, R:S-root-to-shoot ratio

*** Correlation is significant at P value=0.05 ** Correlation is significant at P value=0.01**

produced higher amount of carbohydrates in the plant system registering minimum reduction in number of tubers and overall yield of plants compared to susceptible genotypes (Braun *et al.*, 2016). While the root-shoot ratio indicates that the tolerant genotypes may have efficient root to shoot transport and redistribution of Fe in the plant (Xu *et al.*, 2017). This positive correlation further confirms the involvement of Fe in increasing the photosynthetic apparatus and maintaining the minimal stress in tolerant genotypes to enhance the physiological process under Fe-deficient soils.

CONCLUSION

Under Fe-deficient soil conditions, IDC tolerant genotypes recorded a significantly higher chlorophyll, and peroxidase activity in leaves across all four crop growth stages compared to susceptible genotypes confirming their utility as traits for identification and development of IDC tolerant potato genotypes. Towards developing high-yielding, IDC tolerant potato cultivars for Fe-deficient environments, selection may be practiced for higher tuber weight and root to shoot ratio.

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Original Research Paper

Responses of different okra (*Abelmoschus esculentus*) cultivars to water deficit conditions

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ABSTRACT

A pot experiment was conducted to evaluate the adverse effects of drought on different okra cultivars and to identify the most suitable okra cultivar for growing in drought conditions. Five okra cultivars namely Pusa Green, Clemson, Sabz Pari, Pusa Swani and Mehak Pari were subjected to three drought levels i.e., control (100% Field capacity),50% and 25% Field Capacity (FC). Physiological parameters like fresh and dry weight of plant and plant height were recorded along with biochemical attributes such as chlorophyll content (a, b, total) carotenoids, total protein, proline content, and Membrane stability index (MSI%). Results showed that drought significantly reduced all the studied parameters and at maximum drought (25% FC) lowest values of fresh weight (12.42g), dry weight (1.22g), plant height (7.86cm), chlorophyll a (9.02mg/g FW), chlorophyll b (18.69mg/g FW), total chlorophyll (27.71mg/g FW), carotenoids (11.80mg/g FW), total protein (2.73mg/g FW),whereas maximum Proline (21.36µg/g FW), and MSI (72%) were observed under the same drought. The results concerning responses of okra cultivars under drought conditions showed that maximum. Fresh weight (15.25g) and Dry weight (2.74g) was observed in Pusa green while maximum Plant height (13.77cm), Chlorophyll a (14.38mg/g FW), Chlorophyll b (24.41mg/g FW), Total Chlorophyll (38.80mg/g FW), Carotenoids (18.57mg/g FW), Total Protein (5.44mg/g FW), Proline (27.78µg/g FW), and MSI (56.33%) were produced by Sabz Pari. Hence it can be concluded that drought causes significant variation on physical and biochemical attributes of okra whereas Sabz Pari showed resistance towards the applied stress and produced better results.

Keywords: Drought, Okra, Oxidative Stress and Proline content

INTRODUCTION

Okra (*Abelmoschus esculentus* L.), belongs to the family Malvaceae, is an important vegetable crop grown during summer season in Pakistan for its nutritious edible pods. It is favored for soft and tender green pods, which are commonly consumed as curries and boiled vegetables (Mounir *et al*., 2020). Despite their astonishing beautiful flowers, okra pods are rich source of nutrients and medicinal properties. The fresh okra fruit contains carbohydrates (9.6%), protein (2.25%), fiber (1.1%), fat (0.2%) and minerals such as magnesium iron, potassium, calcium, sodium, zinc, nickel and manganese (Khan and Rab 2019). Fiber found in okra pods reduces cholesterol and risk of cardiac diseases and promotes healthy digestive track. Okra helps in slow absorption of sugar, hence can be consumed as anti-diabetic food (Nawaz *et al*., 2020).

Many environmental conditions including droughts have much harmful effects on the growth and yield of agricultural crops (Ayub *et al*., 2018). During plants exposure to drought, many physiological and biochemical alterations occur inside plants on cellular level. These changes include accumulation of ABA, reduction in leaf area and closing of stomata (Meise

et al., 2018). Drought also decreases the rate of leaf growth by making cell walls sclerotic and reduced plant biomass. Like any other abiotic stresses, under drought stress proline accumulates inside plant which helps plants to with stand under stress conditions (Lintunen *et al*., 2020). Plants which are exposed to drought stress also exhibits lower levels of carbohydrates and starch (Qu *et al*., 2019). During drought stress protein degradation starts and reduction in chlorophyll takes place (Dawood *et al*., 2019). Drought stress mostly causes accumulation of ROS (Reactive Oxygen Species) which leads to oxidative stress in chlorophyll and disrupts normal working of plants cells (Stanley and Yuan 2019). ROS also damages lipids, terpenoids, carbohydrates and nucleic acids (Guo *et al*., 2018).

Okra is one of the most important summer vegetables commonly cultivated in tropical and subtropical plans of Pakistan including Haripur due to its higher nutritional values, ease of cultivation and resistance to harsh environmental conditions. Most of the cultivated lands in Haripur region are rainfed, thus the farming relies mainly on rainfall for water. During the summer followed by low rainfall seasons, these regions face moderate to severe droughts during summer in case of low rainfall. Despite of huge potential of okra production low water availability to the crop causes a great reduction on yield per area and total area under cultivation. Hence this study was conducted to evaluate the adverse effects of drought on different okra cultivars and to identify the most suitable okra cultivar for growing in drought conditions.

MATERIALS AND METHODS

Current study was conducted at Horticulture nursery, Department of Horticulture, The University of Haripur during February-March 2020. For this experiment pots of 20 cm diameter and 15cm depth were used. Each pot was filled with 2kg potting media containing soil, sand and farmyard manure in equal ratio (1:1:1). These pots were kept inside the rainout shelters during night time and during rain to avoid the entry of water to plants. Seeds of five okra cultivars namely Pusa Green, Clemson, Sabz Pari, Pusa Swani and Mehak Pari were collected from National Agriculture Research Council, Islamabad. The experiment was laid out in a split-plot factorial arrangement with Completely Randomized Design with three replications. The drought stress was taken as main plot whereas factorial arrangements of verities were placed as a sub plot.

Imposing drought treatment

Eight seeds of okra were planted in each pot. 10 days after germination plants were thinned to 5 plants per pot. Plants were irrigated up to full field capacity (FC) during 14 days after germination to achieve maximum germination and equilibrium in plant growth, afterward okra plants were subjected to three levels of drought i.e.,100% FC (control/normal irrigation), 50% FC and 25% FC. Field capacity was calculated based on saturation percentage as described by Wilcox (1951).Uniform cultural practices were carried out throughout the research period. After 20 days of drought treatment, plants were uprooted and phenotypic characters viz. plant height, fresh weight, and dry weight were measured as per the standard procedures.

Biochemical analysis

Chlorophyll and carotenoids content was measured by the method explained by Lichtenther (1987). About 0.2 g of grounded leaf sample was extracted with 80% acetone till the residue becomes colourless. Absorbance of the acetone extract was measured at 470, 663 and 646 nm with the help of spectrophotometer (from Germany, Cary-50). Chlorophyll and carotenoids concentration was then measured by following formula.

$$Chla = (12.25A_{663.2} - 2.79A_{646.8})$$

$$Chlb = (21.21A_{646.8} - 5.1A_{663.2})$$

$$ChlT = chla + chlb$$

$$Carotenoids = \frac{1000A_{470}1.8chla85.02chlb}{198}$$

Soluble protein content in the okra leaf sample was measured by the method described by Lowry *et al.* (1951).

Method of Bates *et al.* (1973) was implemented to evaluate the proline content. 5g plant grounded plant sample was mixed in 3% sulfosalicylic acid (aqu) and the mixture was centrifuged at 10,000 rpm. The supernatant was mixed with 2 mL of ninhydrin) and 2 mL of glacial acetic acid, and the solution was boiled for 1h at 100 °C. The reaction is allowed to take place in ice bath and after the completion of

reaction 4mL toluene to extract the mixture and absorbance at 520nm were read by using spectrophotometer.

MSI was measured as per Premachandra *et al.* (1991). Fresh leaf material (1.0 g) was cut into small discs, washed with deionized water and placed in glass test tubes along with blank. 10 mL of deionized water was added to each test tube so that leaf discs get submerged. The test tubes were kept in a water bath for 30 min at 45 °C. After cooling, the electrical conductivity of water (C1) was measured using the conductivity meter. Water was again poured back to the same leaf discs and kept in the water bath at 100 °C for 10 min. The final electrical conductivity was measured. Percent conductivity was used to calculate membrane stability index using the following formula.

MSI (%) = [1 – (C1/C2)] *100

where,

C1 = Initial electrical conductivity
C2 = Final electrical conductivity

The experimental data were subjected to analysis of variance (ANOVA) using windows software Statistix 8.1 with two-factor factorial arrangements. Each treatment was replicated three times. The effects of drought on okra varieties were determined by the Least Significant Difference test (LSD) at pd"0.05, where the F test was significant (Steel and Torrie 1960).

RESULTS

Study was conducted to evaluate the adverse effects of a drought on different okra cultivars and to identify the most suitable okra cultivar for growing in drought conditions. The results obtained for various plant phenotypical and biochemical parameters studied are given in the Table 1.

Table 1: Comparison of okra varieties and drought stress on growth and biochemical attributes

Okra Cultivars	Plant Height	Fresh Weight	Dry Weight	Chlorophyll a	Chlorophyll b	Chlorophyll total	carotenoids	Protein	Proline	MSI
Pusa Green	11.66c	15.25a	2.74a	12.26b	23.30b	35.56b	16.31b	5.44a	23.58b	51.88b
Clemson	12.22bc	14.82b	2.65a	9.33c	20.50c	29.88c	13.30c	5.44a	20.34c	55.22a
Sabz Pari	13.77a	14.23c	2.47c	14.38a	24.41a	38.80a	18.57a	5.44a	27.78a	56.33a
Pusa Swani	13.33a	14.00c	2.41b	8.51d	16.52d	25.03d	10.20d	5.33a	15.78d	55.88a
Mehak Pari	12.88ab	13.93c	2.45b	5.43e	13.51e	18.94e	6.91e	4.55a	11.57e	55.77a
LSD	1.0448	0.3645	0.1166	0.2278	0.4419	0.2790	0.2371	0.7861	0.3912	0.9740
Drought										
Control	17.73a	16.63a	3.70a	10.98a	20.39a	31.37a	14.28a	8.06a	18.47c	35.93c
50% FC	12.73b	14.28b	2.72b	9.99b	19.60b	29.85b	13.09b	5.00b	19.61b	56.86b
25% FC	7.86c	12.42c	1.22c	9.02c	18.69c	27.71c	11.80c	2.73c	21.36a	72.26a
LSD	0.8093	0.2823	0.0903	0.1765	0.1976	0.2161	0.1837	0.6089	0.3030	0.7545
LSD (C×D)	1.8097	0.6313	0.2019	0.3946	0.2551	0.4833	0.4107	1.3615	0.6776	1.6871

The results are presented as mean based on n=3, LSD Least significant differences at ($p<0.05$). Different letters within column indicate significant differences between okra varieties and drought stress ($p<0.05$)

Plant height (cm)

Plant height is the major character significantly affected due to drought. In the present study, maximum plant height (17.73cm) was observed in control whereas lowest plant height (7.86cm) was recorded in plants subject to 25% FC. Okra cultivar Sabz Pari exhibits highest height (13.77cm) whereas lowest height (11.66 cm) was recorded in Pusa green (Table 1). The combined effects of drought and variety showed that Sabz Pari exhibited highest plant height among all other cultivars under all droughts. Maximum plant height 18, 15 and 8.33cm was recorded in Sabz Pari at 100%, 50% and 25% drought stress respectively,

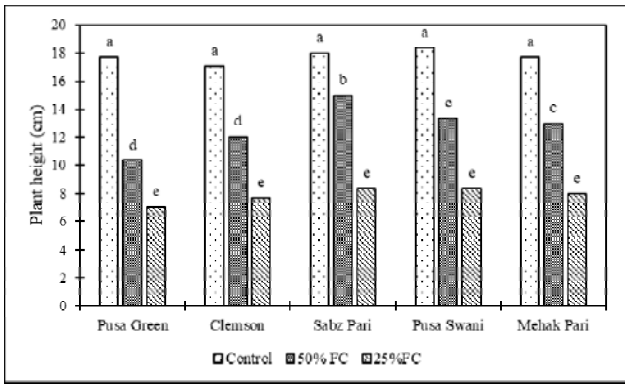

Fig. 1: Effect of drought stress on plant height of okra cultivars

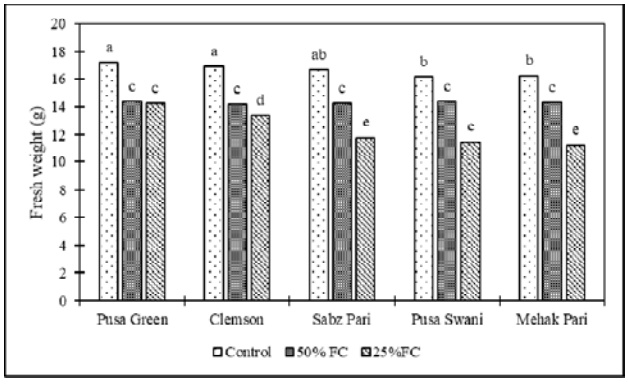

Fig. 2: Effect of drought stress on fresh weight of okra cultivars

whereas lowest plant height 17.66, 13 and 8 cm was observed in Mehak Pari under 100%, 50% and 25% drought. (Fig. 1).

Fresh weight (g)

Highest plant fresh weight (16.63g) was recorded at control (100%FC) whereas, lowest values (12.42g) were observed in plants subjected to water stress of 25% FC. Analysis of variance showed highly statistical difference ($p<0.01$) among okra cultivars for fresh weight and applied drought. Okra cultivar Pusa green showed maximum fresh weight (15.25g) whereas minimum fresh weight (13.93g) was obtained by Mehak Pari (Table 1). Results regarding interaction of drought and okra cultivars indicates that all okra cultivars showed reduction in their fresh weight as drought stress is elevated. Highest fresh weight (17.20g) was recorded in okra cultivar Pusa Green and Clemson under control condition (100% FC) whereas 25% FC drought stress significantly reduced the fresh weight and lowest values of fresh weight (11.26g) were observed in okra cultivar Mehak Pari, meanwhile under same drought level okra cultivars Pusa Green while Clemson retained maximum biomass and showed maximum fresh weight of 14.20g and 13.3g, respectively (Fig. 2). Results of our experiment correspond with findings of Idrees *et al*. (2010), Singh and Usha (2003) and Munir *et al*. (2016) who observed similar reduction in fresh weight of lemon grass, wheat and okra, respectively, when exposed to elevating drought.

Dry Weight of Plants (g)

Results regarding plant dry weight indicate that highest plant dry weight (3.70g) was recorded at control (100% FC) whereas lowest dry weight (1.22g) was observed in plants subjected to maximum water stress of 25% FC. Okra cultivar Pusa green showed maximum dry weight (2.74g) whereas minimum dry weight (2.41g) was obtained for Pusa Swani (Table 1). The interaction effect between drought and okra cultivars suggested a prominent reduction in dry weight for all cultivars. At 100% FC (normal irrigation) and maximum dry weight (3.86g) was recorded in Pusa Green while under same condition minimum dry weight (3.5g) were recorded in Pusa Swani. Meanwhile at highest drought stress (25%FC) least dry weight (0.76g) was recorded by Mehak Pari while under same drought Pusa green produced highest dry weight (1.6g) (Fig. 3). Similar results were observed by Idrees *et al.* (2010),Singh and Usha (2003) and Munir *et al.* (2016)in lemon grass, wheat and okra respectively.

Chlorophyll a (mg/g FW)

It was noted that maximum Chlorophyll-a content (10.98 mg/g FW) were recorded in 100% FC whereas minimum Chlorophyll-a content (9.0 mg/g FW) were observed in 25% FC. Okra cultivar Sabz Pari had highest Chlorophyll-a content (14.38 mg/g FW) while lowest values (5.43 mg/g FW) were recorded in Mehak Pari (Table-1). The combined results of drought and okra varieties revealed that there is a gradual reduction in chlorophyll content with increase in drought stress. At 100% FC (normal irrigation), okra cultivars Sabz Pari had maximum chlorophyll (15.23mg/g) while Mehak Pari had lowest chlorophyll content (6.46mg/g) ,. At 25% FC, Sabz Pari

maintained highest chlorophyll content (13.76mg/g) while Mehak Pari produced lowest values of chlorophyll (4.56mg/g) (Fig. 4). A reduction in chlorophyll content was also reported in drought stressed cotton (Massacci *et al*., 2008) and *Catharanthus roseus* (Jaleel *et al*., 2009).

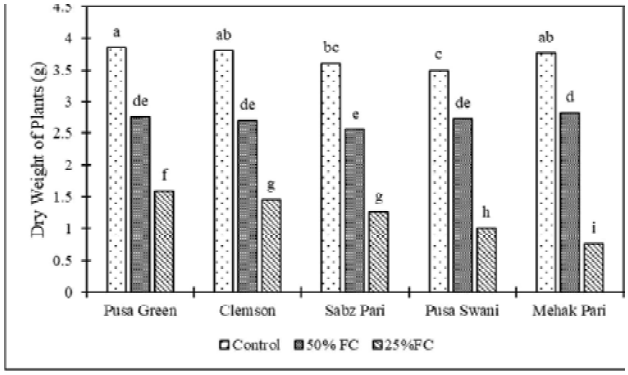

Fig. 3: Effect of drought stress on Dry weight of okra cultivars

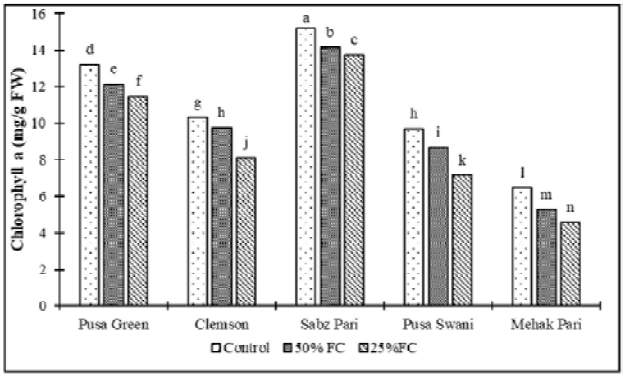

Fig. 4: Effect of drought stress on Chlorophyll a content of okra cultivars

Chlorophyll b (mg/g FW)

Maximum Chlorophyll b content (20.39 mg/g FW) was recorded in 100% FC (normal irrigation) whereas minimum Chlorophyll b content (18.6 mg/g FW) were observed in 25% FC drought. Okra cultivar Sabz Pari had highest Chlorophyll b content (24.41 mg/g FW) while lowest values (13.51 mg/g FW) was recorded in Mehak Pari (Table 1). The combined results of drought and okra varieties revealed that there was a gradual reduction in chlorophyll content with increase in drought stress. At 100% FC (normal irrigation) okra cultivars Sabz Pari showed maximum chlorophyll b (25.7mg/g) while a same irrigation lowest chlorophyll b content (14.56mg/g) were observed in Mehak Pari, on the other hand at highest drought (25% FC) Sabz Pari maintained highest chlorophyll b content (13.76mg/g) while Mehak Pari produced lowest values of chlorophyll b (4.56mg/g) (Fig. 5). The chlorophyll content decreased to a significant level at higher water deficits sunflower plants (Kiani *et al*., 2008) and *Vaccinium myrtillus* (Tahkokorpi *et al*., 2007).

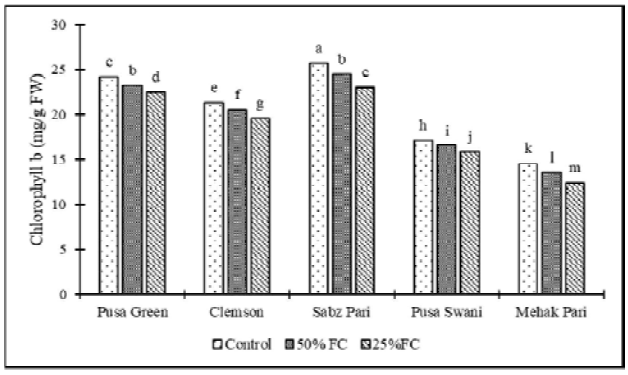

Fig. 5: Effect of drought stress on Chlorophyll-b content of okra cultivars

Total chlorophyll content (mg/g FW)

Effects of drought on total chlorophyll content indicate that maximum Total chlorophyll content (31.37 mg/g FW) was noted under no drought stress (normal irrigation) whereas minimum total chlorophyll (27.71 mg/g FW) was recorded under 25% FC drought (Table 1). Results concerning total chlorophyll content of okra cultivars reveled that Sabz Pari produced highest total chlorophyll content (38.80 mg/g FW) while lowest values (18.94 mg/g FW) were recorded in Mehak Pari (Table 1). The combined results of okra and drought stress revealed that significant reduction was observed with increase in drought stress. Highest total chlorophyll of 40.93, 38.66 and 36.6 mg/g was recorded in Sabz at 100%, 50% and 25% FC conditions, while lowest chlorophyll content 21.03, 18.83 and 16.96 mg/g was obtained by Mehak Pari at 100%, 50% and 25% FC (Fig. 6). Ram *et al*. (2014) and Amin *et al*. (2009) also observed that under drought conditions, reduction in chlorophyll content was noted in watermelon and okra respectively.

Carotenoids (mg/g FW)

Results regarding carotenoids (mg/g FW) of okra plant depicts highly significant statistical difference ($p<0.01$) among okra cultivars, applied drought and their interaction (Table 1; Fig. 7). It was noted that

maximum carotenoids (14.28mg/g FW) were recorded in 100% FC (control) whereas minimum carotenoids values (11.08 mg/g FW) were observed in 25% FC drought stress. Among the okra cultivars studied maximum carotenoids (18.57 mg/g FW) were observed in Sabz Pari while lowest concentration of carotenoids (6.91 mg/g FW) was recorded in Mehak Pari (Table 1). The combined effect of drought stress and okra cultivars for carotenoids suggested that okra cultivar Sabz Pari showed highest carotenoids (20.43, 18.4 and 16.9 mg/g at 100%, 50% and 25% FC drought, respectively), while lowest carotenoids (5.26,7.23 and 5.26 mg/g at 100%, 50% and 25% FC respectively) were observed in Mehak Pari (Fig. 7). Similar findings were previously obtained by Altaf *et al.* (2015) in okra, Idrees *et al.* (2010) in lemon grass and by Ram *et al.* (2014) in water melon.

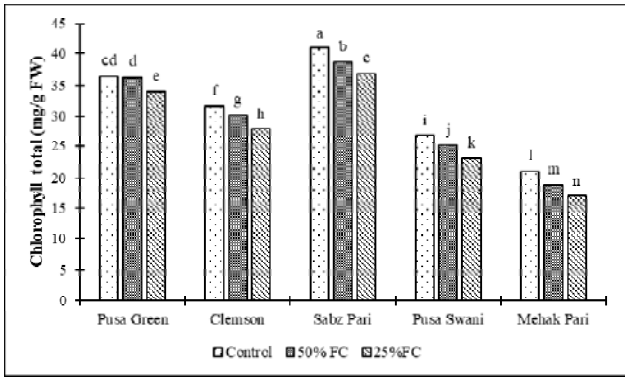

Fig. 6: Effect of drought stress on total Chlorophyll content of okra cultivars

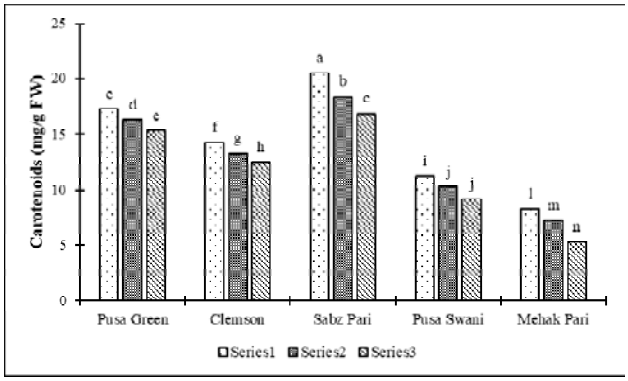

Fig. 7: Effect of drought stress on Carotenoids of okra cultivars

Total Protein (mg/g FW)

It was noted that maximum proteins (8.06 mg/g FW) were recorded in 100% FC whereas minimum protein (2.73 mg/g FW) were observed in 25% FC drought. Results regarding okra cultivars suggested non-significant variation for protein content, maximum protein (5.44 mg/g FW) were observed in Pusa green, Clemson, Sabz Pari and in Mehak Pari while lowest concentration of protein (4.66 mg/g FW) was recorded in Pusa Swani. The combined results of drought stress and okra cultivars for protein showed that okra cultivar Pusa green showed highest protein (9.0 mg/g FW) at control condition, while lowest protein (2.33 mg/g FW) was observed in Pusa Swani at highest drought stress i.e., 25% FC (Fig. 8). Amin *et al.* (2009) obtained similar results in okra and Kabiri *et al*. (2014) in *Nigella sativa.*

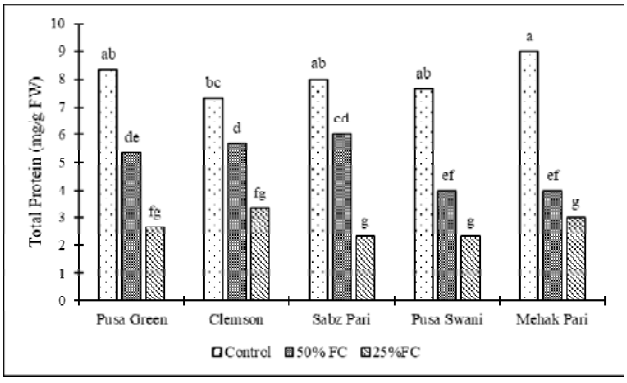

Fig. 8: Effect of drought stress on total protein of okra cultivars

Proline content (µg/g FW)

Maximum proline content (21.36µg/g) was noted in those okra plants which were exposed to 25% FC drought whereas minimum proline content (18.47µg/g) was recorded in control plants (100% FC). Okra cultivars showed significant variations in respect to proline content, okra cultivar Sabz Pari had highest proline content (27.78µg/g) while lowest proline value (11.57µg/g) was recorded in Mehak Pari (Table 1). The combined results of drought stress and okra cultivars suggested an increase in proline content of all okra cultivar with increase in drought stress and among all okra cultivar Sabz Pari showed highest proline content of 29.5, 27.63 and 26.23µg/g at 25%, 50% and 100% FC drought respectively, while okra cultivar Mehak Pari showed lowest proline content of 13.1, 11.4 and10.23µg/g were observed in Mehak Pari at 125%, 50% and 100% FC drought respectively (Fig. 9). Similar observation was recorded by Rokhzadi (2014) in chick pea, Amin *et al.* (2009) in okra and Idrees *et al*. (2010) in lemon grass.

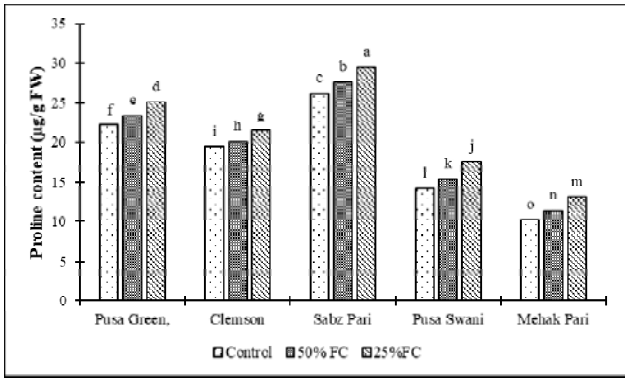

Fig. 9: Effect of drought stress on proline content of okra cultivars

Membrane stability index (%)

Effects of drought on membrane stability index (MSI) indicated that maximum MSI (72.26%) was noted at 25% drought whereas minimum MSI (35.93%) was recorded in control plants. Okra cultivars showed non-significant variations in respect to MSI, okra cultivar Sabz Pari produced highest MSI(56.33%) while lowest MSI values (51.88%) were recorded in Pusa green. The interaction of drought and okra varieties on MSI showed that okra cultivar Mehak Pari produced maximum MSI (73.33%) when grown in 25% FC drought, while least MSI (31.66%) were produced by Pusa green when grown at normal irrigation (Fig. 10). The results of the present study agree with Idress *et al*. (2010) for lemon grass and Sakhabutdinova *et al.* (2006) for wheat.

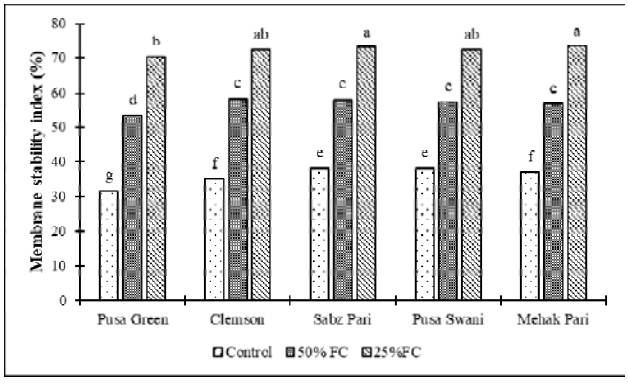

Fig. 10: Effect of drought stress on Membrane Stability index of okra cultivars

DISCUSSION

Reduction in plant biomass and other morphological parameter is clear indication of drought stress on plants and indicates sensitivity towards water deficiency. Our study also confirms that at higher water stress the decrease in plant growth parameters like plant height, fresh weight, and dry weight was observed. Wilting, closing of stomata to prevent transpiration and reduction in cell growth are some key unique responses of plants under drought stress which are produce due to lesser water content, reduced turgor pressure and lower water potential which causes reduced dry and fresh weight and plant height (Guo *et al*., 2018). Water stress also affects cell division, cell differentiation and growth which might be the reason for a cause for reduced plant height, low dry and fresh weight of okra plants. Lesser availability of water under drought stress to cells might have reduced the photosynthesis due to which plants are unable to acquire desirable biomass and height as suggested by Tanveer *et al.* (2019).

In case of effects of drought on chlorophyll content of okra it was observed that an increase in drought significantly reduces the chlorophyll pigment concentration and at highest drought the lowest values were observed. Chlorophyll (a, b and total) pigments are responsible for collection and conversion of sunlight into food and energy. Structural and functional integrity of both pigments is directly related to water availability, which is also confirmed by this study that at higher drought a decrease in photosynthetic pigments were observed as suggested by Peiró *et al.* (2020) and Hussain *et al*. (2019). Decrease in photosynthetic pigments can be attributed to lesser relative water content of leaves and lower water potential (Trueba *et al.,* 2019). Similarly, stomatal impairment in water deficit plants is responsible for reduced chlorophyll pigment content (Dąbrowski *et al.,* 2019). Drought stress also destabilizes the integrity of protein complexes and increases the activity of chlorophyllase, an enzyme which is responsible for chlorophyll degrading, this eventually leads to reduced chlorophyll concentration. This decrease in chlorophyll content might have caused a reduction in photosynthesis rate, which can lead to lesser availability of food and energy required for development and growth of new tissues and organs, which lead to reduced fresh and dry weight and plant height of okra plants under drought conditions.

Drought stress causes a series of physical and bio-chemical changes in plant organs and tissues, carotenoids reduction is one of them. This reduction can be related to severity of drought, duration of

exposure and phase of plant growth and genetic resistance capacity of plants towards drought stress (Plazas *et al.,* 2019). Khan *et al*. (2019) also proposed that the reduced carotenoids levels in plants under stress can be due to chloroplast degradation, photo oxidation of chloroplast, chlorophyll synthesis inhibition and increased chlorophyllase activity. Ahmad *et al*. (2019) suggested that activation of LOX and degradation of β-carotene caused the degradation of carotenoids under drought conditions.

Water deficiency under drought condition causes decrease in protein content of plants because water shortage seriously affects the nitrogen metabolism inside plants. The finding of our experiment regarding protein content showed a decrease in protein content with increase in drought stress; this can be attributed to the fact that under drought conditions reduction in polysomal complexes was noted in plant tissues because of lower tissue water content (Khan and Rab 2019). Also, the production of ROS (Reactive Oxygen Species) caused the collapses of protein structure, hence causing an oxidative stress which might be responsible for reduced protein content in stress affected okra plants.

During this study an increase in proline content was recorded in plants subjected to drought. Proline synthesis is greatly associated with the plants response towards stress. Production of osmolytes by plants is a common mechanism adopted to lower the stress; proline is one of these osmolytes which in case of drought stress act as organic reservoir (Zhang *et al*., 2014). It is reported that increase inproline can protect turgor pressure and prevents membrane damage on plants. So, proline accumulation is an adaptation of plants which amplify the tolerance toward drought stress (Singh *et al*., 2019).

Membrane stability index helps to assess the injury occurred to cell membrane due to a-biotic stress. The integrity of cell membrane allows plant to survive during the continuous or random water deficiencies (Oraee and Tehranifar 2020). Decrease of MSI percentage in plants indicates the tolerance of plants towards drought stress (Jafarnia *et al*., 2018). The increase in MSI of okra plants can be due to production of ROS, and oxidation of cell membrane which caused damage to membrane stability and integrity (Meena *et al*., 2017).

CONCLUSION

From this experiment it was observed that drought have significant effect on the overall growth and caused serious reduction in biochemical characters of okra cultivars and at higher drought levels maximum reductions in all studied parameters was observed. On the other hand, okra cultivars subjected to different drought levels exhibits prominent variations in all parameters but okra cultivar Sabz Pari showed promising results and showed increased chlorophyll concentration, lower Membrane stability index and higher protein content as compared to other cultivars, Mehak Pari failed to withstand the applied stress and is more sanative to the drought stress.

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Original Research Paper

Induced variability for yield and its attributing traits in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] through gamma irradiation

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ABSTRACT

Gamma ray is an effective mutagen which creates useful variability for crop like cluster bean where the natural variation is very meagre and creation of variability by conventional methods is cumbersome. In the present study, cluster bean cv. Pusa Navbahar was treated with different level of gamma rays from 50 Gy to 600 Gy with 50Gy interval and obtained M_2 population was subjected to variability estimation for yield and its attributing traits in augmented block design. The variance between the control and the mutants was found to be significant for plant height, number of pods per cluster, number of pods per plant, pod length, pod width, pulp to seed ratio. For these traits, PCV ranged from 14.28 per cent (pod width) to 31.99 per cent (pulp to seed ratio) whereas, GCV ranged from 10.10 per cent (pod width) to 24.16 per cent (pulp to seed ratio). The heritability in broad sense ranged from 50 per cent (pod width) to 79.99 per cent (Plant height). Genetic advance expressed as percentage over mean ranged from 2.06 per cent (pod width) to 222.44 per cent (plant height). The traits like plant height, pod length, pod width, pulp to seed ratio showed sufficient variability due to induced mutation. All these traits showed medium to high heritability and high genetic advance hence selection for these traits will be effective.

Key words **:** Cluster bean, Gamma irradiation, Mutation and Variability

INTRODUCTION

Cluster bean (*Cyamopsis tetragonoloba* L. Taub) is an underutilized vegetable from the family Fabaceae. But its nutritional value, hardiness and ease in growing makes it a suitable crop for future. Presently, it is being grown widely in north-western states of India for guar gum production which has a high industrial value. Whereas, in south India, it is grown mainly for vegetable purpose. In Karnataka, it is grown year-round in northern districts like Dharwad, Belagavi, Vijayapura and Haveri. Despite its nutritional importance, the area under vegetable cluster bean cultivation is very small due to non-availability of suitable variety. For developing an improved variety, genetic variability for desired traits is a pre-requisite. Cluster bean is a self-pollinated crop with a very narrow genetic base and creation of variation through manual hybridization is further difficult and not economical, owing to small flowers. Looking into this limitation, efforts were made to create variability in cluster bean using induced mutations and selecting the elite genotypes.

Mutations are the heritable changes which alter the phenotype of an organism. It is created by changes in the sequence of base pairs in the genes or changes in chromosome structure and number. The process of mutagenesis alters the biological organization of an individual. It is desirable to select and screen the mutants in M_2 generation as recessive mutation will express at this stage. The use of mutagens is a valuable supplemental strategy to plant breeding in cluster bean (Yadav *et al.,* 2014; Akhtar *et al*., 2015). It creates variability both in terms of qualitative and quantitative traits which are needed for selection. Mutations are very helpful particularly when it is aimed to improve one or two easily identifiable characters in a well-adapted variety. The present investigation was carried out to generate variability in one of the widely cultivated commercial variety 'Pusa Navbahar' which has reached a plateau in terms of yield and attributing traits.

MATERIAL AND METHODS

The present study was conducted using cluster bean variety *Pusa Navbahar* which was treated with 12 different doses of gamma rays from 50 Gy to 600 Gy with 50Gy interval at the Gamma irradiation facility available at ICAR- Indian Institute of Horticultural Research, Bengaluru. The M_1 seeds were planted in a replicated trial in field and all the survived M_1 plants (605 lines) were harvested separately to form the M_2 generation seeds. These M_2 seeds were sown in plant to progeny rows along with parent Pusa Navbahar and 3 other check varieties (MDU 1, Swarna and Sonali) in an augmented block design. The whole plot was divided into 5 blocks and in each block 122 lines were sown except for block 1 where 121 lines were sown. Seeds were sown on raised beds of length 5 m and width 1 m with a spacing 30 x 15cm between rows and plants (December 2018 to April 2019). All the recommended package of practices were followed and from each treatment, 10 plants were randomly selected for recording the data on different quantitative characters in M_2 generation. Similarly, 10 plants were picked up from the checks for comparative assessment. Observations were recorded on days to 50 per cent flowering, number of clusters per plant, number of pods per cluster, total number of pods per plant, pod length (cm), pod width (cm), 10 pod weight (g), pulp to seed ratio, yield per plant (g) and plant height (cm). Based on these observations the parameters of variability like GCV, PCV, heritability (h^2) (Allard, 1960) and genetic advance (Johnson *et al*.,1955) were calculated to identify the traits based on which selections can be made. OPSTAT software was used for the estimation of above parameters.

RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed significant variation for most of the traits studied. Mutants exhibited significant variation for plant height, days to 50 per cent flowering, pod length, pod width and pulp to seed ratio while it was non-significant for number of clusters per plant, number of pods per cluster, number of pods per plant, pod yield and pulp to seed ratio. The variance between the control and the mutants was found to be significant for plant height, number of pods per cluster, number of pods per plant, pod length, pod width, pulp to seed ratio and for only these selected traits, the estimates of variability such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2), genetic advance (GA) was calculated along with mean, range, standard error of mean, critical difference and coefficient of variation (Table 2). Graphical representation of GCV, PCV, h^2 and GA is presented in Fig 1 and 2 respectively.

Table 1: Analysis of variance for yield and its attributing traits in M_2 generation

Source of variation	Mean sum of squares				
	Entries over sample	Control	Mutants	Control Vs. mutants	Error
df	609	3	605	1	12
Plant height (cm)	375.81	1170.21	145.76*	128106.58*	29.16
Number of clusters per plant	1.61	2.91	1.49 ^{NS}	0.98 ^{NS}	1.26
Number of pods per cluster	0.28	4.17	0.22 ^{NS}	26.92*	0.58
Number of pods per plant	41.07	41.73	36.93 ^{NS}	1560.23*	75.02
Days to 50% flowering	5.73	46.32	4.36*	5.12 ^{NS}	1.11
Pod length (cm)	2.39	11.77	2.00*	144.66*	0.49
Pod width (cm)	0.02	0.06	0.02*	0.05*	0.01
Pod yield (g)	418.66	7553.45	258.47 ^{NS}	69623.26*	617.31
10 pod weight (g)	24.16	17.63	18.87 ^{NS}	40.41 ^{NS}	9.78
Pulp to seed ratio	18.79	2.85	18.37*	68.29*	7.89
Powdery mildew	69.27	144.77	67.17*	594.69*	7.08

* denotes significant at $P \leq 0.05$

^{NS} denotes Non-significant at $P \leq 0.05$

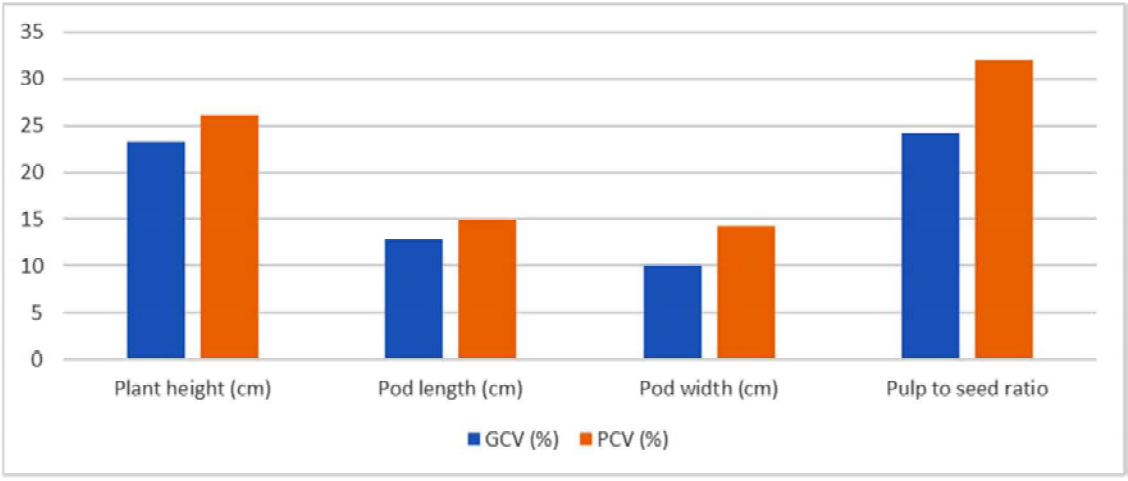

Fig. 1: Genotypic and phenotypic coefficient of variation for yield attributing traits in M_2 generation

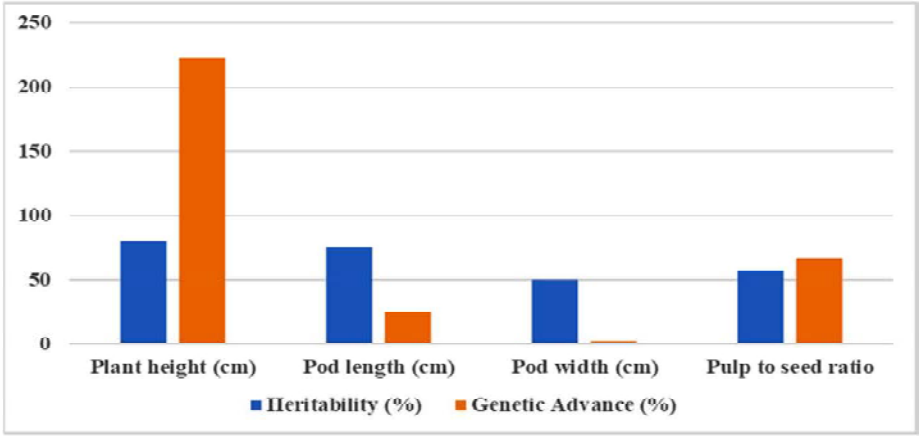

Fig. 2: Heritability and genetic advance for yield attributing traits in M_2 generation

Table 2: Parameters of variability for yield and its significantly attributing traits in M_2 generation

Traits	Mean	Range	SE(m) ±	CD @ 5%	CV (%)	GCV (%)	PCV (%)	h^2 (%)	GA (%)
Plant height (cm)	46.25	20.00 - 142.00	6.39	13.92	11.67	23.35	26.10	79.99	222.44
Number of pods per cluster	4.84	3.97- 8.20	0.90	1.95	15.7	NS	NS	NS	NS
Number of pods per plant	32.53	21.67- 64.67	10.25	22.24	26.63	NS	NS	NS	NS
Pod length (cm)	9.51	5.60 - 15.20	0.83	1.81	7.39	12.92	14.87	75.50	25.31
Pod width (cm)	0.99	0.80 - 1.20	0.07	0.15	5.74	10.10	14.28	50.00	2.06
Pod yield/plant (g)	61.67	28.86- 190.80	29.40	63.80	40.29	NS	NS	NS	NS
Pulp to seed ratio	13.40	5.03 - 28.70	3.32	7.24	20.97	24.16	31.99	57.05	66.69

SE(m) – tandard error of mean
CD – Critical difference
CV – Coefficient of variation
GCV – Genotypic coefficient of variation
PCV – Phenotypic coefficient of variation
h^2 – Broad sense heritability
GA – Genetic advance as per cent of mean

For all the traits, PCV showed higher values compared to GCV but small difference between these values indicated less environmental influence. PCV ranged from 31.99 per cent (pulp to seed ratio) to 14.28 per cent (pod width), whereas GCV ranged from 24.16 per cent (pulp to seed ratio) to 10.10 per cent (pod width). Traits like plant height and pulp to seed ratio showed moderate PCV and GCV. According to Khan and Wani (2006) moderate to high phenotypic and genotypic variance in the quantitative traits in all the mutagenic treatments indicates better chances for selection to be successful. However, coefficient of variation alone is not an effective indicator, to determine the amount of variation and it also depends on the heritability and genetic advance of the trait. The heritability in broad sense ranged from 50 per cent (pod width) to 79.99 per cent (Plant height). Genetic advance expressed as percentage over mean ranged from 2.06 per cent (pod width) to 222.44 per cent (plant height). According to Singh (2001), if heritability of a character is very high (>50 %), selection for such traits could be fairly easy. Mishra *et al*., 2020 has also reported in cluster bean that characters like number of pods per plant, pod length, pod weight, plant height, number of clusters per plant, pods per cluster showed high coefficient of variations and high heritability with high genetic gain indicating their suitability for effective selection.This is because there would be a close correspondence between the genotype and the phenotype due to the relatively small contribution of the environment to the phenotype. Johnson *et al*. (1955) suggested that high heritability combined with high genetic advance as per cent mean is indicative of additive gene action and selection based on these parameters would be more reliable. In this study, plant height (Fig. 3) showed moderate GCV but high PCV and high heritability along with very high genetic advance indicating the additive gene. action, thus making it responsive to selection. Likewise, pulp to seed ratio showed moderate GCV but high PCV, moderate heritability but high genetic advance indicating the dominance of additive gene action favouring selection for improvement of this trait.

Whereas, traits like number of clusters per plant, number of pods per plant, ten pod weight and yield per plant revealed non-significant differences among mutants indicating selection for this trait from the mutant population will be ineffective because of predominance of environmental effect. For days to 50 per cent flowering, variation was found significant between mutants but non-significant over checks that means mutants are on par with the checks or they are of late maturing types which is not the objective of the present study. The variation created through mutation for pod length (Fig. 4) was found significant between the mutants and also over checks. It showed low GCV and PCV but high heritability coupled with moderate genetic advance. Selection would be effective for this trait because of higher heritability and moderate genetic advance due to additive gene

Fig. 3: Variation in plant height obtained in M_2 generation (dwarf mutants)

Fig. 4: Variation in pod length obtained through mutation in M_2 generation

action in its background. However, pod width of mutants exhibited low GCV, PCV with moderate heritability along with very low genetic advance. This may be due to higher environmental influence and non-additive gene action. Therefore, selection followed by few generations of selfing would help in breaking the non-additive gene action. It can be concluded here that sufficient variability could be created for traits like plant height, pod length, pod width and pulp to seed ratio through induced mutation in cluster bean. Further improvement of these traits through selection is possibly result in a superior mutant variety/line in cluster bean.

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Original Research Paper

In vitro multiplication protocol for *Curcuma mangga* : Studies on carbon, cytokinin source and explant size

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ABSTRACT

Mango ginger (*Curcuma mangga* Valeton & Zijp.) is an underutilized rhizomatous species that has been valued in the tropical Asian countries as a source of vegetable, spice, salad, medicine and essential oil. This species is hardy and requires less care for obtaining good yields. Rhizomes are the commonly used propagules for the species, which are also the economic part of the crop. Huge quantity of seed rhizomes is required to promote this crop in larger area. Efficient *in vitro* multiplication protocol is one of the options to meet the planting material requirement. Effects of carbon source (glucose, fructose and sucrose) and concentration (1 and 3%, w/v), cytokinins (BAP and *meta* topolin) and concentration (1 mg/ L and 2 mg/L), size of explants (one/ two/ three bud) and IBA treatment (0, 250, 500 and 1,000 mg/L) for concurrent *ex vitro* rooting cum hardening were studied. Results revealed that for facilitating efficient multiplication, medium should be supplemented with glucose (3%) as carbon source and *meta* topolin (1 mg/L) as cytokinin. Two-bud explant should be used for subculture as it promoted superior shoot proliferation. Concurrent *ex vitro* rooting cum hardening was possible even without auxin treatment. The present protocol could be useful for large scale production of quality planting material of this underexploited tropical species.

Keywords: *Ex vitro* rooting, Glucose, Hardening, Inoculum size, Mango ginger and Tropical

INTRODUCTION

Curcuma mangga Valeton & Van Zijp. is an important species of Zingiberaceae family, which is found distributed in Asian countries including Java, India, Thailand, Malaysia and Indonesia (Sirirugsa *et al.*, 2007; Leong-Škorničková *et al.*, 2010; Singh, 2017). In most of the native regions, young shoots and rhizomes of this underutilized species are used as vegetable, salad or medicine. It is known to be a source of curcumin, a key component in drug industries due to its numerous uses (Aggarwal *et al.*, 2007). Curcumin is found in all rhizome parts including mother, primary and secondary rhizomes in the range of 0.34 to 0.45% (Waman *et al.*, 2018). Studies suggested that the species possesses antioxidant, anticancer and anti-inflammatory activities (Liu and Nair, 2012; Malek *et al.*, 2011). Recently, a cream formulation containing *C. mangga* was found to have wound healing and anti-inflammatory activities (Suthasinee and Supinya, 2019). Further, it has been found to be a source of essential oil, which contains β-myrcene (52.4 to 65.3%) as dominant compound (Wahab *et al.*, 2011, Waman *et al.*, 2018). Considering raw mango like aroma of rhizomes, the species has good scope for use as a flavouring agent in processing industries as well.

Mango ginger, like other rhizomatous species, is propagated by using rhizome pieces. During earlier studies, seed rhizomes (15 to 25 g size) have been recommended for obtaining produce suitable for fragrance, pharmaceutical and value addition industries (Waman *et al.*, 2018). However, mango ginger is generally planted at a closer spacing of 25 to 30 cm, thereby requiring higher quantities of planting material. Micropropagation is an efficient means for large scale production of plantlets.

Earlier studies on *in vitro* culture in the species have primarily dealt with optimization of plant growth regulators for direct shoot regeneration and somatic embryogenesis (Hutami and Purnamaningsih, 2003; Raihana *et al.*, 2011; Pikulthong *et al.*, 2016). However, a number of factors are known to determine the ultimate success of a micropropagation protocol (Waman and Bohra, 2018). Carbon sources are generally added in the culture medium for providing necessary energy and to act as an osmoticum (Lipavska and Konradova, 2004). As both kind and concentration of carbon source influence *in vitro* culture response, these aspects were studied in present investigation. Further, new sources of cytokinins such as *meta* topolins have been found to be superior in several species (Escalona *et al.*, 2003; Bairu *et al.*, 2008; Woztania, 2010) and hence, its efficacy was tested in *C. mangga*. Optimization of explant size is required for obtaining maximum shoots per unit inputs (Elboullani *et al.*, 2017) and hence, was also studied here. Process of concurrent *ex vitro* rooting cum hardening (CEVRH, method in which *in vitro* derived shoots are subjected to rooting and hardening simultaneously) has been found to be superior method than *in vitro* rooting and hardening in various species (Bohra *et al.*, 2016). Hence, the present investigation was undertaken for standardization of carbon source, cytokinin, size of inoculum and CEVRH for *in vitro* culture of *C. mangga*.

MATERIALS AND METHODS

Present investigation was carried out in the Plant Tissue Culture Laboratory, Division of Horticulture and Forestry, ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India during 2018-19. Chemicals including salts of MS medium, plant growth regulators, carbon sources, gelling agents *etc*. were procured from M/s. HiMedia Laboratories, Mumbai, India. Borosilicate culture bottles of 200 ml capacity with polypropylene screw caps were used for culture incubation.

Media preparation and culture incubation

For all the experiments, MS medium was used and other inputs were added as per the experiment. pH of the medium was adjusted to 5.7 using digital pH meter (Hanna, HI2211). Medium was gelled using Clerigar (5 g/L) and 30 ml of it was dispensed into each culture bottle prior to sterilizing in autoclave (Optics Technology, Delhi) at 121 °C for 15 min. Culture bottles were wrapped with cling film and were incubated at 24 °C with photoperiod of 16:8 (light: dark) cycle. For maintaining photoperiod, 40 W fluorescent lights (Crompton Greaves, Mumbai) were used.

Collection of explants and aseptic culture establishment

Growing shoots of *C. mangga* (IC-0628643) were collected from experimental fields of the Institute. Shoots were separated after washing of clumps in running tap water to remove adhering soil. Rhizome blocks containing shoot tips were excised from the clumps and used for establishing aseptic cultures. For this, excised tissue blocks were soaked in 0.2% carbendazim (Bavistin) and streptocyclin sulphate (200 mg/L) for 20 min. It was followed by removal of thin layer of tissues from the blocks. In laminar air flow, the excised explants were washed with sterile deionized water, followed by surface sterilization using 0.1% mercuric chloride (12 min.). Such explants were rinsed thoroughly for four times with sterilized deionized water to remove traces of mercuric chloride. Explants were initiated onto basal Murashige and Skoog's (MS) medium supplemented with ascorbic acid (50 mg/L).

Effect of carbon sources and their concentrations on shoot multiplication

Effect of three types of carbon sources (glucose, fructose and sucrose) at 1 and 3% concentration was studied on culture multiplication. Medium was supplemented with 1 mg/L BAP and 0.2 mg/L NAA. Cultures obtained after first subculture were used for the experiment. Besides culture growth parameters, chlorophyll content in leaves of micro-shoots obtained from various treatments was determined by procedure described earlier (Lichtenthaler and Wellburn 1983).

Effect of cytokinins on shoot multiplication

Effect of two cytokinin *viz*. 6- benzylamino purine (BAP) and *meta* topolin (*m*T) at 1 mg/L and 2 mg/L was studied during subculture 4 and 5. Growth regulator free MS medium was used as control. During subculture 6, both levels of *m*T were compared with MS basal medium. Media also contained 0.2 mg/L naphthalene acetic acid (NAA) and glucose (3%) as carbon source. Experiment was

laid in CRD with six replications each during subculture 4 and 5 and nine replications during subculture 6.

Effect of size of explant on culture multiplication

Effect of one, two and three bud explant was studied on culture multiplication. MS medium supplemented with *m*T (2mg/L) + NAA (0.2 mg/L) + glucose (3%) was used for the experiment. Experiment was laid in CRD with ten replications each.

Effect of auxin dipping on CEVRH

Concurrent *ex vitro* rooting cum hardening was studied in which effect of three concentrations of indole-3- butyric acid (IBA, 250, 500 and 1,000 mg/ L) was compared with de-ionized water as control. Micro-shoots of *ca*. 3 cm were excised and washed with water to remove adhering agar. Basal parts of the micro-shoots were dipped in the solution (30 s) and planted in cups of 200 ml capacity filled with coir pith as a substrate. Plantlets were covered with polythene bags to maintain humidity during hardening. Experiment was laid in CRD. Survival and growth parameters were recorded after 7 weeks of transplanting. For establishment, two replications of ten plants each were used, while for growth observations, ten plants were used.

Experimental design, data collection and statistical analysis

All the experiments were laid out in completely randomized design (CRD). All the values were presented as mean ± standard error of mean. Data collected from all the experiments was subjected to analysis of variance using Web Agri Statistical Package (WASP v. 2.0, ICAR- CCARI, Goa, India). Mean separation was done using least significant difference.

RESULTS AND DISCUSSION

Effect of carbon sources and their concentrations

Influence of three carbon sources at two levels was studied on *in vitro* shoot multiplication in mango ginger (Table 1), which revealed significant differences among the treatments. Sucrose is mainly employed as a source of energy in culture medium. However, kind and concentration vary depending upon the species (Sharmin *et al.*, 2013). During the present investigation, glucose (3%) was found to support significantly superior multiplication (6.3 shoots/ culture). Though higher number of shoots was produced in media supplemented with 3% concentration than those with 1% sugars; except for glucose, none of the treatments were statistically significant. Positive effects of glucose on culture multiplication have been reported in *C. longa* (Salvi *et al.*, 2001) and banana (Bohra *et al.*, 2016).

Alteration of carbon source or its content had no influence on number of leaves per micro-shoot (3.8 to 5.8) and mean shoot length (3.6 cm to 5.5 cm). Interestingly, despite producing highest number of shoots, medium supplemented with glucose (3%) could not improve leaf production and shoot length. It was probably due to utilization of energy for shoot bud proliferation rather than for leaf production. Previous studies on banana have also suggested significant role of carbon source (sucrose/dextrose/ fructose, when used alone) on shoot multiplication,

Table 1: Effect of carbon source and their concentration on shoot multiplication in *C. mangga*.

Carbon source	No. of shoots/ explant	No. of leaves/ shoot	Mean shoot length (cm)
Fructose (1%)	1.8 ± 0.48 b	5.8 ± 0.25 a	3.8 ± 0.90 a
Fructose (3%)	3.0 ± 0.58 b	5.0 ± 0.34 a	4.8 ± 0.38 a
Glucose (1%)	2.3 ± 0.63 b	4.4 ± 1.20 a	3.6 ± 0.94 a
Glucose (3%)	6.3 ± 1.25 a	3.8 ± 0.25 a	4.5 ± 0.39 a
Sucrose (1%)	2.0 ± 0.00 b	5.0 ± 0.20 a	4.0 ± 0.27 a
Sucrose (3%)	2.8 ± 0.25 b	4.6 ± 0.31 a	5.5 ± 0.40 a

Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

while there was no significant difference in mean shoot length (Madhulatha *et al.*, 2006).

Differences in chlorophyll content in micro-shoots were influenced by treatments (Fig. 1). Highest values of photosynthetic pigments *viz*. Chl a (1.77 mg/g), Chl b (0.54 mg/g) and total chlorophyll content (2.31 mg/g) were reported from shoots cultured on medium containing 3% glucose. This means, treatment D_3 not only produced a greater number of shoots, but also had higher accumulation of photosynthetic pigments in them. Such improvement in chlorophyll concentration with increment in quantity of carbon source in the medium has been reported by Capellades *et al.* (1991) in rose. On the contrary, the lowest values for photosynthetic pigments (0.58 mg/g, 0.15 mg/g and 0.73 mg/g) were observed in shoots derived from cultures grown on F_3. This suggested specificity of carbon source required for *C. mangga* in the present study.

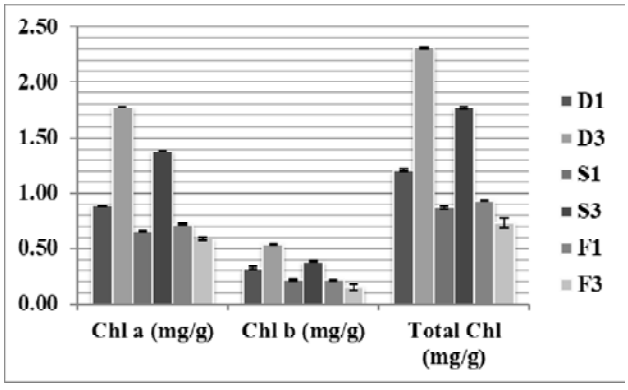

Fig. 1: Chlorophyll components (mg/g) in leaves of micro-shoots cultured on media supplemented with different carbon sources (D_1: Glucose 10 gL^{-1}; D_3: Glucose 30 gL^{-1}; S_1: Sucrose 10 gL^{-1}; S_3: Sucrose 30 gL^{-1}; F_1: Fructose 10 gL^{-1}; F_3: Fructose 30 gL^{-1}). Values are expressed as mean ± SE.

Effect of cytokinins on shoot multiplication

Cytokinins are added in culture medium to facilitate shoot proliferation and its requirement needs to be standardized for each species. In present study, performance of BAP and *m*T was tested in comparison with growth regulator free medium as control during fourth and fifth culture cycles. Even without cytokinins, culture multiplication of 4.0 and 8.2 shoots/ inoculum was possible (Table 2). However, Raihana *et al.* (2011) reported mere 1.2 to 2.3 shoots per explant in growth regulator free medium. This variation could be attributed to differences in the genotypes used in both studies, probably indicating higher concentration of endogenous cytokinins in genotype used in present study.

Though, addition of BAP supported shoot multiplication up to 7.0 (1 mg/L) and 7.7 (2 mg/L) shoots/ culture during fourth subculture, the cytokinin was not effective in inducing multiple shoot buds in subsequent cycle and values remained on par with MS basal medium (Table 2). Similar observations have been reported by Raihana *et al.* (2011) in *C. mangga*, who observed non-significant differences among cultures grown on basal medium and those grown on media supplemented with up to 11 mg/L BAP. Hence, from findings of both present and earlier report, considering the low efficacy of BAP, it should be avoided for obtaining mass multiplication in *C. mangga*. Similarly, Wojtania (2011) had encountered similar conditions in which BAP did not improve,

Table 2: Effect of cytokinins on shoot multiplication in *C. mangga* during subculture

Treatment	No. of shoots/ inoculum		No. of leaves/ shoot	
	SC 4	SC 5	SC 4	SC 5
Basal	4.0 ± 0.68 b	8.2 ± 1.30 b	2.8 ± 0.37 a	4.1 ± 0.18 a
*m*T (1 mg/l)	8.0 ± 0.73 a	13.3 ± 0.84 a	0.8 ± 0.11 c	2.4 ± 0.31 b
*m*T (2 mg/l)	9.3 ± 1.05 a	15.2 ± 1.56 a	1.0 ± 0.11 c	2.4 ± 0.16 b
BAP (1 mg/l)	7.0 ± 0.45 a	7.0 ± 0.45 b	2.1 ± 0.18 b	4.0 ± 0.29 a
BAP (2 mg/l)	7.7 ± 1.15 a	9.2 ± 1.40 b	2.0 ± 0.25 b	3.9 ± 0.35 a

SC – Sub culture, Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

Table 3: Effect of *meta* topolin on shoot multiplication in *Curcuma mangga* during sub-culture 6

Treatment	No. of shoots/ inoculum	No. of leaves/ shoot	Mean shoot length (cm)
Basal	7.9 ± 1.05 b	4.5 ± 0.38 a	8.1 ± 0.31 a
*m*T (1 mg/l)	11.9 ± 0.79 a	3.9 ± 0.26 ab	6.4 ± 0.56 b
*m*T (2 mg/l)	13.7 ± 1.39 a	3.4 ± 0.14 b	5.9 ± 0.11 b

Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

rather hampered the shoot proliferation process in *Pelargonium* hybrids.

Meta topolin was found to be highly efficient cytokinin in the present study. As high as 9.6 (subculture 4) and 15.2 (subculture 5) shoots/ inoculum were reported in cultures grown on medium supplemented with 2 mg/L *m*T. These values remained statistically similar with shoot multiplication observed on medium supplemented with 1 mg/L *m*T. Present findings suggested the efficacy of *m*T in mango ginger even at low concentrations. The results are in conformity with studies in *Pelargonium* hybrids, wherein *m*T was found to be more promising cytokinin whereas BAP did not show positive signs on multiplication (Wojtania 2011). Interestingly, number of leaves/ shoots showed reducing trend as shoot multiplication increased. This was more prominent in case of *m*T as these treatments induced higher bud proliferation.

During subculture 6 also, similar trend was maintained in which 13.7 and 11.9 shoots/ culture were obtained in medium supplemented with 2 mg/ L and 1 mg/L *m*T as against 7.9 shoots/culture in MS basal medium (Table 3). Superiority of *m*T in long term culture has previously been reported (Wojtania 2011), thereby suggesting the superiority of *m*T observed in present study. About 4.5 leaves/ shoot were produced in control, which remained on par with shoots obtained from 1 mg/L of *m*T (3.9 leaves/ shoot). Longest shoots (8.1 cm) were noticed in cultures grown onto basal medium and increase in *m*T concentration resulted in decrease in shoot length to 6.4 cm and 5.9 cm.

Effect of explant size on culture multiplication

Optimum explant density is one of the key determinants for success of a micropropagation protocol. In the present study, culture multiplication improved from 7.1 (one bud) to 12.9 (two bud) and 12.8 (three bud) (Fig. 2 and 3). As there was no significant difference between two and three bud explant, it is recommended to use two bud inoculum as it would reduce the explant requirement without compromising the multiplication ratio. Influence of explant density on culture multiplication has been reported in turmeric and globe artichoke (El-Hawaz *et al.*, 2015; El Boullani *et al.*, 2017).

Fig. 2: Effect of inoculum density on *in vitro* culture multiplication in *C. mangga*;
(one bud, two bud and three bud - from left to right)

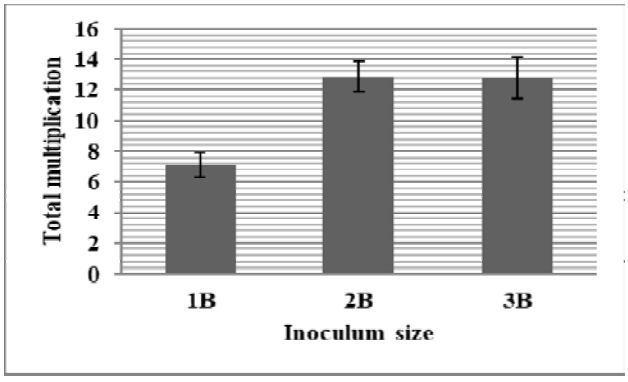

Fig. 3: Effect of explant size (1B: one bud; 2B: two bud and 3B: three bud) on culture multiplication in *C. mangga*. Values are expressed as mean ± SE.

Effect of auxin dipping on CEVRH

Single step *ex vitro* rooting cum hardening has been considered as an efficient method to reduce time, labour and other operational costs (Ranaweera *et al.*, 2013). This is particularly relevant in cases wherein planting material requirement per unit area is higher as in case of present species. During present study, root induction and hardening was possible without external auxin application (Fig. 4). In fact, auxin dip had adverse effect on these parameters. All the micro-shoots treated with deionized water (control) showed 100% establishment and rooting percentage got reduced to 60% at the highest (1,000 mg/L) concentration of IBA (Fig. 5). Being a rhizomatous species, each micro-shoot carried a small part of rhizome at the base and when transferred to the substrate *ex vitro*, it might have given the stimulus for root induction as seen in case of rhizome sett grown plants.

Fig. 4: Concurrent *ex vitro* rooting cum hardening (CEVRH): multiplied cultures (a), isolated micro-shoots (b) and micro-shoots planted in cups for hardening

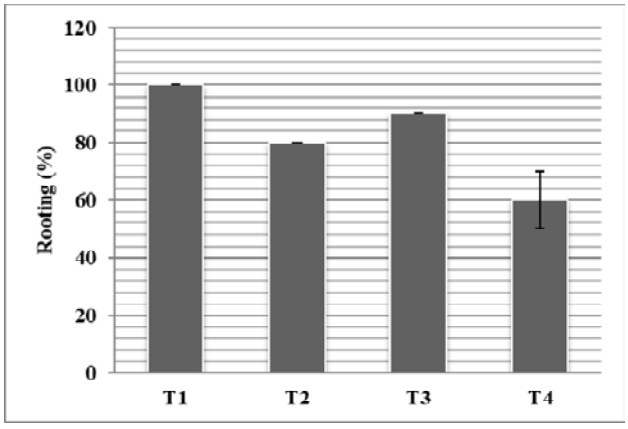

Fig. 5: Root induction percentage as influenced by auxin (IBA) dipping treatments (T_1: 0 mgL^{-1}; T_2: 250 mgL^{-1}; T_3: 500 mgL^{-1}; T_4: 1000 mgL^{-1}) during concurrent *ex vitro* rooting cum hardening (CEVRH) in *C. mangga*

Growth parameters as influenced by CEVRH have been presented in Table 4. Values amongst the treatments varied between 4.0 ± 0.46 cm and 5.0 ± 0.70 cm (shoot length), 0.50 ± 0.03 cm and 0.50 ± 0.05 cm (collar thickness), 4.0 ± 0.57 and 4.9 ± 0.55 (number of primary roots), 2.6 ± 0.21 cm and 3.2 ± 0.45 cm (mean root length), 5.2 ± 0.70 and 5.5 ± 0.71 (number of leaves per plantlet). As none of the treatments could improve the plant growth over control, it could be recommended to use *in vitro* grown micro-shoots for *ex vitro* rooting and hardening without any auxin dip. Hardened plants were transplanted in pots and all the plants grew normally.

CONCLUSION

In the present study, an efficient micropropagation protocol was developed for *C. mangga*. Various factors studied showed profound influence on the *in vitro* multiplication of the species. Use of MS medium supplemented with glucose (3%) as carbon source and *meta* topolin (1 mg/L) as cytokinin was recommended to promote superior shoot proliferation. Single step *ex vitro* rooting cum hardening was successful even in the absence of auxin treatment.

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Table 4: Shoot and root growth parameters in mango ginger plantlets as influenced by auxin dipping during CEVRH

Auxin (IBA) concentration	Shoot Length (cm)	Collar thickness (cm)	No. of Primary roots	Mean root length (cm)	No. of leaves/ plantlet
0 mg/L	4.5 ± 0.73	0.5 ± 0.04	4.9 ± 0.55	3.2 ± 0.45	5.5 ± 0.43
250 mg/L	4.0 ± 0.46	0.5 ± 0.04	4.0 ± 0.57	2.6 ± 0.21	5.5 ± 0.71
500 mg/L	5.0 ± 0.70	0.5 ± 0.05	4.8 ± 0.70	2.6 ± 0.42	5.6 ± 0.56
1,000 mg/L	4.2 ± 0.61	0.5 ± 0.03	4.5 ± 0.43	3.1 ± 0.20	5.2 ± 0.70

Values presented as mean ± SE.

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Original Research Paper

Effect of fungicide and essential oils amended wax coating on quality and shelf life of sweet orange (*Citrus sinensis* Osbeck)

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ABSTRACT

Laboratory research was conducted to study the effect of wax amended coating on the shelf life of *Citrus sinensis* Osbeck during 2017-18 at Rampur, Chitwan. The experiment was conducted in single factor Completely Randomized Design (CRD) with nine treatments and four replications. The treatments consisted of carbendazim and three essential oils *viz*. lemongrass, mentha and eucalyptus oil at two different concentrations of 0.1% and 0.5%, all of them infused with 10% wax emulsion. The wax treatment devoid of fungicide and essential oils served as control. The application of essential oils with wax improved shelf life and enhanced juice retention, firmness, titratable acidity, vitamin C and disease reduction. But total soluble solid was found higher in fruits treated with wax emulsion only. The highest shelf life and disease control was obtained with wax with 0.5% carbendazim but waxing with 0.5% eucalyptus oil and 0.5% lemongrass oil can be better alternatives considering their superior performance in environmental aspects, consumer preferences and quality parameters like juice retention, firmness, titratable acidity and vitamin C.

Keywords **:** Carbendazim, Eucalyptus oil, Green mold, Lemongrass oil, Post-harvest

INTRODUCTION

Sweet Orange (*Citrus sinensis* Obseck) is an economically important citrus fruit of the mid hill region of Nepal. The mid hill region of Nepal (1000 to 1500 masl altitude) has a comparative advantage in the production of sweet orange over traditional crops (rice, wheat, maize *etc*). Sweet orange is the second most grown citrus crop in Nepal after Mandarin in terms of area and production (MOALD, 2020). The oranges in the Nepalese agricultural market have to compete with products coming from neighboring countries like India and China. The cost of production of sweet orange is higher due to high input costs, the need for hybrid budded and grafted saplings and intensive labor requirements to grow the crop which has forced the grower to think about improving postharvest management practices. The lack of suitable storage and preservation techniques forces the farmers to sell sweet oranges before their horticultural maturity and just after picking. The unaffordable postharvest preservation has led to a negative effect on the citrus enterprises in Nepal (Kaini, 2013).

Green mold (*Penicillium digitatum* Sacc.) and blue mold (*Penicillium italicum* Wehmer) are the most economically important postharvest pathogens of sweet orange causing significant losses (Abd-El-Khair and Hafez, 2006; El-Otmani *et al*., 2011; Papoutsis *et al*., 2019). Currently, the control of green and blue mold is accomplished by pre-and postharvest application of chemical fungicides such as carbendazim, imazalil, thiabendazole, pyrimethanil, fludioxonil, prochloraz and and guazatine (Danderson, 1986; Ismail and Zhang, 2004; Smilanick *et al*., 2006; Smilanick *et al*., 2008; Berk, 2016; Joshi *et al*., 2020). Broadly, such fungicides inhibit the ergosterol synthesis, mitochondrial electron transport and synthesis of multi-site enzymes, protein and nucleic acid thereby kill or inhibit fungi or fungal spore

germination (Yang *et al*., 2011). However, synthetic fungicides are used as the conventional ways of reducing postharvest rots which have many drawbacks including high cost, handling hazards, concern about pesticide residue on fruit and a threat to human health and environment (Tzortzakis, 2009). Various synthetic fungicides were identified as toxic and carcinogenic by various researchers (Rouabhi, 2010; Singh *et al*., 2016). Pathogens also developed resistance against extensive use of synthetic fungicides resulting in declining fungicidal efficiency (Fogliata *et al*., 2000; Hao *et al*., 2011).

The application of essential oil amended coatings has been developed as a novel and eco-friendly approach to control postharvest microbes, maintain fruit quality and improve shelf life (Alam *et al*., 2017; Jhalegar *et al*., 2015). The essential oils do not have only anti-fungal properties, but the secondary metabolites also have antioxidant and bio-regulatory properties (Du Plooy *et al*., 2009; Jhalegar *et al*., 2014; Bagamboula *et al*., 2004; Hendel *et al*., 2016). The increasing demand for organic fruits encourages replacing synthetic fungicides with safer alternatives. The volatility, ephemeral nature and biodegradability of essential oils make it comparatively advantageous for the treatment of postharvest citrus disease (Ameziane *et al*., 2007). The synergism between the components in volatiles may be the reason behind the fungitoxic property of essential oils. Therefore, there is a minimal possibility of resistance. The application of essential oils with wax increases its longevity and reduces the amountof essential oils required per fruit. Therefore, the study was made to compare various wax amended treatments, their efficacy and their impact on postharvest parameters.

MATERIALS AND METHODS

Experimental site and fruit material

The present investigation was carried out at Agriculture and Forestry University (AFU), Rampur, Chitwan during the year 2017-2018. The location of the site is 27°40' N and 85°19' E with an elevation of 228 meter above sea level. The experiment was conducted in a cool and humid winter season. The local variety of sweet orange handpicked from the farmers orchard of Sindhuli was transported to Chitwan for the experiment. The fruits were kept in the tagged plastic trays during the storage period at room temperature.The average weight of fruit was 144.56 g. The average seed number was 10. The average juice content of fruit was 84.19 ml.

Experimental design

The experiment was laid out in single factor Completely Randomized Design (CRD) with nine treatments and four replications. There were a total of 36 experimental trays having 12 fruits per tray. The treatments were finalized based on the findings of Tripathi *et al.* (2004), Jhalegar *et al.* (2015) and Rokaya *et al.* (2016).

Treatments details

T1: 10% (w/v) wax emulsion with 0.1% (v/v) Lemongrass (*Cymbopogon flexuosus*) oil

T2: 10% (w/v) wax emulsion with 0.5% (v/v) Lemongrass (*Cymbopogon flexuosus*) oil

T3: 10% (w/v) wax emulsion with 0.1% (v/v) Mentha (*Mentha arvensis*) oil

T4: 10% (w/v) wax emulsion with 0.5% (v/v) Mentha (*Mentha arvensis*) oil

T5: 10% (w/v) wax emulsion with 0.1% (v/v) Eucalyptus (*Eucalyptus sp.*) oil

T6: 10% (w/v) wax emulsion with 0.5% (v/v) Eucalyptus (*Eucalyptus sp.*) oil

T7: 10% (w/v) wax emulsion with 0.1% (v/v) Carbendazim (Bavistin)

T8: 10% (w/v) wax emulsion with 0.5% (v/v) Carbendazim (Bavistin)

T9: Control (dipped in 10% wax emulsion only)

Preparation of 10% wax emulsion

Paraffin wax (58-60°C, Solid LR-Grade) was used for preparing wax emulsion. Five hundred milliliter of water was boiled in a vessel and 50 g of wax was heated in another vessel. Fifteen milliliter of triethanolamine and ten milliliter of oleic acid was added in water as emulsifier and stabilizers. The molten wax was gradually poured into heated water with constant stirring. The stirring was rigorously done until the solution turns milky color. The milky color indicates well prepared emulsion. It was ensured that the heated wax and heated water were at same temperature while mixing. The prepared emulsion was then allowed to cool.

Preparation and application of essential oils and fungicide

The essential oils used in the experiments were prepared at Herbs Production and Processing Corporation Limited (HPPCL), Koteshwor, Kathmandu. The respective herbs collected from the Terai region of Nepal were dried, wilted and steam

distilled to produce oils. One milliliter and five milliliter of essential oils were added in one liter of 10% wax emulsion to prepare 0.1% and 0.5% essential oils with wax emulsion. Similarly, the fungicidal solution of carbendazim was prepared by dissolving 1 g and 5 g of carbendazim (Carbendazim 50% WP) in 1000 ml of distilled water. One milliliter and five milliliter of fungicidal solution were added in one liter of 10% wax emulsion to prepare 0.1% and 0.5% carbendazim with wax emulsion. The fruits were then dipped in the designated solutions for a few seconds, until a glossy film of wax was formed on the surface of fruits.

Table 1: Chemical constituents of essential oils used in experiment

Essential oils	Chemical constituents
Cymbopogan Flexuosus	Geranial, Neral, Limonene, Caryophyllene, Geranyl acetate, Linalyl acetate, Citral, Isogeranial, *p*-cymene, Linalool
Mentha arvensis	Menthol, Menthone, Isomenthone, Menthyl acetate, Limonene
Eucalyptus sp.	Eucalyptol, Limonene, Aromadendrene, Phellamdrene, Terpinolene, Alpha terpineol

Source: HPPCL website

Data collection and analysis

The data about the Juice content, fruit firmness, Total Soluble Solids (TSS), Titratable Acidity (TA), vitamin C (ascorbic acid) and disease severity scoring was taken at every 5th day interval. The physical (fruit firmness) and chemical (juice recovery percentage, TSS, TA and vitamin C) properties of fruits were measured by destructive sampling technique. The fruit firmness was measured by penetrometer (effigy oil model having 8 mm tip) and TSS was measured by hand refractometer. Acidity and vitamin C was determined as per the procedure outlined by AOAC (2005). The juice recovery percentage was calculated by following formulae;

$$\text{Juice recovery (\%)} = \frac{\text{Volume of the juice obtained}}{\text{weight of the fruit}} \times 100$$

The shelf life was evaluated based on the appearance and spoilage of fruits. Fruits were considered to have reached the end of shelf life when fruits showed visible signs of decay irrespective of diameter of symptom (Obagwu and Korsten, 2003).

Disease scoring and identification

Disease scoring was done on 0-5 scale. The assessment was based on the rotted area with respect to total surface area of the sweet orange and expressed in percentage [0 = no infection (fruits are healthy), 1 = infection starts (0-5% rotting), 2 = 6-10% rotting, 3 = 11-15% rotting, 4 =16-20% rotting, 5=>20% rotting] (Obagwu and Korsten, 2003; Abd-El-Khair and Hafez, 2006). The rotted fruits from each replication were removed and counted. Disease severity index of decay fruits by pathogen was calculated by following formulae;

Disease severity index (%) = $\frac{\Sigma(nxr1).....(nxr5)}{MN} \times 100$ (Abd-El-Khair and Hafez, 2006)

Where, n = number of decayed fruits per category, r_1. r_5= severity score
M= maximum rating scale number (5), N= total examined fruits

The infected fruits after treatment with fungicide and essential oils were transferred to the pathology lab of AFU for the isolation of fungi. Isolation was carried out on Martin's medium (Bridson,1995). Small pieces (1-1.2 cm thickness) of rotted fruits were sterilized by dipping into 2% sodium hypochlorite solution for 5 minutes and then washed several times with distilled water and finally dried on sterile filter paper (Abd-El-Khair and El-Mougy, 2003). The fully sterilized pieces were then transferred onto the surface of the medium in sterilized Petri-plates. Inoculated plates were incubated at 25°C for 3-5 days. Hyphal tip technique was followed for purification of the isolated fungi. Barnett and Hunter technique was used to identify fungal cultures (Barnett and Hunter, 1987).

The temperature and relative humidity of the experimental room was recorded daily. The average minimum temperature was recorded 12.88°C while the average maximum temperature was recorded 16.16°C. The average minimum humidity was 86.04% while the average maximum

humidity was 91.73%. The climate was mostly cloudy during the experiment with a few instances of drizzles. The data were entered into Microsoft Excel 2016 and analysis was carried out by using R- Studio version 4.0.2. Both descriptive and inferential analysis was carried out. Interpretations were made based on results, which were assisted by qualitative and quantitative data/information.

RESULTS

Juice recovery percentage

Juice recovery percentage decreased significantly in all treatments with the advancement of storage time (Table 2). On the day of the experimental setup, the juice recovery percentage was found to be 58.24%. The juice recovery percentage was not significant between treatments for the storage period time of 5 days and 10 days. At 30 days after storage, maximum juice recovery percentage was observed in wax coating with 0.5% lemongrass (42.86%), which was statistically at par with the wax coating with 0.5% eucalyptus (42.81%). The lowest juice recovery percentage was seen in control fruits (33.49%).

Table 2: Effect of postharvest treatments on juice recovery percentage of sweet orange fruits

Treatments	Percent juice content of fruits on days indicated						
	1	**5**	**10**	**15**	**20**	**25**	**30**
T1	58.24	57.04	50.17	47.04^{abc}	45.05^{c}	43.82^{bc}	41.45^{cd}
T2	58.24	56.95	49.56	47.81^{a}	46.21^{a}	44.10^{ab}	42.86^{a}
T3	58.24	56.73	49.60	46.67^{bc}	45.30^{bc}	43.69^{c}	41.18^{c}
T4	58.24	57.38	51.70	47.92^{a}	45.93^{ab}	44.25^{a}	41.63^{c}
T5	58.24	57.10	50.60	47.60^{ab}	45.46^{abc}	43.64^{c}	42.29^{b}
T6	58.24	57.23	50.82	47.55^{ab}	46.16^{a}	44.10^{ab}	42.81^{a}
T7	58.24	56.98	49.80	46.42^{c}	43.50^{d}	39.71^{e}	38.50^{e}
T8	58.24	57.20	51.20	46.12^{c}	44.10^{d}	42.02^{d}	41.16^{d}
T9	58.24	56.59	50.63	46.08^{c}	40.67^{e}	36.10^{f}	33.49^{f}
LSD		0.60^{ns}	0.85^{ns}	1.06**	0.70***	0.31***	0.28***
CV		0.73	1.47	1.55	1.08	0.51	0.47
Mean		57.01	50.45	47.02	44.71	42.38	40.59

LSD = Least Significant Difference, CV= Coefficient of Variation, Means within the column followed by same letters do not differ significantly at 5% level of significance by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05

Fruit firmness

The fruit firmness decreased with the advancement of the storage period in all treatments (Fig. 1). On the day of the experimental setup, the fruit firmness was found to be 5.35 kg/cm^2. On the 30th day after storage, firmness was highest for wax with 0.5% eucalyptus (3.50 kg/cm^2) and lowest in control (2.25 kg/cm^2) followed by wax with 0.1% carbendazim (2.75 kg/cm^2).

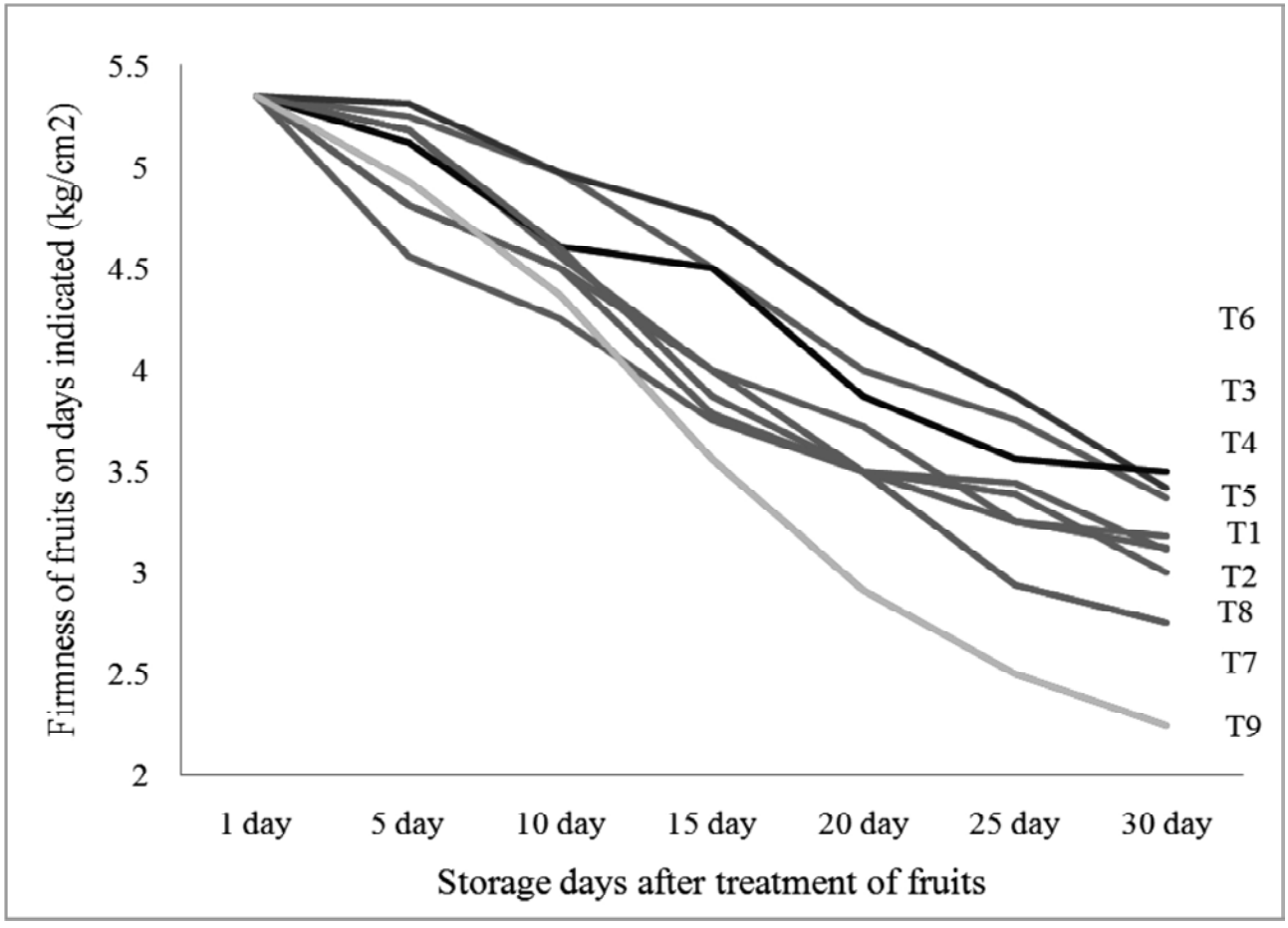

Fig.1 : Effect of postharvest treatments on firmness of sweet orange fruits

Total soluble solids (TSS)

Total soluble solid directly influences the taste of sweet orange. The TSS of fruit on the first day of storage was 11.20 °Brix. TSS increased with increment in the storage period in all treatments from 10 days onwards (Table 3). However, TSS was found to decrease on the 5th day of storage for the treatment wax with mentha. There was no significant difference between treatments on the 5th and 10th day of storage. The highest TSS was observed in control fruits (12.41° Brix) followed by wax with 0.1% carbendazim (12.28° Brix) while the lowest TSS was observed in wax with 0.5% lemongrass (11.97° Brix) at 30th day after storage.

Titratable Acidity (TA)

The titratable acidity is an important factor that is directly related to organic acid present in the fruit and also determines the quality of sweet orange. The TA was 1.12 on the first day of the experiment. The effect was significant only after the 10th day of treatment. There was a gradual decrease in TA of sweet orange along with the storage time. At the end of storage life i.e. 30th day, TA was highest for wax with 0.5% lemongrass (0.94%), which was statistically at par to wax with 0.1% lemongrass (0.92%) and 0.5% carbendazim (0.91%). The lowest TA was shown by control (0.72%) at the end of storage life (Table 4).

Table 3: Effect of postharvest treatments on TSS of sweet orange fruits

Treatments	TSS of fruits on days indicated						
	1	5	10	15	20	25	30
T1	11.20	11.25	11.30	11.40^{bcd}	11.69^{b}	11.82^{e}	12.21^{c}
T2	11.20	11.25	11.30	11.40^{bcd}	11.60^{c}	11.72^{f}	11.97^{d}
T3	11.20	11.00	11.20	11.41^{abc}	11.65^{b}	11.97^{bc}	12.22^{bc}
T4	11.20	11.00	11.30	11.45^{ab}	11.78^{a}	11.8^{e}	12.19^{c}
T5	11.20	11.25	11.30	11.37^{cd}	11.65^{bc}	11.9^{d}	12.20^{c}
T6	11.20	11.25	11.30	11.40^{bcd}	11.61^{c}	11.8^{d}	12.10^{d}
T7	11.20	11.20	11.25	11.34^{d}	11.65^{bc}	12.02^{b}	12.28^{b}
T8	11.20	11.25	11.30	11.37^{cd}	11.61^{c}	11.95^{c}	12.19^{c}
T9	11.20	11.25	11.30	11.47^{a}	11.78^{a}	12.19^{a}	12.41^{a}
LSD		0.20^{ns}	0.08^{ns}	0.06**	0.04***	0.04***	0.06***
CV		1.82	0.53	0.37	0.27	0.23	0.35
Mean		11.18	11.28	11.40	11.66	11.91	12.20

LSD = Least Significant Difference, CV= Coefficient of Variation, Means within the column followed by same letters do not differ significantly at 5% level of significance by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05.

Table 4: Effect of postharvest treatments on TA of sweet orange fruits

Treatments	TA of fruits on days indicated						
	1	5	10	15	20	25	30
T1	1.12	1.05	1.03^{bc}	1.02^{ab}	0.95^{ab}	0.93^{ab}	0.92^{ab}
T2	1.12	1.04	1.05^{b}	1.03^{ab}	0.99^{a}	0.95^{a}	0.94^{a}
T3	1.12	1.03	0.95^{de}	0.92^{de}	0.91^{c}	0.84^{d}	0.84^{d}
T4	1.12	1.04	0.99^{d}	0.94^{d}	0.93^{bc}	0.88^{c}	0.82^{d}
T5	1.12	1.05	0.95^{de}	0.9^{e}	0.86^{d}	0.85^{d}	0.82^{d}
T6	1.12	1.02	1.04^{bc}	0.98^{c}	0.95^{abc}	0.91^{b}	0.86^{cd}
T7	1.12	1.02	1.05^{bc}	1.01^{b}	0.96^{ab}	0.93^{ab}	0.87^{bcd}
T8	1.12	1.03	1.08^{a}	1.04^{a}	0.97^{ab}	0.93^{ab}	0.91^{abc}
T9	1.12	1.03	0.89^{e}	0.85^{f}	0.76^{e}	0.74^{e}	0.72^{e}
LSD		0.00^{ns}	0.12**	0.01***	0.03***	0.02***	0.05***
CV		4.86	1.85	1.30	2.75	2.01	4.00
Mean		1.03	1.00	0.96	0.92	0.88	0.85

LSD = Least Significant Difference, CV= Coefficient of Variation, Means within the column followed by same letters don not differ significantly at 5% level of significance by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05.

Ascorbic Acid (Vitamin C) content

Vitamin C content is an important nutritive parameter in citrus fruits and it was decreased gradually during the advancement of storage days (Fig. 2). On the first day of storage, the vitamin C content was measured to be 40 mg/100ml of orange juice. On the 30th day, the highest vitamin C was found in fruits coated with wax and 0.5% eucalyptus (30.31 mg/100ml), followed by wax with 0.5% mentha (29.50 mg/100ml) and 0.1% mentha (29.18mg/100ml), while the lowest vitamin C was observed in control fruits (24.5 mg/100ml).

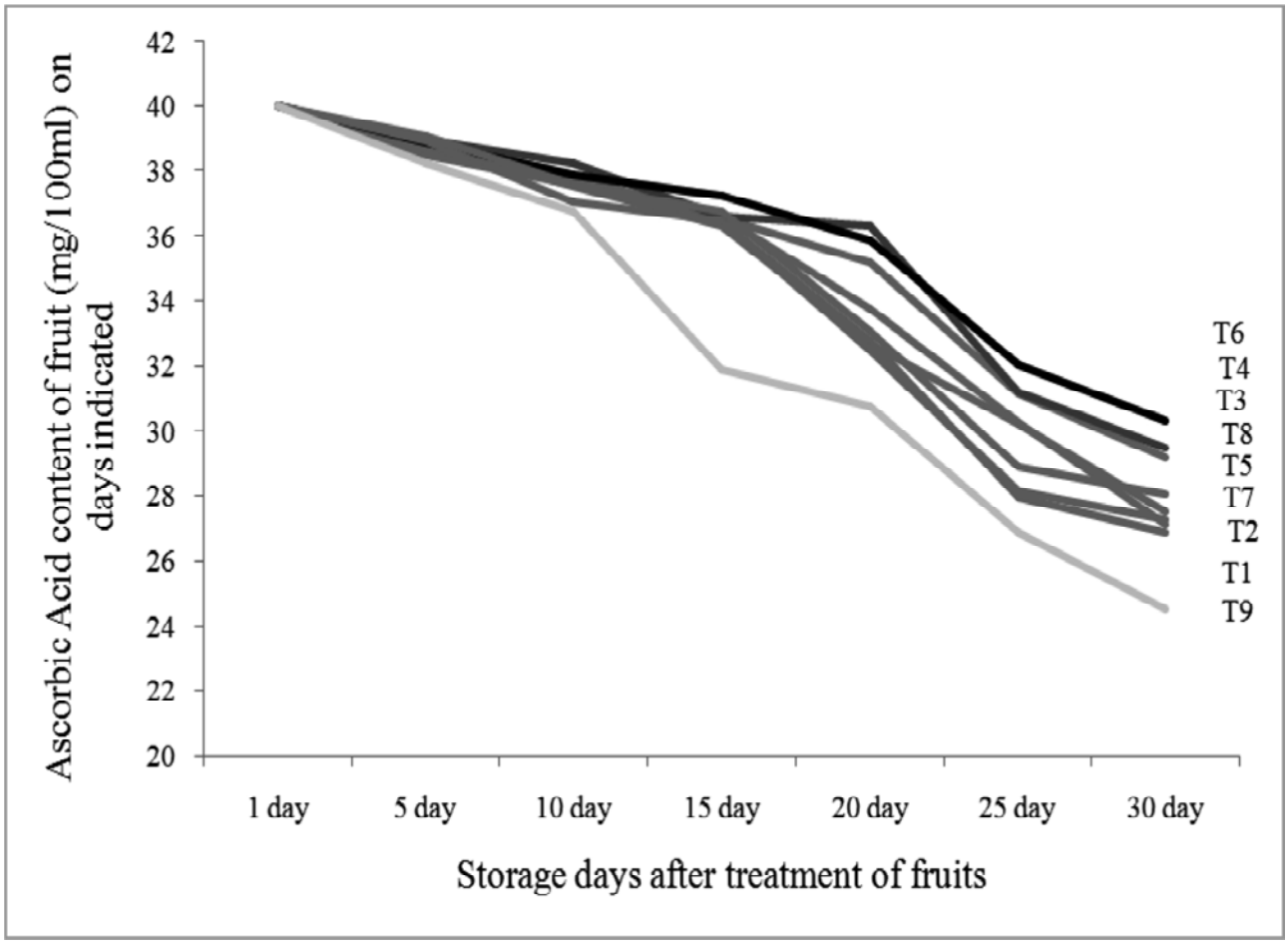

Fig. 2: Effect of postharvest treatments on Ascorbic Acid content of sweet orange fruits

Disease severity index

The disease occurrence in sweet orange was increased with the storage days (Table 5). The green mold (*P. digitatum*) was confirmed through the lab culture of a pathogen. The fruits treated with essential oils and fungicide were found to be more resistant to postharvest fungal diseases. On the 30th day of storage, almost all treatments exhibited noticeable disease occurrence, control being the highest infected (0.372%) followed by wax with 0.1% mentha (0.180%). The treatment of wax with 0.5% carbendazim (0.004%) was most effective against fungal pathogen and wax with 0.5% eucalyptus oil (0.025%) and 0.5% lemongrass oil (0.025%) being the most effective essential oils.

Table 5: Effect of postharvest treatments on disease severity index in sweet orange fruits

Treatments	Disease severity index of fruits on days indicated						
	1	5	10	15	20	25	30
T1	0.00	0.00	0.00	0.00	0.025^{bc}	0.075^{b}	0.123^{b}
T2	0.00	0.00	0.00	0.00	0.004^{de}	0.012^{cd}	0.025^{d}
T3	0.00	0.00	0.004^{b}	0.012^{b}	0.038^{b}	0.075^{b}	0.180^{b}
T4	0.00	0.00	0.00	0.00	0.017^{cd}	0.046^{bc}	0.114^{bc}
T5	0.00	0.00	0.00	0.012^{b}	0.038^{b}	0.058^{b}	0.114^{bc}
T6	0.00	0.00	0.00	0.00	0.008^{de}	0.012^{cd}	0.025^{d}
T7	0.00	0.00	0.00	0.00	0.00	0.000	0.033^{cd}
T8	0.00	0.00	0.00	0.00	0.00	0.000	0.004^{d}
T9	0.00	0.00	0.042^{a}	0.054^{a}	0.096^{a}	0.207^{a}	0.372^{a}
LSD			0.009***	0.013***	0.013***	0.038***	0.080***
CV			121.76	105.94	35.646	48.471	50.13
Mean			0.005	0.008	0.025	0.054	0.110

LSD = Least Significant Difference, CV= Coefficient of Variation, Means within the column followed by same letters do not differ significantly at 5% level of significance by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05.

Shelf life

The wax treatment with carbendazim and essential oils had a significantly better shelf life as compared to the control treatment (Table 6). Wax with 0.5% carbendazim (28.25 days) being the highest and significantly better than other treatments. It was followed by wax with 0.1% carbendazim (25.75 days), wax with 0.5% lemongrass oil (20.00 days) and wax with 0.5% eucalyptus oil (19.75 days). The control fruits (8.25 days) were observed to have the lowest shelf life.

Table 6: Effect of postharvest treatments on shelf life in sweet orange fruits

Treatments	Shelf life
T1	17.25^{d}
T2	20.00^{c}
T3	10.00^{f}
T4	16.75^{d}
T5	14.25^{e}
T6	19.75^{c}
T7	25.75^{b}
T8	28.25^{a}
T9	8.25^{g}
LSD	0.92***
CV	3.55
Mean	17.80

LSD = Least Significant Difference, CV= Coefficient of Variation, Means within the column followed by same letters don not differ significantly at 5% level of significance by DMRT, Significance codes ***at 0.001, **at 0.01, *at 0.05.

Discussion

A significantly lower juice recovery percentage of control fruits might be due to the fact that the essential oils act as a barrier which checks the loss of moisture from the fruit surface due to the clogging of natural openings (Castillo *et al*., 2014). Additionally, the lower incidence of disease in essential oils and fungicides treated fruit ensure lower metabolism, which might have contributed to a higher juice recovery percentage. The present finding was supported by (Bisen *et al*., 2012). The control fruit also had a wax coating and the transpiration process was very slow, so there was an insignificant difference in juice recovery percentage between treatments before 15th day of storage. The moisture

loss was found significantly lower in fruit treated with essential oil enriched coatings (Du Plooy *et al*., 2009; D Antunes *et al*., 2012; Castillo *et al*., 2014).

In general, coating formulations that minimize weight loss are also better at maintaining firmness, since this attribute is highly influenced by water content. Fruit firmness decreased gradually and significantly along with increasing storage period in all treatments. The decelerated damage may be due to the anti-microbial properties of essential oils. The lowest fruit firmness in control fruit might be due to the rapid degradation of cell walls due to the action of wall-degrading enzymes such as pectinesterase, pectinmethyl-esterase and polygalacturonase which are produced by fungi. Essential oil amended coating maintains cell wall carbohydrate metabolism during storage which is related to decreased susceptibility to infection by fungal pathogen and therefore improves quality. The essentials oils together with commercial wax coating maintain the organoleptic integrity along with firmness as mentioned by Jhalegar *et al*. (2014). The essential oils affect the portioning of the lipids of the plasma membrane and changing of its integrity, permeability and inorganic ion equilibrium due to their hydrophobic nature (Lambert *et al*., 2001) which might be the reasons for greater firmness in the fruits treated essential oils. The present findings were supported by Chafer *et al*. (2012) on the firmness of Navel Powell orange and Castillo *et al*. (2014) on lemon fruits.

The gradual increase of TSS with extending of the storage period might be attributed to concentrated juice content results from dehydration and hydrolysis of polysaccharides.The increased respiration rate due to microbial spoilage, degradation of fruits and increased ethylene production ultimately increased the TSS during ripening and senescence which might be the reason for slightly higher TSS in control fruits as compared to other treatments. The present result was in agreement with the findings of Chafer *et al*. (2012) on Navel Powell orange and Castillo *et al*. (2014) on lemon and Tao *et al*. (2014) on Satusma mandarin, as the essential oils did not show a significant effect on TSS. The present finding was also inconsistent with Asghari *et al*. (2009) who reported insignificant results in TSS while using cumin essential oil on strawberry.

The decrease in titratable acidity with storage is due to the oxidation of organic acids and further utilization in the metabolic process in the fruits (Hafeez *et al*.,2012). A gradual declining trend in titratable acidity content of fruit during storage for any treatment was observed by Ansari and Feridoon (2007) and Obenland *et al*. (2008) in citrus. The decreased in titratable acidity of fruits during storage could be due to the consumption of organic acids in the respiration process as stated by Zokaei *et al*. (2006) and Ishaq *et al*. (2009). Similarly, Baiea (2013) on Washington Navel orange detected a decrease in the acidity of fruits during storage. Fruits treated with essential oils showed higher retention of titratable acidity during the storage period which might due to delayed in physiological ageing and alteration in metabolism. The present results are in line with Mahajan *et al*. (2010) suggesting that organic acids were used in the respiratory process. The higher titratable acidity in wax with lemongrass treatment is aligned with the finding of Fatemi *et al*. (2012) who reported that the thymol oil delayed the changes in titratable acidity of Valencia orange. The present finding was also supported by Jhalegar *et al*. (2014) on Kinnow mandarin. Abd El wahab *et al*. (2014) also reported bergamot oil delayed the changes in titratable acidity during cold storage of Crimson seedless grape.

Adisa (1986) stated that vitamin C decreased over time in storage which is similar to the experimental outcome. The decreased in ascorbic acid content of fruits during storage could be due to the conversion of dehydroascorbic to diketogulonic acid by oxidation as reported by Ishaq *et al*. (2009). Under stress, such as a pathogen or chemical exposure, ascorbate oxidase levels were increased, which decreased the level of vitamin C (Loewus and Loewus,1983; Loewus *et al*.,1987). The maximum retention of vitamin C was observed with essential oils treatments due to the antioxidant property of essential oils which prevent ascorbic acid from oxidation (Shao *et al*., 2013). The result was similar to Lin *et al*. (2011) who found that the decrease in vitamin C level was associated with a reduced capacity of preventing oxidative damage which is triggered by the incidence of physiological disorders during storage. The degradation of vitamin C was highest in control fruits which might be due to fruit senescence accompanied by rapid respiration, ethylene production and decay. These results are similar to those reported on the effect of thyme and

clove oil in maintaining ascorbic acid as for orange (Zeng *et al*., 2012; Baiea and Ei-Badawy, 2013).

The result was similar to the report of Abd-El-Khair and Hafez (2006), as they reported the lemongrass and eucalyptus essential oils significantly reduced the incidence of fungus *P. digitatum* in Washington navel orange during storage. Abdolahi *et al*., (2010) and Al-Samarrai *et al.,* (2013) found various plant extracts including lemongrass extract could inhibit the mycelial growth of pathogenic fungus *P. digitatum*. The phenolic compounds and their derivatives of essential oils altered the microbial cell permeability by interacting with membrane proteins which would cause deformation in cell structure and function and permit the loss of macromolecules from their body (Fung *et al*., 1997; Rattanapitigorn *et al*., 2006) which might be the reasons of lower microbial growth in the essential oils treated fruits compare to control. Amit and Malik (2010) indicated that the vapoursof lemongrass oil damaged the cell membrane mainly due to membrane deformation. However, the variation in the antifungal effect of the essential oils depends on the solubility and capacity to interact with the cytoplasmic membrane (Tripathi and Shukla, 2007). The efficacy of lemongrass was also found superior by Jhalegar *et al*. (2015). Similar results were reported by Du Plooy *et al*. (2009), Fan *et al*. (2014), Jhalegar *et al*. (2014) and Gandarilla-Pacheco *et al*. (2020) in citrus fruits.

Table 7: Summary of studies on the effect of essential oils on major post-harvest pathogens of citrus

Fruit	Target pathogen	Essential oils	References
Orange cv. Tomango	*P. digitatum*	Mentha oil	Du Plooy *et al*. (2009)
Orange, Lime	*P. italicum*	Mentha oil	Tripathi *et al*. (2004)
Washington Navel Orange	*P. digitatum*	Lemongrass oil, Eucalyptus oil	Abd-El-Khair and Hafez (2006)
Valencia Orange	*G. citri-aurantii*	Lemongrass oil	Regnier *et al*. (2014)
Kinnow Mandarin	*P. digitatum, P. italicum*	Eucalyptus oil	Jhalegar *et al*. (2014)
Kinnow Mandarin	*P. italicum and P. digitatum*	Lemongrass oil, Eucalyptus oil	Jhalegar *et al*. (2015)

The wax with 0.5% carbendazim with its prominent disease resistance had the longest storability. The superiority of shelf life of 0.5% lemongrass and eucalyptus oils treated fruits might be due to the antifungal properties of essential oils (Tzortzakis and Economakis, 2007; Jhalegar *et al*., 2014; Jhalegar *et al*., 2015). In addition to this, postharvest decay is positively correlated with ethylene production and respiration rate which were found to be decreased by the application of essential oils (Jhalegar *et al*., 2014; Jhalegar *et al*., 2015). The present result was inconsistent with Tripathi *et al*. (2004) and Tavakoli *et al*. (2019).

The green mold is the major postharvest pathogen in sweet orange. The chemical fungicides have been found effective against such pathogens, but the health hazard of such pesticides is alarmingly high. The use of essential oils as an alternative for chemicals can be an environment friendly technique for prevention of the health hazards. The shelf life of sweet orange can be extended by infusing wax with carbendazim or essential oils. But the superiority of essential oils especially wax with 0.5% eucalyptus oil and 0.5% lemongrass in qualitative parameters as well as in consumer's preferences, organic requirements and environmental aspects make them a better alternative.

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Original Research Paper

Post-harvest quality and quantification of betalains, phenolic compounds and antioxidant activity in fruits of three cultivars of prickly pear (*Opuntia ficus-indica* L. Mill)

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ABSTRACT

Postharvest quality, quantification of betalains, phenolic compounds and antioxidant activity of peel, pulp and juice of fruits of three prickly pears (*Opuntia ficus-indica* L. Mill.) cultivars of Colegio de Postgraduados in México, were measured. The red and orange cultivars showed outstanding features of postharvest quality (size, texture, TSS and pulp and juice content), highest content of betalains and phenolic compounds. Therefore, highest antioxidant activity. In general, highest content of bioactive compounds was detected in peel, besides the content in pulp and juice did not show statistically significant differences. Phenolic content is very high in comparison with other fruits. Antioxidant activity was measured by three assays: FRAP, ABTS and DPPH. Three cultivars showed high correlation between antioxidant activity and phenolic compounds. The methodologies used in this work are a very useful tool for the quantification of bioactive compounds in *O. ficus-indica* fruit tissues.

Keywords **:** Betalains, Flavonoids, *Opuntia ficus-indica*, Phenolic compounds and Prickly pear

INTRODUCTION

Prickly pear (*Opuntia ficus-indica* L. Mill.) is the species of cacti with the greatest economic importance in the world (Bravo, 1978); (Kiesling, 1999); (Griffith, 2004); (Feugang *et al*., 2006). It is cultivated in several continents, but is native to America, where, there are more than 93 species of *Opuntia* (Hunt, 1999). In the southern highlands of Mexico, there are more than 243 varieties, used as fodder, vegetables and fruit. Most of the prickly pear cactus is collected from the wild, since there are only approximately 20,000 commercial plantations of prickly pear cactus. The semiarid regions of central Mexico hosted the greatest genetic diversity, as well as the largest cultivated area of prickly pear cactus in the world. Variability is found in both cultivated and wild populations. Prickly pear has become an important fruit crop in the semi-arid lands of Mexico, where it plays a strategic role in subsistence agriculture (Pimienta, 1994). The prickly pear has been recognized for its numerous nutritional virtues, nutritional and functional properties. Recent data have revealed the high content of some chemical components, which can give added value to this fruit. High levels of betalains, taurine, calcium, magnesium and antioxidants stand out. In addition, some of the components show promising characteristics in terms of functionality (Piga, 2004).

The diversity of betalains found in these prickly pear cultivars, indicate the potential value of Opuntia cactus pear fruit, as a good source of pigments, and their potential industrial exploitation for drinks and food products. Therefore, consumption of cactus pear fruit may provide nutritional and health benefits (Castellanos & Yahia, 2008). Flavonoids have been reported by several authors (Feugang *et al*., 2006); (García *et al*., 2019). Also, Kuti (2000) reported about the presence of phenolic compounds in fresh prickly pear fruits. (Lee *et al*., 2002) also reported the antioxidant effects of *Opuntia* extracts. There is few information on the quantification of betalains and

phenolic compounds in different fruit tissues, and juice of *Opuntia ficus-indica* cultivars. The purpose of the following work was to evaluate the postharvest quality, quantification of betalains, phenolic compounds and antioxidant activity of fruit tissues of three prickly pears (*Opuntia ficus-indica* L. Mill.) cultivars grown at Colegio de Postgraduados.

MATERIALS AND METHODS

Plant material

Three *O. ficus-indica* cultivars colors red, white and orange developed in the fruticulture experimental field of Colegio de Postgraduados, located in Montecillo, State of Mexico (coordinates 19°272 513 N 98°542 153 O, altitude 2250 msnm), high altitude, temperate climate, the driest of the sub-humid, with rainfall in summer, precipitation 572. 25 mm and mean annual temperature of 15.3 ºC (García, 1988) were selected for the study according to flesh color, identified as CP1 (red), CP3 (white) and CP4 (orange). For fruit harvesting, the criteria established were the flattening of the floral cavity and the moment when the glochids or thorns fell (Cantwell, 1995).

Color characteristics

The fruit color was measured by CIELAB system. The epicarp color was measured on two opposite sides of the equatorial zone of the fruit, with a Hunter-Lab model D-25 reflection colorimeter (Reston, Virginia, USA); CIELAB parameters L*, a*, b* were recorded and the hue angle (°h=tan-1(b^*/a^*) and saturation index (Chroma (C) (a^2+b^2) $^1/_2$) were calculated (McGuire, 1992).

Postharvest quality

A total of 50 fruits per cultivar were harvested and measured for size, structural components (peel, pulp and seeds), epicarp (peel) color, texture, juice content, total soluble solids, juice pH, betalains, flavonoids, phenols contents and antioxidant capacity. Size was determined based on longitudinal and equatorial diameter, measured with a trupper-14388 digital vernier on a total of 15 fruits; data were reported in millimeters (mm). The structural components evaluated were the proportion of peel, pulp and seeds, determined on a weight basis with an Ohaus Scout-Pro electronic balance with a sensitivity of 0.1 mg and the percentage of peel, pulp and seeds was calculated; in addition, the number and area (mm^2) of seeds was determined using an Epson Scan scanner with WinSeedle TM 2013 software. Firmness was measured based on the deformation of the fruit when a force of 1 kg was applied with a Chantillon texturometer (Wagner Force Five model FDV-30) with a flat strut; the results were expressed in Newtons/cm^2 (N/cm^2).

Juice extraction

To determine the juice content, the juice was extracted from a total of 15 fruits separately with an Oster ® FPSTJE317 centrifugal extractor; for the calculations, the equation % juice= (juice weight/pulp weight) x100 was applied. Total soluble solids (%) and pH were measured according to the methods of the (AOAC, 1990) using a portable refractometer Palette Atago, PR-320 (0-.32%) and a Corning Model 12 potentiometer, NY, USA, respectively.

Obtaining prickly pear tissues

Samples of the epicarp (30g), mesocarp and endocarp (30 g), as well as juice from the pulp (15 mL) were obtained separately by hand using an Oster ® FPSTJE317 centrifugal extractor. All samples were kept in Ultrafreeze at -65°C and subsequently freeze-dried for 3 days at -45°C and 1.3 × 10^{-3} MPa in a Labconco Freezone 2.5 L equipment. The freeze-dried samples were homogenized using a Nutribullet Nb-101b to obtain a fine particle. Finally, they were preserved in airtight aluminum bags for storage at -18°C until analysis.

Extraction procedure of freeze-dried prickly pear tissues

Extraction was performed by placing 1 g of freeze-dried prickly pear sample in 50 mL of methanol: water (80/20, v/v) and mixed by vortex for 3 min, subsequently pH was adjusted to 3 with hydrochloric acid, and put in an ultrasonic bath (Bransonic™ CPXH series) for 15 min. After that, the samples were rotated for 30 min at 150 rpm and 27°C. Finally, they were centrifuged for 15 min (3500 rpm) and the supernatant was separated. The extracts were stored at -18°C in dark for further analysis.

Spectrophotometric quantification of total betalains and phenolic compounds

For the determination of total betalains and phenolic compounds, the prickly pear extracts mentioned above were used. Betalain content was measured

according to the method of (Castellanos & Yahia, 2008) using a Sinergy 2 microplate multidetector equipped with Gen 5 Data Analysis Software (Biotek Instruments Inc., Winoosky, VT USA). The absorption spectrum was obtained from 200 to 700 nm to obtain the absorption maximum and an OD <1. Readings were obtained for each extract in triplicate. The betalain content was expressed as: µg betanin equivalents for betacyanin content (BC) and µg indicaxanthin equivalents for betaxanthin content (BX). The calculation was made using the following formula: BC or BX (mg/g) = [A(Df)(Mw)(Vd)/ ε(L)(Wd)] where A is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, Vd is the dried pulp solution volume (mL), Wd is the dried pulp weight (g), and L is the path-length (0.38 cm) of the cuvette. The molecular weight (Mw) and molar extinction coefficient (ε) of betanin [Mw) 550 g/mol; ε) 60,000 L/(mol cm) in H2O] were applied in order to quantify the betacyanins. Quantitative equivalents of the major betaxanthins (Bx) were determined by applying the mean molar extinction coefficient [ε) 48,000 L/(mol cm) in H^2O]. In all cases, water extracted the highest level of pigments.

The total flavonoid determination was conducted according to the colorimetric method defined by Chang *et al*. (2002) with modifications. The prickly pear extract was mixed with 100 µL of potassium acetate, 100 µL of 10% aluminum chloride and 4.7 mL of distilled water. After incubation at room temperature for 30 min in darkness, the absorbance of the reaction mixture was measured at 415 nm in a microplate multidetector mentioned in section 2.7 placing 200 µL of sample and reagent blank in respective microwells. The amount of 10% aluminum chloride was substituted by the same amount of methanol: water (80/20, v/v) in blank. Quercetin (0.4 – 1.6 µg/mL) was used to make the calibration curve and the results were expressed as mg quercetin equivalents per g dry weight (mg EQ/ g dry weight).

The total phenolic determination was expressed as µg gallic acid equivalents per g of dry weight (mg GAE g dry weight), according to the Folin-Ciocalteau assay which detects electron transfer by measuring the reducing capacity of the sample and can therefore also be considered as antioxidant activity assay (Cano *et al*. 2017).

Antioxidant activity

The antioxidant activity of each cultivar of prickly pear was determined using three assays: FRAP, ABTS and DPPH which have been widely applied in the analysis of food samples (Re *et al*., 1999). The FRAP assay was performed according to the methodology (Benzie & Strain, 1996) with some modifications. The FRAP solution includes 10 mL of 300 mM acetate buffer at pH 3.6, 1 mL of 10 mM TPTZ and 1 mL of 20 mM $FeCl_3 6H^2O$. The prickly pear extracts (20 µL) were allowed to react with 180 µL of FRAP solution and 60 µL of distilled water for 10 minutes in dark conditions. Readings were taken at 595 nm. The calibration curve was linear between 50 and 600 µM Trolox. Results were expressed in µM Trolox equivalents (µM TE)/g dry weight. For ABTS assay, the procedure of (Re, 1999) was followed with some modifications. The $ABTS^{-+}$ radical solution included 7.4 mM $ABTS^{-+}$ and 2.6 mM sodium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react in the dark for 16 hours. The solution was then diluted by mixing 600 µL of $ABTS^{-+}$ solution in 9.4 mL of methanol. The prickly pear extracts (20 µL) were allowed to react with 180 µL of ABTS solution for 10 minutes in dark conditions. Readings were taken at 734 nm. The calibration curve was linear from 50 to 500 µM Trolox. Results are expressed in µM Trolox equivalents (µM TE/g dry weight).

DPPH assay was done according to the method of Williams *et al*. (1995) with some modifications. The DPPH stock solution was prepared by dissolving 19.7 mg of DPPH in 100 mL of 80% methanol. Prickly pear extracts (200 µL) were allowed to react with 50 µL of DPPH solution for 30 min in dark conditions. Readings were taken at 515 nm. The calibration curve was linear from 50 to 500 µL of Trolox. The results were expressed in µM Trolox equivalents (µM TE/g dry weight). Additional dilutions were made when the values obtained from the samples were outside the linear range of the calibration curve.

Statistical analysis

The compositional data were expressed as mean ± standard deviation of at least five independent determinations. Significant differences between results were calculated by one-way analysis of variance

(ANOVA), followed by a post hoc Tukey's test. A level of $p < 0.05$ was considered a significant difference**.** To investigate the relationship between main phytochemicals, a bilateral Pearson correlation analysis was performed with a significance of $p < 0.01$ and $p < 0.05$. All statistical analyses were executed with SAS Institute, Inc 9.4.

RESULTS AND DISCUSSION

Morphological characterization

The morphological and physical characteristics of three prickly pear cultivars are directly influenced by selection (Table 1). Fruit length averages (mm) were significantly different among them, with CP4 and CP3 obtaining the highest and lowest values (97.15 and 73.2 mm, respectively). Regarding diameter, no significant differences were found between selections with averages of 52 and 55 mm respectively. The values of both lower and upper limits are very similar to those reported by Parish and Felker (1997) with average ranges of 73 to 88 mm for length and 56 to 57 mm for diameter. Cerezal & Duarte (2005) evaluated prickly pears harvested in the Andean highlands of the 2nd Region of Chile, reporting average length values of 62 to 78 mm and 46 to 52 mm in diameter. Karababa *et al.* (2004) reported fruit length values ranging from 66 to 71 mm and diameter values from 45 to 52 mm for a variety harvested in five locations in Turkey. On the other hand, Singh (2003) reported length and diameter values lower than those found in this study for prickly pear clones from the USA and introduced to India with average ranges of 55 to 76 mm in length and 33 to 46 mm in diameter.

CP1 and CP4 had values of epicarp firmness of 32 and 36.5 N/cm^2 respectively, higher than CP3 (26.6 N/cm^2). Weight of fruit of CP3 was significantly lower (124 g) compared to CP1 and CP4 (160 and 164 g respectively). There are other published works about the size of fruit, weight, TSS, pH and number of seeds (Cerezal & Duarte, 2005); (Karababa *et al*., 2004); (Parish & Felker, 1997); (Singh, 2003).

Table 1: Morphological, physical and physico-chemical characteristics of fresh fruits of three prickly pear cultivars (*Opuntia ûcus-indica* L. Mill.)

	CP1 (red)	CP3 (white)	CP4 (orange)
Size of fruit (mm)	87.18±6.64^{b}	73.19±8.72^{c}	97.15±6.62^{a}
Diameter (mm)	55.39±2.95^{a}	54.02±6.7^{a}	52.22±4.24^{a}
Firmness (N/cm^2)	31.99±9.54^{a}	26.63±6.94^{b}	36.51±6.98^{a}
Total weight of whole fruit (g)	159.91±23.81^{a}	123.95±33.55^{b}	154.26±17.67^{a}
Peel content (%)	37.19±4.15^{b}	40.9±3.33^{a}	39.67±3.51^{ab}
Pulp content (%)	62.3±4.18^{a}	57.57±5.2^{b}	60.54±2.93^{ab}
TSS of pulp (%)	15.53±1.22^{a}	12.59±1.73^{b}	11.4±0.78^{ab}
Juice content (%)	74.99±5.36^{a}	66.57±7.11^{b}	67.72±5.64^{b}
pH	7.31±0.16^{a}	6.55±0.15^{c}	7.03±0.13^{b}
TSS of juice (%)	13.52±0.86^{a}	12.86±2.33^{a}	11.91±0.54^{a}
Seed content (%)	2.74±0.17^{ab}	2.08±0.61^{b}	3.09±0.60^{a}
Weight of seeds (g)	4.18±0.86^{a}	3.11±0.39^{b}	4.12±0.69^{a}
Number of seeds	329.67±61.8^{a}	188.11±32.65^{bc}	231.56±50.7^{c}
Average area of seeds (mm^2)	15.75±0.7^{c}	18.41±0.68^{b}	19.592±1^{a}

*Values are the mean of 15 independent determinations ± standard deviation.

*Different letters indicate statistically significant differences (pd" 0.05) between columns.

In contrast with studies of Barbera *et al*. (1994) the biggest fruit (CP4) don´t have the high quantity of seeds, in this case the fruit of CP1 had high quantity of seeds. The cultivar with less number of seeds was CP3 (white), it has been cultivated to produce prickly pear for many years. So, it has had a selection process. El Behi *et al.* (2015); Barbera *et al.* (1994); Mejía & Cantwell (2003) mention in their studies that the relationship between fruit size and seed content is highly variable and influenced by factors such as genotype, crop load and fruit position within the canopy.

Firmness is a mechanical property gives post-harvest quality in fresh fruits. A loss of firmness is caused by loss of cell turgor due to aging or dehydration. Both thinning and softening of the peel contribute to increased susceptibility to physical damage and deterioration of prickly pears during handling (Cantwell, 1995). However, this characteristic is also due to genetic and nutritional issues of the crop. Guerrero (2018) reports firmness values for white prickly pear *Opuntia amyclaea* green mature (36.28 N/cm^2) and mature (26.48 N/cm^2). In this study we obtained values between 23.63 N/cm^2 for CP3 and 36.51 N/cm^2 for CP4.

Red cultivar (CP1) was characterized by the significantly higher content of pulp (62.3%), TSS of pulp (15.53 Brix) and juice (13.52%), juice content (74.99%), and lower content of peel (37.19%). Significant differences in the pH of the three cultivars were observed with values between 6.55 (CP3) and 7.31 (CP1).

This values were higher than reported by Andreu *et al*. (2018) in six cultivars of prickly pears grown in Spain, who showed values of pH between 5.2 and 6.06. Regarding seed content, cultivars CP1 (red) and CP4 (orange) showed higher seed weight (4.18 and 4.12 g respectively), and higher seed quantity (329 and 231 seeds respectively) than CP3 (white). However, CP1 (red) has significantly smaller seeds (15.75 mm) than CP3 and CP4.

Color

Table 2 shows that the three cultivars had L* values less than 50, the CP3 (white) was the closest with (L= 47.5), so it is the one with the least dark color. Between CP1 (red) and CP4 (orange) cultivars, no significant differences were observed for lightness. Hue values suggest that there are three types of shades; white with high hue values (112.27), red with intermediate value (25.72) and orange with low hue values (7.49). The highest chroma values were also presented by CP3 (white) (21.88), CP1 (red) and CP4 (orange) obtained very close values (16.17 and 15.32) respectively.

Table 2: Color of fruit or three prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

	CP1 (red)	CP3 (white)	CP4 (orange)
L*	35.19±2.76^b	47.5±3.96^a	34.33±1.86^b
a	6.2±2.5^b	-8.3±2.3^c	9.6±2.1^a
b	9.3±3.0^b	19.8±1.5^a	11.4±1.2^b
Hue	25.72±9.59^b	112.27±5.52^a	7.49±7.49^c
Chroma	16.17±3.97^b	21.88±2.4286^a	15.32±1.7^b

* Values are the mean of 15 independent determinations ± standard deviation.

* Different letters indicate statistically significant differences ($p \leq 0.05$) between columns.

Quantification of betalains

Betalains are water soluble compounds present in a restricted number of families of plants from the *Caryophyllale* family. They are classified in two chemical families: betacyanins and betaxanthins with 540 and 480 nm absorption maxima. Betalains are powerful radical eliminators in chemical system and act as an efficient antioxidant in biological models (Cano *et al*., 2017).

Betalain content was measured in CP1 (red) and CP4 (orange) cultivars, in the peel, pulp and juice of prickly pear. The CP1 cultivar showed higher betacyanins (BC) and betaxanthins (BX) content than CP4 (orange) with values of 1181 and 1137 µg/g d.w in peel, respectively for CP1 (red) and values of 161 and 408 µg/g d.w in peel for CP4 (orange), respectively. These compounds are responsible for the red and orange shades respectively.

Betacyanins appear to be in higher concentration in the peels of both prickly pear cultivars (red and orange), however, betaxanthins are observed evenly distributed in both peel, pulp and juice in the CP4 (orange) cultivar. This is consistent with the findings of (Cano *et al.*, 2017).

On the other hand, no significant differences are shown between BC and BX content in pulp and juice

for both selections (Table 3). In this sense, we could assume that no significant betalain content is lost during the juice extraction process.

Table 3: Betalain content in two prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

		CP1 (red)	CP4 (orange)
BC[1]	Peel	1181.67±151.3^{aA}	161±6.08^{bA}
	Pulp	496±30.51^{aB}	69.67±0.58^{bB}
	Juice	472.33±12.74^{aB}	65.67±5.69^{bB}
BX[2]	Peel	1137.67±169.82^{aA}	408±2.65^{bA}
	Pulp	552.67±26.65^{aB}	435.33±58.77^{aA}
	Juice	398±19^{aB}	457±21.07^{aA}

* Values are the mean of 3 independent determinations ± standard deviation.

* Lowercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

* Uppercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

BC[1]: Betacyanins expressed as µg of betanin equivalents per gram of dry weight.

BX : Betaxanthins expressed as µg of indicaxanthin equivalents per gram of dry weight.

Castellanos & Yahia (2008) reported values of betacyanins of 5290 µg/g dw in Camuesa cultivar, followed by 2060 µg/g in Roja Pelota, 2040 µg/g dw in Cardona and much lower contents in the Reyna variety (50 µg/g dw). Betaxanthins were found in the yellow prickly pear varieties Naranjona, 2651 and 21441 with values of 160, 140 and 120 µg/g dry weigt, respectively. These values differ greatly from those found in this work.

García *et al.* (2019) reported betacyanin values of 1670 µg/g d.w and 450 µg/g and betaxanthin values of 730 and 370 µg/g in the pulp of Mexican varieties of purple and red prickly pear, respectively. The values reported for red tuna are more consistent with what was found in this study.

Quantification of total phenols (TP) and total flavonoids (TF)

Some of the published works on the chemical composition of prickly pear showed information about the main compounds with antioxidant activity (Fernández *et al.*, 2010). Phenolic compounds are known as bioactive or functional compounds that serve as protectors against certain diseases (Butera *et al.*, 2002), which are mainly characterized by their antioxidant activity (Andreu *et al.*, 2018).

Table 4 shows the content of total phenols in the peel, pulp and juice of the three cultivars evaluated. CP1 (red) and CP3 (white) presented the highest Total phenols content (TP) in peel (7225.67 and 7486.67 µg GAE. g^{-1} dw, respectively), which was significantly different for CP4 (orange), which obtained 59.39% with respect to the CP3 (white) cultivar. No significant differences were found in the total flavonoid content in the peel of the three selections studied (2505, 2114 and 2239 µg QE g^{-1} d.w.) respectively.

The Total Flavonoids content (TF) in pulp and juice of the three cultivars did not show significant differences with average values of 2121, 1422.5 and 1911 µg GAE. g^{-1} dw for CP1, CP3 and CP4, respectively). García *et al*. (2019) found values of 2067 µg GAE. g^{-1} dw for red prickly pear fruit pulp and 3501 µg GAE. g^{-1} dw in peel. This value is close to that we found in this study for the orange selection (4446 µg GAE. g^{-1} p.s.).

TP and TF were found in 70 and 83% higher concentrations in peel than in pulp and juice in CP1 (red). In 82 and 83% in CP3 (white) and 62 and 93% in CP4 (orange). This corresponds with the findings of several authors, giving clear evidence that the highest antioxidant contents are present in the peel of the fruit (Andreu *et al.,* 2018); (García *et al*., 2019); (Morales, 2009).

CP1 presented the highest content of TP and TF in pulp (2149 µg GAE. g^{-1} dw and 558 µg QE g^{-1} dw respectively). In addition, cultivars CP1 and CP4 had the highest TP in juice (2092 and 2138 µg GAE. g^{-1} dw and CP3 had the highest TF in juice (555.33 µg QE g^{-1} dw).

The content of total phenols in prickly pear is very high compared to other fruits. The TP ranges (µg GAE. g^{-1} dw) are 140 to 1020 in nectarines, 210 to 110 in peaches and 420 to 1090 in plums (Gil *et al.*, 2002). On the other hand, the results are close to other fruits with high antioxidant capacity such as guava, which obtained values of 1700 to 3000 µg GAE. g^{-1} dw in a study carried out on pink-fleshed clones (Thaipong *et al.*, 2006).

Table 4: Content of total phenols, total flavonoids and antioxidant activity (FRAP, ABTS y DPPH) in three prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

		CP1 (red)	CP3 (white)	CP4 (orange)
Total Phenols[1]	Peel	7225.67±198.07^{aA}	7486.67±461.24^{aA}	4446.67±295.5^{bA}
	Pulp	2149.33±211.05^{aB}	1529.67±163.09^{bB}	1683.33±54.37^{bB}
	Juice	2092.67±132.08^{aB}	1315.33±155.58^{bB}	2138.33±127.45^{aB}
Total Flavonoids[2]	Peel	2505.33±194.54^{aA}	2114.67±78.56^{aA}	2239.67±176.52^{aA}
	Pulp	558±55.51^{aB}	249±21.52^{bC}	168±5.57^{bB}
	Juice	425.67±68.38^{bB}	555.33±25.66^{aB}	148.67±2.08^{cB}
FRAP[3]	Peel	17.68±0.74^{aA}	14.94±0.48^{bA}	19.13±0.35^{aA}
	Pulp	8.63±0.75^{aB}	6.83±0.84^{aB}	7.71±0.32^{aB}
	Juice	7.48±0.49^{aB}	5.23±0.16^{bB}	8.14±0.17^{aB}
ABTS[4]	Peel	20.61±0.74^{aA}	20.49±0.32^{aA}	19.08±0.35^{aA}
	Pulp	18.34±1.34^{aA}	7.39±0.45^{bB}	7.65±0.32^{bB}
	Juice	14.38±1.21^{aB}	6.09±0.19^{cC}	8.09±0.17^{bB}
DPPH[5]	Peel	16.03±4.23^{bA}	32.38±1.61^{aA}	19.82±5.65^{aA}
	Pulp	8.96±0.74^{aAB}	6.56±0.89^{bB}	2.41±0.24^{cB}
	Juice	5.05±0.37^{aB}	2.89±0.15^{bC}	2.38±0.22^{bB}

* Values are the mean of 3 independent determinations ± standard deviation.

* Lowercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

* Uppercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

[1] expressed as µg of gallic acid equivalents per gram of dry weight.

[2] expresado as µg of quercetin equivalents per gram of dry weight.

[3,4,5] expressed as µmol de trolox equivalents per gram of dry weight.

*DPPH (2,2-difenil-1-picrilhidrazilo), ABTS (ácido -3 etilbenzotiazolino-6-sulfónico) y FRAP (Ferric Reducing Antioxidant Power).

On the other hand, other species such as xoconostle (*Opuntia matudae*) have shown higher values of these compounds (TP) with values of up to 8590 and 9180 µg GAE. g^{-1} dw in pulp and peel (Morales, 2009). Similarly, values from 4950 to 9800 µg GAE. g^{-1} dw have been reported in blueberry (Wada, 2002) and from 526 to 6819 µg GAE. g^{-1} dw at different maturity stages in garambullo (Felix, 2018).

Antioxidant activity (AOA)

Antioxidant activity, is one of the main mechanisms in which vegetables and fruits provide health benefits to humans (Andreu *et al.,* 2018). Several studies have established inverse correlations in the consumption of fruits and vegetables and cardiovascular, inflammatory, cancer and age-dependent diseases (Willet, 2001).

The use of a single technique to determine antioxidant activity may prove to be unrealistic and not as useful, however there are a large number of published techniques that purport to measure antioxidant activity *in vivo* (Wuang *et al.,* 2005). The measurement of antioxidant activity in prickly pear fruits was evaluated based on three spectrophotometric assays; DPPH, ABTS and FRAP. The results are shown in Table 4. As with total phenols and flavonoids, the highest antioxidant activity was clearly observed for the three assays and three cultivars (CP1, CP3 and CP4) in the fruit peel, except in the peel and pulp of CP1 (red) by ABTS.

For the FRAP assay, CP1 (red) and CP4 (orange) show higher antioxidant activity (17.6 and 19.13 µmol ET g^{-1} dw) than CP3 (white) in peel. CP3

shows the lowest antioxidant activity in this assay in the three tissues (14.9, 6.8 and 5.2 µmol ET g^{-1} dw) in peel, pulp and juice, respectively. In the ABTS assay, CP1 (red) showed higher antioxidant activity in pulp and juice with values of 18.34 and 14.38 µmol ET g^{-1}dw, respectively. There are no significant differences in the antioxidant activity of the three cultivars in peel. In DPPH, CP3 (white) and CP4 (orange) showed higher antioxidant activity in peel 32.3 and 19.8 µmol ET g^{-1}dw, respectively. On the contrary, CP1 (red) showed higher antioxidant activity in juice and pulp than the other cultivars with values of (8.96 and 5.05 µmol ET g^{-1}dw), respectively.

FRAP technique estimates the reducing activity of Fe(III), which is not necessarily relevant for calculating its antioxidant capacity (Ou, *et al*., 2002). Taking into account that not all antioxidants reduce Fe(III) as fast as required (Pulido *et al.,* 2000), their antioxidant capacity could be underestimated. The ABTS technique is considered to be highly sensitive (Kuskoski *et al.,* 2005), however, the working solution for this technique needs to be kept in the dark for 12 hours to generate free radicals. As the reacting solution is not always of the same age, this can lead to differences in values depending on the determination times (Thaipong *et al.*, 2006).

The DPPH assay has been a widely used method to detect the ability of compounds to scavenge free radicals or the antioxidant activity of extracts (Hou, *et al*., 2003). Sanchez suggested that 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is an easy and accurate method to measure the antioxidant capacity in fruit and vegetable extracts (Sánchez, 2002).

As concluded by Frankel and Meyer, these assays differ from each other in terms of substrates, probes, reaction conditions and quantification methods, making it very difficult to compare the results obtained between them (Frankel & Meyer, 2000).

A single method is not sufficient to determine the antioxidant capacity of plant extracts; more than one type of AOA determination is required to represent the different modes of action of antioxidants. The methods used are basically classified into two types: assays based on hydrogen atom transfer (HAT) and assays based on electron transfer (ET) (Dudonné *et al.*, 2009). In this study, AOA was determined by two HAT-type assays: ABTS and DPPH, as well as Fe reduction capacity, using the FRAP assay.

The presence of phenolic compounds in plant extracts contributes significantly to their antioxidant potential (Dudonné *et al.*, 2009). Part of this AOA comes from flavonoids, low molecular weight polyphenolic compounds distributed in fruits and vegetables (Hertog *et al*., 1992). For their part, betalains are powerful free radical scavengers that act as efficient antioxidants in biological models (Cano *et al*., 2017).

Antioxidant capacity varies considerably from one type of fruit to another. (Wuang *et al*., 2005) and coworkers conducted a study in which the antioxidant capacity of 12 fruits and 5 commercial juices was measured by ORAC assay, resulting in strawberry having the highest AOA (15.36), followed by plum (9.49), orange (7.50), grape (7.39), kiwi (6.02) and melon (0.97 µmol ET g^{-1} fresh fruit).

(Andreu *et al*., 2018) and coworkers reported high levels of antioxidant capacity in prickly pear fruits of different cultivars for peel and pulp showing higher values than those found in this study in the three methods. By the ABTS technique, they reported the lowest AOA value in peel for cultivar NA (14.7) and the highest value for cultivar NA (14.7 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NJ (6.4) and the highest value by NT (30 µmol ET g^{-1} dw). By the DPPH technique, the lowest AOA value in peel was obtained by cultivar NE (54.8) and the highest value in cultivar FR (60 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NO (57.4) and the highest value by NT (60 µmol ET g-1 dw). Finally, measured by FRAP, the lowest value of AOA in peel was obtained by cultivar NE (40.2) and the highest value by cultivar NA (116 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NA (15) and the highest value by FR (32 µmol ET g-1 dw).

This exceeds the results found for antioxidant capacity in this study for the three selections, with

the highest values found by the DPPH technique for CP3 peel (32.3) and for CP1 pulp by the ABTS technique (18.3 µmol ET g^{-1} dw).

Some authors have reported results consistent with this study, finding a higher antioxidant capacity in fruit peel than in the pulp of pomegranate (Calín *et al.*, 2013), guava (Marquina *et al.*, 2008) and berries (Oszmiański *et al*., 2016).

Correlation between tests

To determine the linear relationship between the antioxidant capacity methods performed and the phenolic compounds and betalains, Pearson's correlation coefficient was calculated. Table 5 shows high correlations between the three methods and phenolic compounds (Phenols and Flavonoids). The correlation coefficient between total phenols and flavonoids and the AOA measured by the FRAP assay was 0.85 and 0.93, respectively. The correlation between total phenols and flavonoids and AOA measured by ABTS was 0.79 and 0.81 and by DPPH was 0.85 and 0.77, respectively.

The correlation coefficients of betalains (betacyanins and betaxanthins) and AOA by FRAP were lower, with values of 0.41 and 0.48, respectively, 0.67 and 0.51 by ABTS and 0.50 and 0.466 by DPPH.

All techniques used for the determination of antioxidant capacity (AOA) showed a high correlation with TP and TF for three evaluated cultivars (CP1, CP3 and CP4). This may be because phenolic compounds, known as hydrophilic antioxidant compounds, are the most abundant secondary metabolites in plants (Gil *et al*., 2002). This corresponds with what has been found by other authors such as (Thaipong *et al.,* 2006) in guava extracts (r=0.97) using the FRAP technique and by (Dudonné *et al.,* 2009) in Pinus bark (r=0.96) using the ABTS technique. In addition, high correlations have been reported between total phenols and antioxidant activity by FRAP in fruit juices (Gardner *et al.*, 2000). Kuti also reports similar correlations to those found in this work between total flavonoids and the antioxidant capacity of four varieties of prickly pear with values ranging from 0.76 to 0.88 using the ORAC technique (Kuti, 2000).

Table 5: Pearson correlation matrix

	FRAP	ABTS	DPPH
BC	0.415*	0.670**	0.504*
BX	0.489*	0.516*	0.466*
FT	0.854**	0.798**	0.853**
FL	0.938**	0.811**	0.775**

*,**= significant ($p \leq$ 0.05 y 0.01 respectively).

BC: Betacyanins, BX: Betaxanthins. TP: Total phenols, TF: Total Flavonoids.

FRAP= total antioxidant capacity determined using Cu (III) complex as oxidant. ABTS= total antioxidant capacity determined with the 2, 2'-azino-bis-3-ethylbenzothiazoline6-sulfonic radical (ABTS•+); DPPH= total antioxidant capacity determined with the radical 2,2-diphenyl-1-picrylhydracil (DPPH •).

The high correlation shown by both TP and TF, as determined by the three techniques, indicates that both compounds are important contributors to the antioxidant activity of prickly pear fruit.

In the case of betalains, low correlations were found with the three techniques, ranging from 0.41 to 0.67: the lowest correlation was by the FRAP technique and the highest by ABTS. This may be attributed to the assays used, considering the fact that individual antioxidants may, in some cases, act by multiple mechanisms depending on the reaction system (Fernández *et al.,* 2010). Cano and collaborators reported a negative correlation of total betalain content and antioxidant capacity determined by the DPPH technique (-0.08) (Cano *et al.,* 2017).

The body's defense system is composed of several antioxidant components. Supplementation with one or few antioxidants may not be as effective. Fruits contain a group of natural antioxidants that could have not only high antioxidant activity, but also a good combination or mixture of antioxidants (Wuang *et al*., 2005).

CONCLUSIONS

The present study provides information on physicochemical characterization and antioxidant properties of three selections of prickly pear (*Opuntia ficus-indica* Mill) grown at the Colegio de Postgraduados, Mexico. The results show that prickly pear has considerable levels of phenolic compounds that play an important role against oxidation. The highest content of these compounds is found in the peel of the fruit and there are no significant differences between the content in pulp

and juice. Therefore, prickly pear peel has a great potential for obtaining bioactive compounds, antioxidants. These natural antioxidants can be formulated to give nutraceuticals, which can help prevent oxidative damage from occurring in the body.

In relation to quality and physicochemical characteristics, CP1 (red) and CP4 (orange) were outstanding in aspects of size, weight, greater resistance to deformation, higher total soluble solids content, greater quantity of pulp and juice, and smaller seed.

All these aspects make the CP1(red) and CP4(orange) selections interesting materials for both fresh and processed products. Further research is needed to find alternatives to take full advantage of the compounds found in all parts of the fruit, as well as to understand the role played by betalains in the antioxidant activity of the fruit.

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Original Research Paper

Soil microbial community dynamics as influenced by integrated nutrient management practices in sweet basil (*Ocimum basilicum* L.) cultivation

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ABSTRACT

An experiment was conducted to study the effect of integrated nutrient management practices on the microbial community dynamics of soils under sweet basil (*Ocimum basilicum* L.) at ICAR - Indian Institute of Horticultural Research, Bengaluru during Kharif season of 2015 and 2016. There were nine treatments replicated thrice in randomized complete block design. The results indicated that integrated application of FYM (10 t/ha) + 100% recommended N through FYM + bio-fertilizer i.e., T_2 recorded the highest population of heterotrophic free-living N_2 fixers (40.66 and 63.33 CFU $\times 10^3$/ g), phosphate solubilizing bacteria (5.6 and 6.6 CFU $\times 10^3$/ g) and fungal (6.4 and 5.33 CFU $\times 10^3$/ g) while T_9 with application of NPK (160:80:80 kg /ha) + FYM (10 t/ha) recorded the highest population of actinomycetes (29.93 and 44.56 CFU $\times 10^3$/ g) in soil during 2015 and 2016, respectively. Application of recommended dose of FYM (10 t/ha) in T_7 resulted in reduction in population of heterotrophic free-living N_2 fixers (26.13 and 34 CFU $\times 10^3$/ g) and actinomycetes (20 and 30.5 CFU $\times 10^3$/ g) whereas, the application of recommended dose of chemical fertilizer in T_8 recorded the lowest population of phosphate solubilizing bacteria (3.9 CFU $\times 10^3$/ g) and fungal (3.6 and 2.5 CFU $\times 10^3$/ g) during 2015 and 2016, respectively. Highest organic carbon (0.63 and 0.66 %) content in the post-harvest soil samples was recorded with application of NPK (160:80:80 kg /ha) + FYM (10 t/ha) while, the lowest organic carbon value (0.52 and 0.53%) was recorded in T_8 during 2015 and 2016, respectively. Application of recommended FYM (10 t/ha) along with recommended NPK (160:80:80 kg/ha) in T_9 recorded maximum herbage yield in the main crop (41.59 and 38.31 t/ha) and ratoon (20.97 and 17.77 t/ha) during 2015 and 2016, respectively. The results obtained from this study clearly demonstrated that integrated nutrient management can maximize soil microbial community dynamics which is considered as driving force behind regulating soil processes that support sustainable sweet basil cultivation.

Keywords **:** Chemical fertilizers, Bio-fertilizer, Farm yard manure, Soil microbial community and Sweet basil.

INTRODUCTION

Soil biota refers to the organisms both animals (fauna/ micro-fauna) and plants (flora/microflora) that determines overall quality, fertility and stability of the soils. Further, the process of soil formation, structural stabilization, nutrient cycling is largely regulated by these soil organisms. Hence, they are most important in achieving the soil sustainability. The fact is that soil contains a vast number and wide range of organisms which are important in the myriad of biochemical reactions and intricate biological processes that take place within the soil (Bajracharya, 2011). Koopmans and Smeding (2008) state that learning how to manage beneficial soil biological processes as the key step towards developing sustainable agricultural systems.

Maintenance of soil fertility reflects positively on the crop yield (Mbonigaba, 2007). This can be achieved by practicing integrated nutrient management including application of organic manures that results in a general improvement in the soil organic matter (SOM) which represents the main reservoir of energy for

microorganisms and nutrients supply for plants (AL-mansour *et al.*, 2019). Microorganisms such as bacteria, fungi and other micro fauna representatives are responsible for the energy and nutrients cycling (Bot and Benites, 2005). So it represents important component in the evaluation of soil quality and can be used as biological indicators for production systems (Franchini, 2007) and it has strong correlation with the soil organic matter, which in turn reflects in crop yield (Gundale, 2005). Increase in the microbial population have been linked to increase in soil carbon and ecological buffering capacity and in response to organic management, as well as various organic amendments application such as livestock manure (Ling *et al*., 2014). A 19-year long-term experiment conducted to evaluate the effects of fertilization regimes on soil organic carbon (SOC) dynamics indicated that the SOC content in the top 20cm soil layer remained unchanged over time under the unfertilized control plot whereas it significantly increased under both organic, bio and NPK fertilizers and combined manure treatments (Yang *et al*., 2011).

Regular/recommended application of organic manures such as FYM that increase soil aggregation is therefore vital because most soils rely on aggregation of particles to maintain favorable conditions for soil microbial and faunal activity, plant growth and yield (Yu *et al.*, 2012). An experiment was conducted to study the effect of FYM, bio-fertilizers, mineral NPK fertilization on vegetative growth, oil production and chemical composition of basil plant. The results obtained by (Zeinab, 2005) indicated that the application of FYM at high level (25t/ha) significantly increased the studied parameters compared with other fertilization including the control. The interaction between the main-plots (FYM treatments) and sub-plots (bio, and NPK treatments) had significant effect on the yield parameters.

The rise in agricultural systems studies concerning soil quality and microbial properties are a reflection of the importance of soil to the understanding of agricultural sustainability. How management practices impact the soil is fundamental in evaluating the sustainability of an agricultural system. More than just a substrate for supporting root structure, the soil has its own complexes ecosystem in which microorganisms are the dominant form of life and are responsible for performing functions vital to soil productivity. Sweet basil (*Ocimum basilicum* L.) belonging to the Lamiaceae family, cultivated around the world (Baritaux *et al.*, 1992) is considered as an important source for food and medicine (Palada *et al.*, 2002). However, the studies on integrated nutrient management in basil are meager. Hence, the study was conducted with different combination of inorganic fertilizers, organic manure (FYM) and bio- fertilizer to find out their effect on dynamics of soil microbial population and organic carbon in sweet basil (*Ocimum basilicum* L.) cultivation.

MATERIAL AND METHODS

Experimental location and treatment details

Field experiments were conducted in a randomized complete block design with three replications in an experimental field of ICAR-Indian Institute of Horticultural Research (IIHR), Bangalore during the kharif season of 2015 and 2016. The experimental station is situated at an altitude of 890 m above mean sea level and 13°58" North latitude and 77°29" East longitudes. The nine treatments of the experiment consisted of different combinations of organic manure (FYM), bio-fertilizers and chemical fertilizers (NPK) : T_1- (FYM (10 t/ha) +100% recommended N through FYM), T_2 - (FYM (10 t/ha) + 100% recommended N through FYM + Arka Microbial Consortium @ 5 kg/acre), T_3 - (FYM (10 t/ha) + 75% recommended N through FYM), T_4 - (FYM (10 t/ha) + 75% recommended N through FYM + Arka Microbial Consortium @ 5 kg/acre), T_5 - (FYM (10 t/ha) + 50% recommended N through FYM), T_6 - (FYM (10 t/ha) + 50% recommended N through FYM + Arka Microbial Consortium @ 5 kg/acre), T_7 - (recommended FYM (10 t/ha) only), T_8 - (recommended NPK(160:80:80 kg/ha) only), and T_9 - (recommended FYM (10 t/ha) + recommended NPK (160:80:80 kg/ha). Estimated N content of FYM used in this experiment was 0.64%. Arka Microbial Consortium (AMC) is a carrier-based product which contains N Fixing, P & Zn solubilizing and plant growth promoting microbes as a single formulation. After 15 days of transplanting, recommended dose of AMC @ 5 kg/acre was applied at 2 cm deep to individual plants in treatments T2, T4, T6 and immediately covered by soil. Similar method of application was followed for ratoon crop after harvest of main crop.

Land preparation

The land was brought to a fine tilth by ploughing and harrowing. The experimental site was divided

into plots having dimensions of 4.8 m long and 4.0 m wide with the spacing of 40 cm between the plants and 60 cm between the rows. There was a space of 0.5 meter between plots and 0.5 meter between replications. Basil seeds were sown in two nursery beds of 6.0 m in length with 0.1 m in width and 10 cm height. Forty days old (40 days) healthy and uniformly rooted seedlings of sweet basil were transplanted to the field. Weeding was done manually and drip irrigation was given daily for half an hour during the early stages of the crop and subsequently irrigation was given depending on the soil moisture condition.

Estimating the fresh herbage yield

Five plants were randomly selected from each plot for recording the observations and the crop was harvested at full bloom stage before setting the seed. Fresh herbage harvested from each plot was weighed and converted to per hectare and expressed in tonnes (t).

Microbial population of the soil

Microbial population of the soil under different treatments was enumerated by standard plate count technique. Total bacterial count in soil was determined by serial dilution method. For the study, initial soil samples prior to the start of the experiment and after harvest were collected from the surface layer (0-15 cm) according to different treatments with three replications. Exactly 5 gm of soil sample was taken into 50 ml of sterile distilled water and shaken for 15 minutes. A series of 9 fold dilutions were prepared and 0.1 ml of each dilution was spread on media plates. To enumerate fungal, azotobacter, phosphate solubilising bacteria and actinomycetes population, potato dextrose agar (PDA), Jensen's media, Pikovskaya Agar and Kenknight media were used, respectively. After 3-5 days of incubation microbial population was counted following the spread plate technique and expressed as CFU $\times 10^3$/ g of soil.

Organic carbon estimation (%)

The organic carbon content of the soil was estimated by Walkley and Black wet oxidation method as described by Jackson (1973).

Statistical Analysis

The data generated from the experiment were analyzed using SAS 9.3 version of the statistical package (SAS Institute Inc, 2011). Analysis of variance (ANOVA) was performed using SAS PROC ANOVA procedure. Means were separated using Fisher's protected least significant difference (LSD) test at a probability level of $p<0.01$

RESULTS AND DISCUSSION

Population of heterotrophic free-living N_2 fixers

The data in **Table 1** indicated significant difference among the treatments with respect to population of heterotrophic free-living N_2 fixers (CFU $\times 10^3$/g of soil). While, maximum population of the colonies in the soil after cropping (40.66 and 63.33 CFU $\times 10^3$/ g) was recorded in T_2 with application of FYM (10 t/ha) +100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre during 2015 and 2016, respectively. Whereas, the treatment i.e. T_7 recorded the lowest counts (26.13 and 34 CFU $\times 10^3$/ g) during first and second year, respectively. The addition of organic manure greatly influences the microbial populations which expected to cause changes in the organic matter content of soil that directly influenced microbial dynamics of soil (Deforest *et al*., 2012). Application of bio-fertilizer stimulates the native soil microorganisms and reactivates the biogeochemical cycles leading to increase in the organic material that significantly increases the bacterial populations. The results are on line with Watts *et al*., (2010), Krishnakumar *et al*. (2005) and Lalfakzuala *et al*., (2008).

Population of phosphate solubilizing bacteria

The data on the population of phosphate solubilizing bacteria (CFU $\times 10^3$/ g of soil) after cropping given in Table 1 indicated that there was no significant difference between the treatments during first year (2015). The application of FYM (10 t/ha) + 100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre i.e., T_2 recorded the highest population of PSB (5.6 CFU $\times 10^3$/ g) while, the lowest PSB (3 CFU $\times 10^3$/ g) was recorded in T_8. However, there were significant differences among the treatments in respect to population of

phosphate solubilizing bacteria in the soil after cropping was observed during second year (2016). Similar to first year, application of FYM (10 t/ha) + 100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre recorded the highest population of PSB (6.6 CFU $\times 10^3$/ g) in soil while, the application of recommended dose of chemical fertilizer recorded the lowest population of PSB (3.9 CFU $\times 10^3$/ g).

Growth of P solubilizing microorganisms is generally accompanied by decrease in pH of the soil (Mishra, 1985). Reduction in pH due to application of FYM along with bio-fertilizer is a result of the production of organic acids which include citric, gluconic, fumaric, malic, oxalic, lactic, 2- ketogluconic, malonic acids etc. (Vassilev, 1996). Although chemical fertilization has resulted in increases in crop yield, this application was not sufficient in triggering a significant improvement in the soil microbial properties. Similar results were obtained by Wang *et al*., (2011). The addition of fertilizers enriched the soil microbial biomass and soil enzymes by enhancing the soil physico-chemical properties and soil organic matter, especially through the addition of FYM. Root exudates augmented the soil microbes in general by the crop growth and that could explain the increase of soil population at harvest time comparing with the initial soil.

Population of fungi

Fungal population in the soil after cropping in two years of the experiment was affected significantly by the treatments involving different levels of organic manure with and without bio-fertilizers and inorganic fertilizers. As showed in **Table 2** application of FYM (10 t/ha) +100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre (T_2) recorded the maximum fungal population (6.4 and 5.33 CFU $\times 10^3$/ g) in the soil while, the application of recommended dose of chemical fertilizer (T_8) recorded the lowest fungal population (3.6 and 2.5 CFU $\times 10^3$/ g) in the soil after cropping in 2015 and 2016, respectively.

Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.,* 1997). Fungi constitute an essential component of biological characteristics in soil ecosystems. Organic carbon level in the soil and precipitation play pivotal role in fungal growth and sporulation. Greater microbial populations in FYM treated plots along with bio-fertilizer as compared to chemically amended plots due to enhancing the organic carbon in the soil. Similar kind of results was reported by Venkateswarlu and Srinivasa Rao, (2000). Application of farm yard manure can be viewed as an excellent way to recycle nutrients and provide a steady source of organic C to support the microbial community resulting in higher fungal populations compared to NPK- treated plots.

Lower fungal population in soil with application of chemical fertilizers alone is attributed to lack of organic amendment input. The microbial population dynamics is governed by interactions between plant type, climate, and management practice. So that, the low temperature that prevailed in the first season could have influenced the proliferation of fungi, which require low temperature for their growth; Song *et al*. (2007) indicated that difference in the establishment of field leads to alteration of microbial communities.

Population of actinomycetes

The data in Table 2 on actinomycetes population of the soil after cropping (CFU $\times 10^3$/ g of soil) indicated significant differences between the treatments. The highest population of actinomycetes (29.93 and 44.56 CFU $\times 10^3$/ g of soil) was recorded in T_9 with application of recommended NPK (160:80:80 kg/ha) + FYM (10 t/ha) during 2015 and 2016, respectively, while application of recommended dose of FYM (10 t/ ha) in T_7 resulted in minimum population (20 and and 30.5 CFU $\times 10^3$/ g of soil) during 2015 and 2016, respectively.

Actinomycetes are one of the predominant members of soil microbial communities and they have beneficial roles in soil nutrients cycling and agricultural productivity (Elliot and Lynch, 1995). Organic matter, salinity, relative moisture, temperature, pH and vegetation are important factors which control abundance of actinomycetes in soil (Mcarthy and Williams, 1992). The density of actinomycetes is opposite to the hydrogen ion concentration, that could justify increasing its population with application of NPK along with

Table 1: Heterotrophic free-living N_2 fixers and phosphate solubilizing bacteria population (CFU $\times 10^3$/ g of soil) as influenced by different levels of N through FYM, bio-fertilizers and inorganic fertilizer

Treatments		Soil microbial population			
		Heterotrophic free living N_2 fixer		phosphate solubilizing bacteria	
T1	FYM (10 t/ha) +100% Rec. N through FYM	34.96ABC	41C	4	5.48ABC
T2	FYM (10 t/ha) +100% Rec. N through FYM + BF	40.66A	63.33A	5.6	6.6A
T3	FYM (10 t/ha) +75% Rec. N through FYM	31.83BCD	36.83C	3.6	4.8BCD
T4	FYM (10 t/ha) +75% Rec. N through FYM + BF	36.42ABC	54.5 B	4.5	5.6 AB
T5	FYM (10 t/ha) +50% Rec. N through FYM	29.5CD	39.4C	3.3	4.08CD
T6	FYM (10 t/ha) +50% Rec. N through FYM+BF	34ABC	40C	4	4.9BCD
T7	Rec. FYM (10 t/ha) only	26.13D	34C	3.7	4.06CD
T8	Rec.NPK (160:80:80 Kg /ha)	36.26AB	35C	3	3.9D
T9	Rec. NPK (160:80:80 Kg /ha)+ Rec. FYM (10 t/ha)	39A	55B	4.5	5.32ABC
General Mean		34.26	44.67	4.04	4.99
CV% 10.99		10.33	26.54	17.07	
LSD at 5%		6.52	7.99	NS	1.47

Table 2: Fungal and Actinomycetyes population (CFU ×10³/ g of soil) as influenced by different levels of N through FYM, bio-fertilizers and inorganic fertilizer

Treatments	Soil microbial population			
	Fungal		Actinomycetyes	
After the experiment	2015	2016	2015	2016
T1 FYM (10 t/ha) +100% Rec. N through FYM	6.26^{AB}	5.16^{AB}	24.33	34.00^{AB}
T2 FYM (10 t/ha) +100% Rec. N through FYM + AMC (5kg/ac)	6.4^{A}	5.33^{A}	26.67	36.33^{AB}
T3 FYM (10 t/ha) +75% Rec. N through FYM	4.1^{CD}	3^{CD}	22.67	31.67^{B}
T4 FYM (10 t/ha) +75% Rec. N through FYM + AMC (5kg/ac)	4.7^{CD}	3.65^{CD}	25.85	35.07^{AB}
T5 FYM (10 t/ha) +50% Rec. N through FYM	4.3^{CD}	3^{CD}	21.67	30.67^{B}
T6 FYM (10 t/ha) +50% Rec. N through FYM+ AMC (5kg/ac)	5.3^{BC}	4^{BC}	24.67	32.50^{B}
T7 Rec. FYM (10 t/ha) only	3.7^{D}	2.66^{D}	20.00	30.50^{B}
T8 Rec.NPK (160:80:80 kg /ha)	3.6^{D}	2.5^{D}	28.33	41.83^{AB}
T9 Rec. NPK (160:80:80 kg /ha)+ Rec. FYM (10 t/ha)	5.1^{BC}	4^{BC}	29.93	44.56^{A}
General Mean	4.80	3.70	24.33	35.24
CV%	1.26	1.26	16.74	12.5
LSD at 5%	15.20	19.72	NS	7.47

FYM (Alexander, 1977) While, increasing the colony's in the second season comparing to first one due to a relatively low level of moisture, this property of actinomycetes might be due to their sporulation capability under stress conditions (El-Tarabily and Sivasithamparam, 2006).

Organic carbon

The treatments effect on organic carbon per cent in the soil are presented in Table 3. Application of FYM (10 t/ha) +100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre i.e., T_2 recorded the maximum organic carbon (0.63 and 0.66 %) in the post-harvest soil samples collected during 2015 and 2016, respectively. While, the minimum value (0.52 and 0.53%) was recorded in T_8 with application the recommended dose of inorganic fertilizer (160:80:80 kg /ha) during 2015 and, 2016, respectively. Organic carbon per cent is fine indicators of soil quality which influence soil function in specific ways (e.g., immobilization–mineralization) and are much more sensitive to change in soil management practices (Saviozzi *et al.,* 2001). The results showed the positive influence of higher level of N through FYM and bio-fertilizer in increasing the organic carbon content that could be because of the effect of FYM and bio-fertilizer in stimulation of soil microbial activity, therefore increasing the C output. Similar results were also found by Halvorson *et al.*, (2002); Su *et al.*, (2006) and Lou *et al.*, (2011).

Fresh herbage yield

The fresh herbage yield of basil differed significantly between the treatments during two years of the experiment. It is evident from the Table 4 that the application of NPK (160:80:80 kg /ha) + FYM (10 t/ha) i.e., T_9 recorded significantly the highest herbage yield in the main crop (41.59 and 38.31 t/ha) and ratoon (20.97 and 17.77 at/ha) during *kharif* 2015 and 2016, respectively. The lowest fresh herbage yield per hectare was recorded with recommended dose of FYM alone in the main crop (28.36 and 17.49 t/ha) and in ratoon (12.59 and 8.93 t/ha) during first and second year, respectively. Similar trend was also reflected in total herbage yield of basil. Application of NPK (160:80:80 kg /ha) + FYM (10 t/ha) i.e., T_9 recorded significantly the highest total herbage yield (62.56 and 56.08) while, the lowest value (40.95 and 26.42 t/ha) was recorded in T_7 during individual years.

Table 3: Organic carbon content (%) in the soil as influenced by different levels of N through FYM, bio-fertilizers and inorganic fertilizer

Treatments		Organic carbon (%)	
Before the experiment		**0.5**	
After the experiment		**2015**	**2016**
T1	FYM (10 t/ha) +100% Rec. N through FYM	0.61 ^{AB}	0.65^{A}
T2	FYM (10 t/ha) +100% Rec. N through FYM + AMC (5kg/ac)	0.63 ^{A}	0.66 ^{A}
T3	FYM (10 t/ha) +75% Rec. N through FYM	0.58^{ABC}	0.62 ^{A}
T4	FYM (10 t/ha) +75% Rec. N through FYM + AMC (5kg/ac)	0.61^{AB}	0.65 ^{A}
T5	FYM (10 t/ha) +50% Rec. N through FYM	0.56 ^{ABC}	0.60^{AB}
T6	FYM (10 t/ha) +50% Rec. N through FYM+ AMC (5kg/ac)	0.58 ^{ABC}	0.64 ^{A}
T7	Rec. FYM (10 t/ha) only	0.55 ^{ABC}	0.57^{ABC}
T8	Rec.NPK (160:80:80 kg /ha)	0.52	0.53^{C}
T9	Rec. NPK (160:80:80 kg /ha)+ Rec. FYM (10 t/ha)	0.54^{BC}	0.54^{BC}
General Mean		0.58	0.60
CV%		5.09	6.13
LSD at 5%		0.02	0.03

Table 4: Fresh herb yield (t/ha) of basil (*Ocimum basilicum* L.) as influenced by different levels of N through FYM, bio-fertilizers and inorganic fertilizer

Treatments		Fresh herb yield (t ha^{-1})					
		First year (2015)			Second year (2016)		
After the experiment		Main crop	Ratoon	Total yield	Main crop	Ratoon	Total yield
T1	FYM (10 t/ha) +100% Rec. N through FYM	33.31^{C}	17.03^{D}	50.34^{E}	25.94^{C}	11.33^{C}	37.27^{C}
T2	FYM (10 t/ha) +100% Rec. N through FYM + AMC (5kg/ac)	37.84^{B}	18.59^{C}	56.43^{C}	27.73^{C}	12.31^{B}	40.04^{C}
T3	FYM (10 t/ha) +75% Rec. N through FYM	32.40^{C}	15.63^{E}	48.03^{E}	21.73^{D}	10.19^{D}	31.92^{DE}
T4	FYM (10 t/ha) +75% Rec. N through FYM + AMC (5kg/ac)	36.19^{B}	17.12^{D}	53.31^{D}	22.84^{D}	11.27^{C}	34.11^{D}
T5	FYM (10 t/ha) +50% Rec. N through FYM	29.93^{D}	13.57^{G}	43.47^{F}	21.02^{D}	9.51^{E}	30.53^{E}
T6	FYM (10 t/ha) +50% Rec. N through FYM + AMC (5kg/ac)	33.08^{C}	14.96^{F}	48.04^{E}	22.32^{D}	10.11^{D}	32.43^{DE}
T7	Rec. FYM (10 t/ha) only	28.36^{D}	12.59^{H}	40.95^{G}	17.49^{E}	8.93^{E}	26.42^{F}
T8	Rec.NPK (160:80:80 kg /ha)	40.39^{A}	19.59^{B}	59.98^{B}	33.28^{B}	14.92^{B}	48.2^{B}
T9	Rec. NPK (160:80:80 kg /ha) + Rec. FYM (10 t/ha)	41.59^{A}	20.97^{A}	62.56^{A}	38.31^{A}	17.77^{A}	56.08^{A}
General Mean		34.79	16.67	51.46	27.05	10.82	37.44
CV%		3.15	2.21	2.19	5.72	3.83	4.58
LSD at 5%		1.89	0.63	1.95	2.54	0.71	2.88

Application of inorganic fertilizers are expected to release greater quantity of nutrients particularly N, P, K at a faster rate and higher level and there by greater uptake by the plants which resulted in higher growth and yield parameters. On the other hand application of FYM along with inorganic fertilizer release nutrients after mineralization. Such controlled but regulated supply of nutrients increased uptake N, P, K which in turn, brought about higher growth and yield. Increase in the yield parameters with combined use of organic and inorganic application reported in earlier reports of Joy *et al*. (2005) in black musli, Kothari *et al*. (2005) in Spilanthus acmella, Rajendran and Gnanavel (2008) in Aloe vera and Ravikumar *et al.* (2012) in coleus. Organic amendments show a slower nutrient release pattern than mineral fertilizer but facilitate an increased soil organic matter (SOM) content (Pinitpaitoon *et al*., 2011). Although Vanlauwe and Giller (2006) claim that organic resources are not sufficient enough to supply crops with the required nutrients, the increased SOM is enhancing productivity due to the improved soil properties (Watson *et al*., 2002). Similar results were obtained by Mohamad *et al.* (2014) and Asieh (2012).

CONCLUSION

The experimental results concluded that the conjunctive use of FYM (10 t/ha) +100% recommended N through FYM + Arka Microbial Consortium @ 5kg/acre is found to have best microbial population dynamics and organic carbon content. However, the highest fresh herbage yield of sweet basil was recorded with integrated use of recommended FYM (10 t/ha) and recommended NPK (160:80:80 kg/ha**).** Further, the study evidently emphasis that the appropriate utilization of manures and bio-fertilizers within the nutrient management systems can enhance the soil microbial activity and diversity that reflected on yield sustainability.

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Original Research Paper

Effect of spectral manipulation and seasonal variations on cut foliage production and quality of *Philodendron* (*Philodendron* 'Xanadu')

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ABSTRACT

Influence of spectral manipulation of light using coloured nets and seasonal variation on cut foliage yield and quality of *Philodendron* 'Xanadu' was evaluated under red, green, white and black coloured shade nets permitting light intensities ranging from 240.50 to 370µ mol m^{-2} s^{-1} (75% shade net), for two consecutive years from 2014-16, at ICAR-IIHR, Bengaluru. The plants grown under white shade net (75% shade) resulted in higher foliage production plant^{-1}month^{-1} (14.53) and were on par with those grown under green shade net. The quality of the cut foliage in plants grown under white shade net with respect to stalk length (24.91 cm) and width of lamina (5.19 cm) was on par with those under green and black shade nets. Coloured shade nets did not influence vase life of the cut foliage. Developmental stages of the foliage under the different coloured nets have indicated that leaves attained the harvestable maturity stage at 29.92 days post emergence under white shade. Cultivation of *Philodendron* 'Xanadu' under white shade resulted in maximum cut foliage yield and quality.

Key words **:** Coloured shade nets, Cut foliage, *Philodendron* 'Xanadu' and Photomorphogenesis

INTRODUCTION

Philodendron 'Xanadu' is a perennial evergreen tropical shrub, which belongs to the family Araceae and is cultivated under partial shade for its glossy green foliage. The cut foliage is used in floral bouquets as fillers and in floral arrangements. Coloured shade nets are used to regulate the crop growth and quality by screening various spectral bands of the solar radiation. According to Shahak (2008), the spectral manipulation by use of coloured nets influences the microclimate to which the plant is exposed, and promotes light-regulated desirable physiological responses, besides the nets providing physical protection against excessive radiation, insect pests and environmental changes. Stuefer and Huber (1998) opined that changes in light composition influence the development and morphogenesis. Photomorphogenesis is influenced by phytochromes (Quail *et al*., 1995), cryptochromes (Ahmad and Cashmore, 1996; Batschauer, 1998), and specialized UV-A and UV-B (Christie and Jenkins, 1996) receptors, about which little is known. Mohr (1994) reported that in many of the photomorphogenetic reactions, more than one photoreceptor is involved. Coloured shade nets induce desirable physiological responses and this is related to the production and quality of the crop. According to Ganelevin (2008), this is an economical and environmentally friendly alternative to labour and chemical intensive crop production and is being assessed in an increasingly growing number of crops, leading to gradual expansion of the commercial use. Many of the cut greens are commercially grown in India under green or black shade nets. A study was conducted to evaluate the coloured shade net most suitable for commercial production of *Philodendron* 'Xanadu' cut foliage, with the objective of exploring the possibility of improving the yield and quality.

MATERIALS AND METHODS

An experiment was conducted at the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during 2014 to 2016 on *Philodendron* 'Xanadu' grown under four coloured polypropylene shade nets (75%) *viz*., red,

black, green and white in randomised block design with six replications. Healthy, uniform suckers of *Philodendron* 'Xanadu' were planted on raised beds at 45 x 45cm spacing. Well decomposed FYM was added to the beds @ 2 kg m^{-2} and was thoroughly mixed. Fertilizers were applied to the plants @ 100:30:60 kg NPK ha^{-1} per year. Entire dose of phosphorous was supplied as basal dose and nitrogen and potash were applied in six equal split doses at bimonthly intervals. Observations on the number of cut foliage $plant^{-1}month^{-1}$, length of lamina, length of stalk, frond width and diameter of stalk were recorded. Based on the importance with regard to marketable standards, some of these parameters were assigned a factor with which it was multiplied and the weighted averages were calculated. Developmental stages of the foliage, epicuticular wax content of the foliage and post harvest qualities under the diffrerent coloured shade nets were recorded. The gas exchange parameters, net photosynthesis (P_N), transpiration rate (E) and stomatal conductance (gs) were recorded between 09:30 h and 11:00 h on fully expanded fourth leaf from shoot apex using portable photosynthetic system (LC pro+, ADC Bioscientific limited, UK). Seasonal influence on the production and quality attributes of the foliage was also analysed. The pooled data were statistically analysed (Gomez and Gomez, 1984) and the results have been presented. The average maximum and minimum temperatures and relative humidity recorded under the different coloured shade nets during the experimental period have also been presented in Figs. 1 a and 1b.

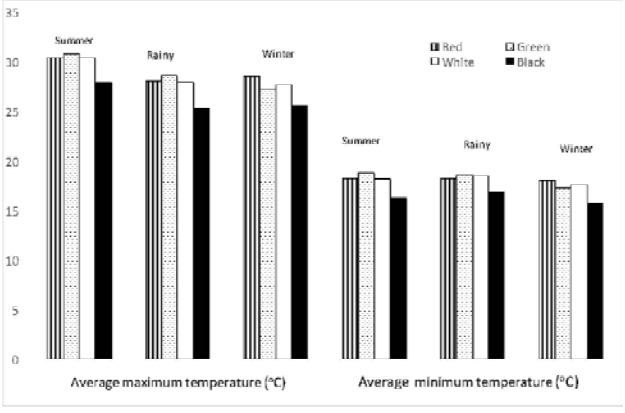

Fig. 1a: Maximum and minimum temperatures (°C) inside coloured shade nets during different seasons

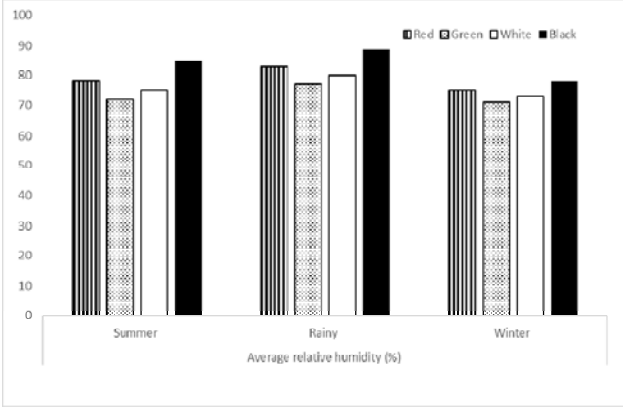

Fig. 1b: Seasonal effect on the relative humidity (%) inside coloured shade nets

RESULTS AND DISCUSSION

Data pertaining to the influence of coloured shade nets on production and quality of the cut foliage of *Philodendron* 'Xanadu' have been presented in Table 1. Plants grown under white shade nets produced the maximum number of cut foliage $plant^{-1}month^{-1}$ (14.53) which was on par with those grown under green shade net (13.71) followed by plants under red net (13.31). Minimum yield of cut foliage $plant^{-1}month^{-1}$ was obtained under black shade net (10.36). However, Stamps (2008) recorded the maximum foliage production under red shade net in *Philodendron* 'Xanadu'. In this study, the foliage production was highest under white shade net which might be due to the higher PAR availability. The foliage quality was significantly influenced by the colour of the shade nets. Plants grown under black shade net recorded maximum length of stalk (26.27 cm), which was on par with green (25.86 cm), white net (24.91) and was minimum in red (22.95 cm). Cultivation of plants under green shade net resulted in the maximum average width of lamina (5.36cm) which was on par with black (5.23 cm), white (5.19) and it was minimum in red (4.97 cm). Cultivation of plants under green shade net resulted in maximum stalk diameter (5.11 mm) and was minimum in black nets (4.49 mm).

The seasonal influence on cut foliage production and quality of *Philodendron* 'Xanadu' during three seasons *viz,* summer (March to June), rainy (July to October) and winter (November to February) was analysed and graphically presented in Figs. 2(a-e).

During rainy season, the cut foliage production $plant^{-1}$ was higher in the plants grown under all the coloured nets as compared to summer and winter

Table 1: Influence of shade net colour on the production and quality of cut foliage in *Philodendron* 'Xanadu'

Treatment (Shade net colour)	Number of foliage/plant/ month	Length of lamina (cm)	Length of stalk (cm)	Average lamina width (cm)	Diameter of stalk (mm)
Red	13.31	15.23	22.95	4.97	4.67
Green	13.71	15.70	25.86	5.36	5.11
White	14.53	15.24	24.91	5.19	4.74
Black	10.36	14.70	26.27	5.23	4.49
SEm ±	0.40	0.30	0.38	0.87	0.10
CD (P=0.05)	1.17	NS	1.12	0.25	0.3

seasons. The cut foliage production plant^{-1} month^{-1} (Fig. 2) was maximum in plants grown under white shade net during rainy season (19.54) and minimum in plants grown under black shade net during summer (9.01). Production of foliage was at par during the summer and winter seasons. Foliage quality was influenced to a certain extent by seasonal variations for characters like length of lamina, length of stalk, average width of lamina and diameter of stalk across the colour of the nets. The quality of cut foliage harvested from plants grown under green shade nets was superior for length of lamina (15.68 cm, 15.77 cm and 15.65 cm during summer, rainy and winter seasons, respectively), length of stalk (28.26cm during rainy season), lamina width (5.52 cm during summer season) and diameter of stalk (7.30 mm during winter season). The quality attributes of the foliage grown under white shade nets in respect of length of lamina (14.97 cm) and length of stalk (25.60 cm) during winter months and width of lamina during summer months (5.18 cm) were at par with that of the green nets. In general, the quality parameters of the foliage were lower under red and black nets depending on the season.

The per cent available Photosynthetically Active Radiation (PAR) inside the green coloured nets was highest (35.45 % during summer, 41.54% during rainy season and 38.16% during winter season), followed by the PAR inside white coloured nets (29.09 % during summer, 31.75% during rainy season and 35.04% during winter season). PAR designates the spectral range of solar radiation from 400 to 700 nanometres that plants are able to use in the process of photosynthesis. Higher PAR might have contributed to increase in photosynthesis by the plants, production of more photosynthates, thereby resulting in increased production of foliage. The quality of foliage like longer lamina, stalk and width of lamina could be correlated with the increase in sink capacity due to production of more photosynthates.

Epicuticular wax content (EWC) of the foliage was estimated and presented in Fig.5. EWC in the foliage of plants grown under green shade net was 871.8 µgdm^{-2} whereas the plants under white nets recorded 800.2 µgdm^{-2}. EW strongly contributes to the maintenance of high photosynthetic rates on leaves, which are able to keep their stomata open for longer periods, with high water use efficiency (Medeiros *et al*., 2017).

The vase life of cut foliage was evaluated under room condition (temperature 24-28 ^{o}C and 50-67% RH) in distilled water and it did not vary significantly among the cut foliage grown under different shade net colours (Fig 3) which was in accordance with the findings of Stamps (2008) in *Philodendron* 'Xanadu'.

The developmental stages of *Philodendron* (Fig. 4) were studied and the days taken by the foliage to attain each of the three critical stages i.e., Stage 1 (leaf tightly rolled in cataphyll), Stage 2 (just expanded tender leaf) and Stage 3 (fully expanded matured leaf) under the different shade nets were recorded. The days taken for progression from the first to second stage did not show marked difference under the different coloured nets. Green shade net (30.83 days) followed by white shade net (29.92 days) was found to have increased time intervals for the attaining the fully expanded mature stage from the tender leaf stage as compared to black (28.00 days) and red

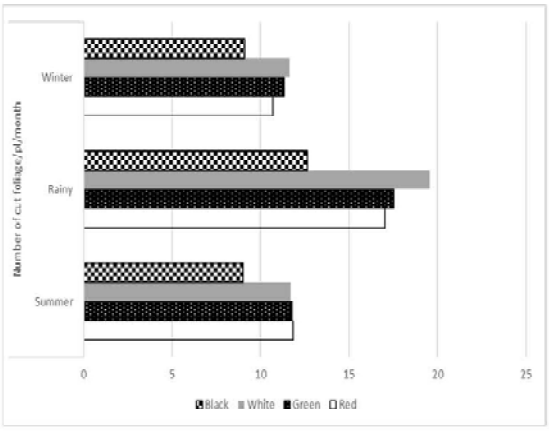

Fig. 2a. Number of foliage plant^{-1}month^{-1}

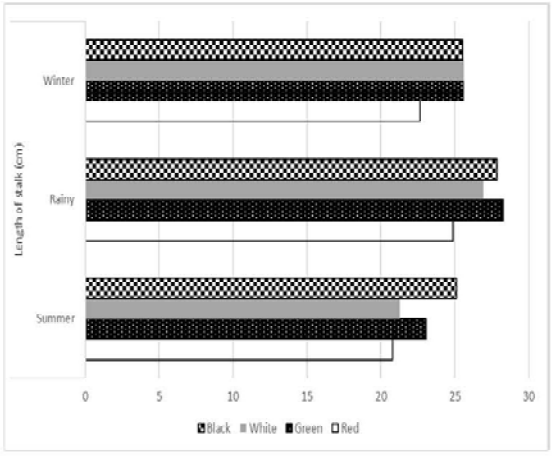

Fig. 2b. Length of stalk (cm)

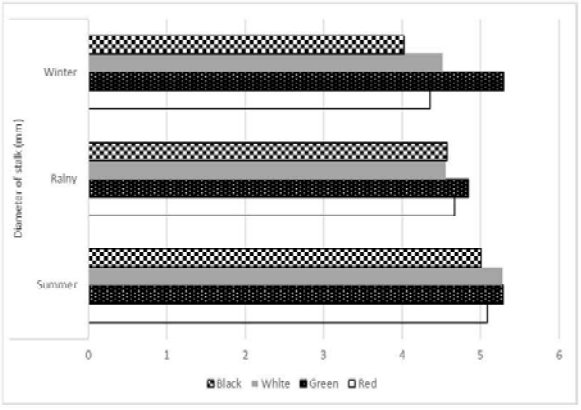

Fig. 2c. Diameter of stalk (mm)

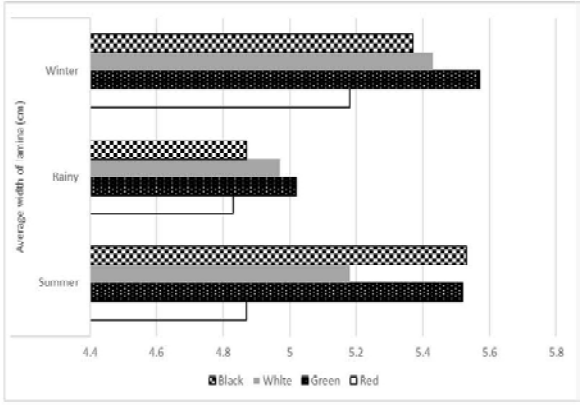

Fig. 2d. Width of lamina (cm)

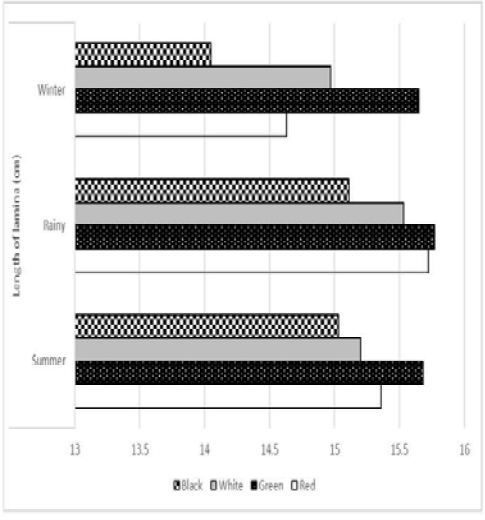

Fig. 2e. Length of lamina (cm)

Fig 2 (a-e). Influence of coloured shade nets and seasons on the cut foliage production and quality of foliage in *Philodendron* 'Xanadu'

shade nets (26.08 days). This can be correlated with the quality of the foliage indicating that with the increase in time taken to attain maturity, the foliage would have accumulated more of photosynthates resulting in superior quality.

Observations on gas exchange characteristics were recorded from the plants grown under different shade net colours. The photosynthesis rate was highest under green shade net during rainy season, which was on par with white shade net (Table 2). During summer and winter seasons, highest photosynthesis rate was observed in the plants under red shade net and was on par with white shade net. Plants under black shade net showed consistently lower photosynthesis rates across the seasons. The per cent PAR availability under different shade nets varied with highest available PAR under the green shade net followed by white shade net and least PAR was available under the black shade net (Table 2). Though definite trend was not observed among the different shade net treatments, overall, the transpiration rate was higher during summer season compared to rainy and winter seasons. The stomatal conductance was higher during rainy season compared to summer and winter seasons. In this study, it was observed that due to lower PAR availability under black shade net compared to other colour shade net, the photosynthesis rate was also lower. Ayala-Tafoya *et al*. (2018) in their study on the influence of coloured shade nets on photosynthesis and yield of cucumber observed that black shade net provided higher level of shade to the plants. The availability of PAR as well as red and blue lights were lower under black shade net compared to the other colour shade net. Black and white shade nets are known to cause reduction in light quantity and provide shade, while red shade net has effect on changes in red light composition (Ayala-Tafoya *et al*., 2011; Lobos *et al*., 2012; Oliveira *et al*., 2016). Though the green shade net transmitted highest PAR among the shade nets, the photosynthesis rate was highest during rainy season but was lower during summer and winter seasons which might be correlated with the temperature coefficient of real assimilation.

The maximum and minimum temperatures recorded inside the coloured nets also varied with green recording the highest average maximum and minimum temperatures in summer and rainy seasons and red net the highest maximum and minimum temperatures during winter season (Fig. 1a). Black shade net recorded the lowest average maximum and minimum temperatures across the seasons. The highest relative humidity was recorded inside the black shade net across the seasons followed by red shade and lowest inside green net (Fig. 1b).

Table 2: Seasonal influence of shade net colour on transpiration rate (m mol $m^{-2}s^{-1}$), stomatal conductance (mol $m^{-2}s^{-1}$), photosynthesis rate (μ mol CO_2 $m^{-2}s^{-1}$) and per cent available PAR

Treatment (Shade net colour)	Transpiration rate (m mol $m^{-2}s^{-1}$)			Stomatal conductance (mol $m^{-2}s^{-1}$)			Photosynthesis rate (μ mol CO_2 $m^{-2}s^{-1}$)			Per cent available PAR		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
Red	6.22	3.36	3.02	0.18	0.30	0.17	9.44	10.44	9.13	26.58	31.69	27.58
Green	4.84	3.58	3.29	0.12	0.31	0.20	7.59	11.64	7.21	35.45	41.54	38.16
White	3.40	2.31	3.33	0.12	0.46	0.25	8.93	11.19	8.49	29.09	31.75	35.04
Black	3.74	3.33	2.43	0.10	0.31	0.17	6.87	5.62	5.13	17.12	31.75	14.80
CV (%)	10.07	13.3	13.63	9.86	12.54	10.4	7.29	11.75	11.39	5.45	10.43	9.77
CD (P= 0.05)	1.82	0.83	NS	0.05	0.08	NS	1.19	2.28	1.70	1.88	4.3	3.39

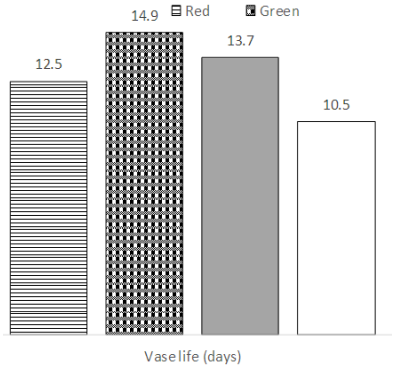

Fig. 3. Vase life of cut foliage of *Philodendron* 'Xanadu' cultivated under coloured shade nets

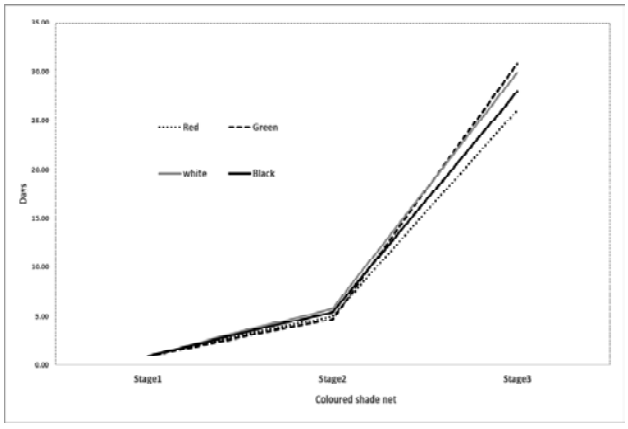

Fig. 4. Developmental stages of *Philodendron* 'Xanadu' foliage under coloured shade nets

Stage 1- Leaf rolled in cataphyll
Stage 2- Just expanded tender leaf
Stage 3- Fully expanded matured leaf

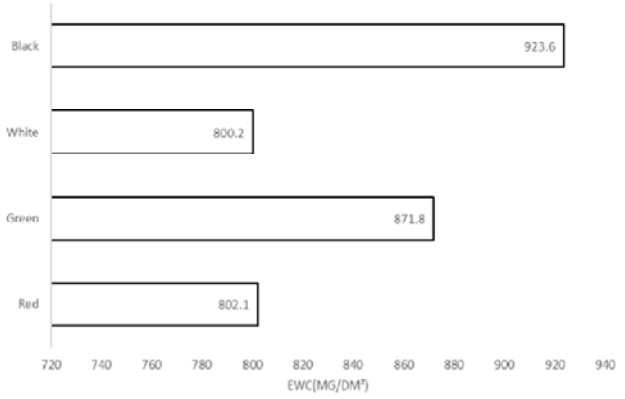

Fig. 5. Epicuticular foliage wax content of *Philodendron* 'Xanadu' cultivated under coloured shade nets

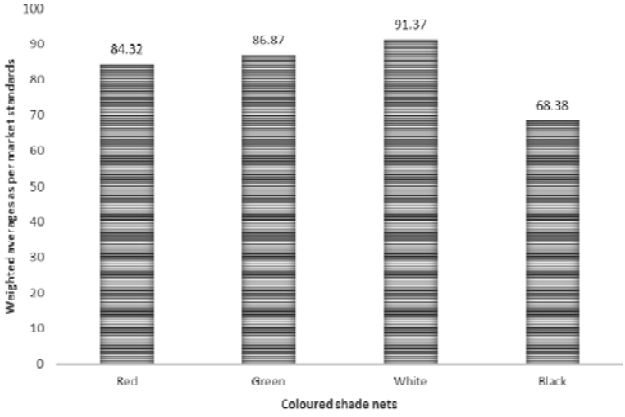

Fig. 6. Weighted averages for cut foliage of *Philodendron* 'Xanadu' cultivated under coloured shade nets

Weighted averages were assigned for the different characters of commercial importance according to the market standards (Fig. 6). White shade net grown cut foliage had the maximum weighted average (91.37) followed by green net (86.87), red net (84.32) and the least for cut foliage grown under black shade net (68.38).

Incidence of pests and diseases was monitored at weekly intervals. The plants remained relatively pest and disease free. Infestation of aphids was observed during the last week of March 2016, with the white shade net recording maximum number (18.35 per leaf) followed by red net (14.38 per leaf). Minimum infestation of 0.38 aphids per leaf was recorded on plants grown under black shade net. Incidence of *Cercospora* Leaf spot was recorded during the months of January- February and PDI was <5 across all the shade net colours.

Among the different coloured shade nets, cultivation of *Philodendron* 'Xanadu' under the white shade net resulted in higher cut foliage production, whereas, the quality parameters and post-harvest keeping quality were on par with plants cultivated under green shade nets. The yield of cut foliage and quality attributes were higher during the rainy season compared to winter and summer. It can hence be concluded that, cultivation of *Philodendron* 'Xanadu'under white shade net (75%) permitting light intensities ranging from 240.50 to 370 µ mol m^{-2} s^{-1} maximised the cut foliage yield, quality and post harvest keeping quality.

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Short Communication

Studies on mutagenic sensitivity of seeds of pummelo (*Citrus maxima* Merr.)

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ABSTRACT

Mutation breeding is a key method of generating large number of heritable variations. Effective dose (LD^{50}) needs to be standardized for inducing sufficient variation in a crop. In the present study, seeds were irradiated with different doses of Gamma rays and found that 66.94 Gy could suppress germination close to 50 per cent (LD^{50}) in pummelo. This 60 Gy gamma dose can effectively be used for raising the mutant populations to identify a desirable mutation in pummelo.

Keywords : Gamma Irradiation, Germination, Gray (Gy), LD^{50}, Mutation and Pummelo

Induced mutation plays a significant role in the crop improvement of horticultural crops. It is an important tool for induction of variation in quantitative and qualitative characters. It can be a supplement to conventional breeding methods when it is desired to improve one or two characters in a well-adapted variety. Induced mutation improving crops cultivars, enhancing biodiversity and Gamma irradiations are safe for human and environment and can be used widely to develop new varieties in fruit crops. In the recent past, mutation using gamma rays is regularly attempted in the banana breeding program (Smith *et al.,* 2006 and Mishra *et al.,* 2007). Among the different strategies to enhance crop improvement programs, induced mutagenesis has contributed immensely by creating mutant varieties with improved and desirable genetic changes in agronomically important traits in crops. Much progress has been made in generating superior genotype with favourable attributes through induced mutations in fruit crops. Pummelo is the largest citrus fruit and known in the western world mainly as the principal ancestor of the grapefruit. Pummelo fruit, like all fruits of the citrus family has several health benefits because of its super – rich Vitamin C and Vitamin B content. It also contains Vitamin A, Vitamin B1, B2 and C, bioflavonoid, healthy fats, protein, fibre, antioxidants and enzymes. It bears medium sized fruits (1-1.5kg) with good TSS (8-10 ^{0}B) value. The pulp texture of pummelo fruit is fleshy and pulp colour is pink. Excessive bitter taste in citrus juice is a major problem in citrus industry worldwide because it reduces the quality and commercial value of the product (Mongkolkul *et al.,* 2006). The bitterness in citrus fruit is affected by limonin and naringin, which are generally recognized as the major two bitter compounds. Limonin is the bitter limonoid found in major citrus cultivars such as grapefruit, the Navel orange, and the Shamouti orange (Guadagni *et al.,* 1973). Naringin is not transported after being synthesized in the fruit or leaves. However, till date, there is no information about availability of sweet pummelo varieties or bitter free pummelo varieties across the globe. Hence, the present investigation is aimed to determine the optimal dosage of gamma irradiation (LD50) for mutation induction in seeds of pummelo genotype "Kallar Selection" (Deep pink pulp with high bitterness) for developing a mutant with desirable horticultural traits.

The present investigation was carried out at ICAR-Indian Institute Horticultural Research, Bengaluru-560089. The seeds of Kallar Selection (Accession-6) pummelo were irradiated with different doses of gamma rays. The fresh and physically pure quality seeds of pummelo fruit were used for the irradiation purpose. In first step of experiment, a preliminary study was conducted to know the sensitivity of pummelo dry seeds to gamma radiation in which, pummelo seeds irradiated with gamma rays at the doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy. In second step of the experiment, based on preliminary results the pummelo fresh seeds were

irradiated with gamma doses of 25Gy, 50Gy, 60Gy, 75Gy and 100Gy to determine the exact LD50. The irradiated seeds along with non-irradiated seeds (control) were sown in protrays filled with cocopeat and each protray was marked with given gamma dose. Thereafter, water was sprinkled over the protray to provide enough moisture for seed germination and it was kept in controlled condition. Different parameters (seed germination per cent, number of seed germinated and number of seed deformities during germination) related to determining LD50 was recorded in 60 days after sowing. The data were subjected to Chi-Square analysis and Chi-Square table was used for the calculation error degrees of freedom.

For any induced mutagenesis programme, it is necessary to fix the LD50 value based on which larger population can be raised to isolate the desirable mutant progeny/progenies. The LD50 value varies according to crop species, varieties, seeds or other planting materials, nature of treatment, method of raising, climate, cultural practices and other parameters (Singh, 1994). In Citrus, several attempts to induce variability with some traits of seedless, thorn less, color changed fruits and juices (Maluszynski *et al*., 2000). The radio-sensitivity (LD50) of acute citrus exposure ranges from 40 to 100 Gy (Sanada and Amano, 1998; Sparrow *et al*., 1968), depending on the species and variety. The bud wood of pummelo Nambangan was irradiated with the dosage of 20, 40, and 60 Gy. The buds then grafted to Japanish Citron rootstocks. After three years of selection based on performance and fruit evaluation on MV2 generation, a mutant plant derived from 20 Gy irradiation treatments were obtained with improved character on the number of seeds. Pummelo Nambangan has more than 40 seeds/ fruit and the mutant has less than 10 seeds/fruit on average and it shows higher volume of juice compared to that in pummelo Nambangan. However, the fruit appearance between mutant plant and parent in term of the fruit shape had no difference, showing a combination of spheroid and pyriform shape. The difference between them was shown prominently when the fruit was cut, indicating the less seed contained and more intense red color of flesh of the mutant fruit compared to that of parent (Mariana *et al*., 2018.) In the initial study, pummelo seeds were treated with 100, 200, 300, 400 and 500 Gy). The effect of different doses of gamma rays ranging from 0 to 100 Gy on seed germination are shown in Table 1 and shown in Fig.1. The percentage of seed germination ranged from 18.31 per cent to 95.07percent with different doses of gamma rays in pummelo (Acc-06) as compared to 97.96 per cent in control.

Table 1: Effect of gamma irradiation in pummelo seed germination

Treatment	No of seeds sown	No of seeds germinated	No of under developed seedlings	Days to germination	Germination percentage
Control	98	96	0	10	97.96
Dosage 25 Gy	98	93	0	11	94.90
Dosage 50 Gy	98	88	0	11	89.90
Dosage 60 Gy	98	46	21	14	46.94
Dosage 75 Gy	98	24	16	20	24.49
Dosage 100 Gy	98	18	14	20	18.37

Gradual reduction/decrease in pummelo seed germination was observed with increase in gamma irradiation dose. The inhibitory effect on seed germination was directly proportional to the dose of gamma radiation. Similar results were reported by Dhatt *et al*. (2000) and Latado *et al*. (2001) with gamma radiation in citrus. Decrease in percent seed germination by gamma irradiation might be due to its effect on genetical and cytological processes coupled with the changes induced in metabolic processes. The decrease in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds (Sjodin, 1962). Sinha and Godward (1972) opined that the reduction in percentage of seed germination was due to the disturbances caused at the physiological level coupled

with chromosomal damage. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments (Kulkarni,2011). Gamma radiation is well known for their action causing point mutations, enzyme inhibitions and chromosomal aberrations. The observed reduction in seed germination in pummelo as a result of gamma radiation might be due to point mutations or the injuries caused to the genetic material. This may eventually lead to decrease the rate of respiration and energy production, which finally caused decrease in seed germination. Days taken to germination and number of seeds with deformity were increased with increasing dose of gamma radiation. Based on probit analysis, 66.94Gy dose was found to be effective as LD50 for irradiation of pummelo seeds (Table 2) to induce sufficient variation. This finding may assist as reference dose for large scale gamma irradiation of pummelo genotypes to induce genetic variation. However, considering the practical difficulty, its better fix 60 Gy as LD50 to induce sufficient mutation to select the desirable one. This result was in line with other studies on the effect of gamma rays in citrus. On lemon, Gulsen *et al*. (2007) obtained most seedless fruit from 50 Gy treatment while Spiegel-Roy *et al*. (2007) successfully obtained seedless fruit with 13.3 Gy. On mandarin, Kafa *et al*. (2015) obtained most mutant plants with seedless fruit with 30 Gy while Montanola *et al*. (2015) obtained seedless fruit with 40-50 Gy.

Table 2: Lethal dose calculation

Pummelo	n^a	LD50 LCL-UCL (95% confidence limit)	LD90 LCL-UCL (95% confidence limit)	χ^2	df
Gamma irradiation	98	66.94 (60.06-74.61)	164.27 (127.03-212.42)	9.4	4

LD50= lethal dose that kill 50% of the population; n^a= population number; LCL=lower confidence limit; UCL= upper confidence limit

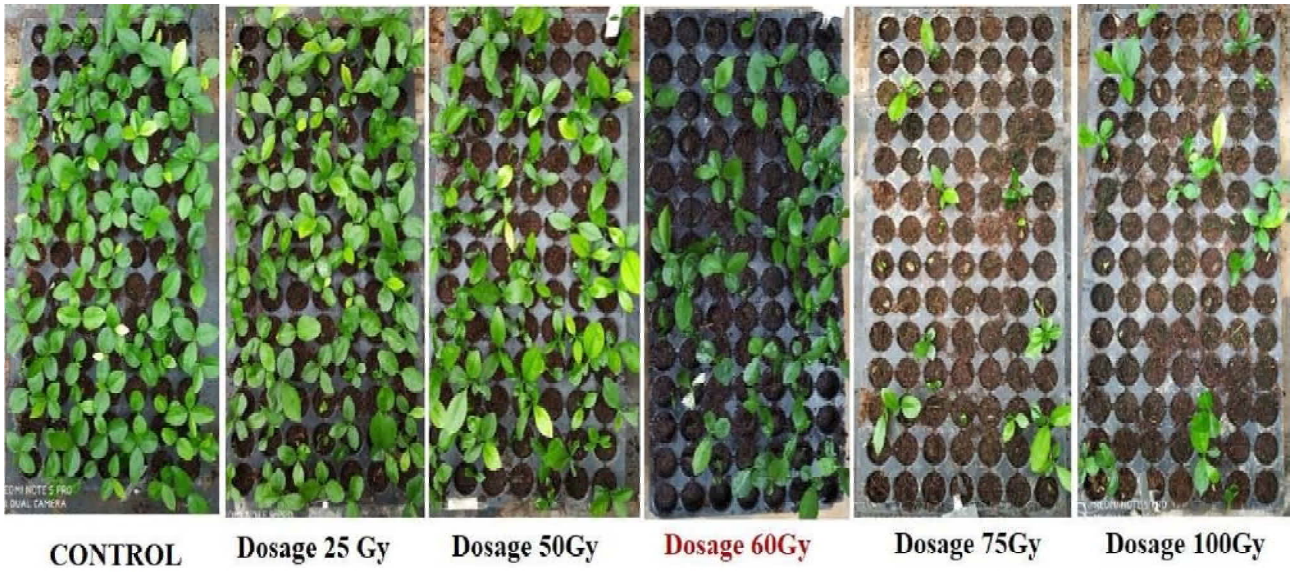

Fig.1: Mutagenic sensitivity of pummelo seedlings to different doses of gamma radiation

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Short Communication

Isolation and characterization of microsatellite markers from *Garcinia indica* and cross species amplification

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ABSTRACT

Garcinia indica **popularly known as '*Kokum*' or *Murugalu*", is a medium sized evergreen tree found in western-ghats of India. This tree species is highly exploited to produce anti-obesity drugs and culinary purposes. Its population is threatened by over exploitation and loss of habitat. Development of microsatellite markers would help in understanding genetic structure and further to develop appropriate conservation strategies. In this study, using next generation sequencing platform Illumina Hiseq 2000, we have sequenced partial genome of *G. indica* and identified 3725 microsatellites. Forty-eight microsatellite markers were analyzed using 30 accessions. Polymorphism information content (PIC) values ranged from 0.718 to 0.968 with a mean value of 0.922. Allele per locus ranged from 3 to 33 per locus. Probability of identity values ranged from 0.00329 to 0.30489. Cross species amplification SSR primers in the related species, showed a moderate transferability from 12.5 % (for *G. morella*) to 18.7%(for *G. gummigutta*)**

Key words : Cross-species amplification *Garcinia indica*; Microsatellite markers and Next-generation sequencing (NGS)

Garcinia indica Choisy (Thouars; Family Clusiaceae), is a perennial tree. *G. indica* is commonly known as a Brindonia Tallow tree or 'Kokum Butter' tree in English. Kokum has many uses in cuisines and an important ingredient in locally prepared medicines. The seeds are a rich source of Kokum butter, which is nutritive, demulcent, agent for smoothening, softening and used for cosmetic, confectionery, culinary purposes. Raw fruits, young leaves and bark are also used as medications against several disorders. The fruit rind is a rich source of Hydroxy Citric Acid (HCA) that prevents accumulation of fat in the human body cells. Therefore, *G. indica* has become the natural source for production of anti-obesity drugs. (Baliga *et al*., 2011). *Garcinia* species are endemic and distributed in tropical rain forests of the Western Ghats. Perceiving the threat of over exploitation, FRLHT (Foundation for Revitalization of Local Health Traditions) and IUCN (International Union for Conservation of Nature) have recognized this species as '*Vulnerable*' and '*Threatened*' category respectively (Hareesh and Vasudeva, 2010). A few studies examined diversity in this species using general DNA markers like RAPD and ISSR markers (Thatte *et al*. 2012; Palkar and Sellappan, 2019). However, so far there are no efforts to develop species specific, highly reproducible microsatellite markers or SSR markers in this species. Keeping this in view, an attempt has been made to develop microsatellite or SSR markers using next generation sequencing

technology. The development of molecular markers would help in studying its diversity, analyzing the genetics of traits, and further help in evolving conservation strategies and improvement.

The plant material was obtained from the germplasm collection of the College of Forestry, Sirsi (University of Agricultural Sciences, Dharwad), Karnataka state, India. Total genomic DNA was isolated from the leaves of *G. indica* genotypes using modified CTAB method (Ravishankar *et al*., 2000). Genomic DNA was sequenced using Illumina HiSeq2000 platform at M/s Genotypic Pvt. Ltd, Bengaluru facility following manufactures instructions. High quality sequence data was used for assembly into contigs. *De novo* assembly of reads into contigs was performed using SOAPdenovo2-src-r240 software (Luo *et al.,* 2012). This has resulted in 92125 contigs. The total assembled size of the contigs is approximately 25.6 Mbp. An SSR survey of genomic sequences using MISA software (http://pgrc.ipk-gatersleban.de/misa), showed that 3590 contigs contained at least one microsatellite (Ravishankar *et al.* 2015). A total of 3725 microsatellite was identified. A total of 1374 microsatellites (ESM1) primers were designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/; Untergrasser *et al*., 2012). From these, randomly 50 loci were selected for initial screening. Finally, 48 SSR primers were selected for genetic analysis based on clear amplification of PCR products. We employed Thirty genotypes of *Garcinia indica* for assessing polymorphism at each locus. The fluorescence based M13 tailed PCR method of Schuelke (2000) was followed to amplify the microsatellites in a quick, accurate and efficient manner. PCR was carried out in the 20µl reaction volume containing 2µl of 10X reaction buffer, 2.0µl of 1 mM dNTPs, 0.9µl (5 pmol) of forward, 0.9µl reverse primers (5 pmol), labeled M13 probe 1.2µl (5 pmol), 5.0 µl (50-75 ng) of template genomic DNA, 0.8 µl (2 U) of Taq DNA polymerase and 7.2 µl of nuclease free water. The PCR cycling profile was: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30sec., 55°C for 30 Sec., 72°C for 1 min and a final extension at 72°C for 5 min. Amplified products were separated on 96 capillary Automated DNA Sequencer (Applied Biosystems, ABI 3730 DNA Analyzer) at M/S Eurofin facility, Bengaluru.

The raw data generated was analyzed and compiled using Peak Scanner V1.0 software (Applied Biosystems, USA) for estimating the allele size in bp**.** The allele size data was used for genetic analysis using Cervus 3.0 software (Kalinowski *et al*. 2007). We have calculated observed heterozygosity, expected heterozygosity, polymorphic information content(PIC). The probability of identity (PI) was calculated using IDENTITY1.0 software (http://www.uni-graz.at/~sefck/: Wagner and Sefc, 1999). Genetic analysis of 48 SSR loci, showed PIC values ranging from 0.718 to 0.968 with a mean value of 0.922. The mean values of observed and expected heterozygosity are 0.2813 (Table 1) and 0.933 respectively (Table 1 and 2). The allele per locus ranged from 13 to 41 with a mean of 16.395. The probability of identity (PI) values ranged from 0.00329 to 0.304896 with a mean of 0.03506. The total probability of identity is 8.132729×10^{-80}. In cross species amplification, out of 48 SSR primers, 6 amplified in *G. morella ,* accounting 12.5 per cent transferability and 9 amplified in *G. gummigutta* accounting 18.8 percent transferability (ESM2). This relatively low cross-species transferability compared to what has been observed in *G. gummigutta* species (Ravishankar *et al*., 2017).

This is the first report of SSR markers for *Garcinia indica*, where 3725 microsatellites were identified and primers were designed for 1374 microsatellites. The genetic analysis showed that the majority of the SSR primers developed have high PIC values indicating high heterozygosity in the species. The low probability of identity values of many SSR loci is useful for molecular characterization. Finally, the SSR developed will be useful in studying genetic diversity, mapping and fingerprinting of *Garcinia indica* and related species.

Table 1: Genetic analysis of microsatellite markers developed for *Garcinia indica*

Locus	Forward Sequence 5¹→3¹	Reverse Sequence 5¹→3¹	Repeat Type	Number of Allele (k)	Allele size range (bp)	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	Polymorphic Information Content (PIC)	Probability of Identity (PI)
GI_KVRa577	TTTGGCGAGGGTGTTGGTGAGT	ACACGTGTAGGCTGACACCAACC	$(GT)^{6}$	20	140-230	0.345	0.924	0.902	0.012828
GI_KVRa614	TGTGAGTTGTTTGGCATGGGTGA	GGAGGGTGAGCAAATCACAGCTCA	$(TG)^{22}$	26	197-290	0.185	0.962	0.941	0.005254
GI_KVRa615	TGTGAGGGGTGAGGTTGAGGCT	ACAAACGCATCCCCACTCTCGG	$(AT)^{6}$	27	283-379	0.259	0.953	0.933	0.006829
GI_KVRa651	TGGGTGGCAAATTTGGGAGGAAA	TGCCGCCCAAGGAGAGAGGAAA	$(AC)^{8}$	24	185-277	0.2	0.971	0.95	0.006622
GI_KVRa723	TGCACCAGGAGGGTCACAGACT	ACAACGAGGCCTTCCAACAGGA	$(AC)^{10}$	21	412-488	0.143	0.926	0.904	0.011916
GI_KVRa747	TGACAGATCGACAGGCTAGACTCGAA	TCGCCCCCGTCTATGTATCAGTC	$(AT)^{6}$	25	432-531	0.192	0.962	0.941	0.006535
GI_KVRa748	TGAATGCCGAGAGCAATTGTGCC	TCACATCACAAGGCTTGCTCAAACA	$(TA)^{6}$	33	140-214	0.519	0.979	0.96	0.003290
GI_KVRa834	GTGCACATGTCGCCATAAAGATGGA	ACCTACCCCTCCATAACATGCCTT	$(AT)^{6}$	16	105-180	0.133	0.853	0.828	0.036897
GI_KVRa861	GGCCCATGGCCTCCTCTCATACAA	TGGGGAAGGACAATTAAGTCGGGA	$(TA)^{6}$	15	103-185	0.138	0.721	0.695	0.087401
GI_KVRa862	GGCACATGTGTCTACACCGCAC	TGTGGACAGGTAGGGTCACAGGT	$(AT)^{7}$	9	233-294	0.143	0.855	0.819	0.037316
GI_KVRa961	CCACACACAAAATGCCACAATTCCA	TGTGCGTGTGTGGTTGACAGGT	$(CA)^{6}$	14	99-124	0.286	0.847	0.816	0.036213
GI_KVRb069	AGACATCCGTCACCGGGCTCAT	TGCCATTTGTATGTGTTGTTGGCGG	$(CA)^{7}$	10	99-125	0.214	0.873	0.841	0.029837
GI_KVRb130	ACCCGCATTCACAATGCACATACA	GTGGCGCTATTGGGAAATGAGTACA	$(CA)^{7}$	8	233-341	0.000	0.86	0.823	0.033681
GI_KVRb131	ACCCCTAACGGTGGGTTCGTCA	TCGAGGGTCCTTGAGTTCTCCCCT	$(AT)^{6}$	13	99-190	0.148	0.905	0.879	0.017689
GI_KVRb132	ACCCCTAACGGTGGGTTCGTCA	TGGCCTTCGGTTGAGTTGTCCC	$(AT)^{6}$	10	117-157	0.429	0.774	0.733	0.067668
GI_KVRb174	ACACCGGTAAGGTGGTGAGAAGGA	ACACACAGAGTACCCCATATACGCACA	$(TG)^{7}$	12	101-148	0.25	0.783	0.749	0.054954
GI_KVRb175	ACACCGGTAAGGTGGTGAGAAGGA	ACACAGAGTACCTCACATACGCACA	$(TG)^{7}$	18	100-165	0.517	0.915	0.891	0.016365
GI_KVRb176	ACACCCGATCCCATTCCGACCT	ACACCAACCACGCTCCCTTCCT	$(TA)^{7}$	24	453-524	0.276	0.945	0.925	0.008223
GI_KVRb200	AACTACCATCAAACATCACCAACACGA	TGGAAGGTGTTGAGGTCGGCCA	$(CA)^{6}$	22	430-514	0.32	0.957	0.934	0.009077
GI_KVRb201	AACGGCTAGCTTTTCAACTGACTGT	TGGTAAGTCGATTGTTGGGCTTCG	$(TA)^{6}$	17	116-179	0.16	0.913	0.887	0.017850
GI_KVRa975	CACCCCATACACAACCACATTCCC	GGTGTATGTGCCTGGATAAATGAAGGT	$(CA)^{6}$	23	201-285	0.103	0.938	0.918	0.009212
GI_KVRa976	CACATCCTTACATGTACACGGTCCAC	CTGACCGGCTAAACATACAAGTTCCA	$(TA)^{7}$	20	316-397	0.083	0.926	0.901	0.016775
GI_KVRa977	CACATAAGGAACAACAACAAGGCCTCA	GCCGGAGGCCGTACAATTGTGTT	$(AT)^{7}$	24	99-171	0.433	0.856	0.835	0.031996
GI_KVRa978	CAATCTCATTCCTAGACAACCTGCACA	AGTTGATCCAGGATTTGGCGAGGGT	$(AC)^{6}$	20	99-148	0.414	0.933	0.912	0.011202
GI_KVRa979	CAAGGCTGCTCGGACGTCGAAT	ATCCCACCGGCTCGAGCAAGAA	$(CT)^{6}$	23	428-582	0.286	0.905	0.883	0.015518
GI_KVRa980	CAACATGCTTCAACCAAGCACATACAA	TGCTACTACCTTAGGAGACATGCATCA	$(TG)^{11}$	21	112-198	0.444	0.942	0.92	0.009296
GI_KVRa981	CAACAAAGGGCATTCATGCACACA	TTGGGGGAGGAACCAAGCAAGT	$(AT)^{6}$	24	313-399	0.633	0.955	0.936	0.006817
GI_KVRb047	AGCGAGGACAAGGGAAAGGACG	TGGCGGATATGTGTGCTTGGCG	$(TA)^{7}$	19	323-365	0.36	0.911	0.885	0.018187

Table 1 Contd....

GI_KVRb048	AGCGAATGCATGCGTGTAGCGA	ACGATCACCTTGGGGACGCTCA	$(AT)^6$	19	472-527	0.261	0.871	0.846	0.031785
GI_KVRb204	AACCCAGTGAGTGTAATGCGAATTGT	TGTTGTTGGCTTATAGCCGAATGTGA	$(CA)^7$	21	102-195	0.107	0.948	0.927	0.007728
GI_KVRb205	AACCCAATGAGTGTAATGCCAGTTGT	ACTGTGGTTGGCTTATGGCCTGA	$(CA)^6$	21	103-197	0.5	0.919	0.898	0.015233
GI_KVRb206	AACAGGACCGGTGTGCGGTTGA	TCCGCACATGTGTCCACACCAA	$(TA)^8$	21	201-341	0.423	0.909	0.885	0.016389
GI_KVRb207	AACACGTGGCAGACGCTCAAGG	TGGTGAGGTCGGTCCAAACAGGA	$(AT)^6$	8	117-178	0.233	0.793	0.757	0.070882
GI_KVRb208	AACACGCGCGAGGACATACTGC	CCAAGCCTCCTCTCCCATTTGTGC	$(TA)^6$	7	154-171	0.679	0.774	0.72	0.077586
GI_KVRb209	AACACCTGCACGGGTTTCGTGG	ACTTTCCATCTCGACCACGCCG	$(TA)^7$	10	330-413	0.000	0.89	0.86	0.023726
GI_KVRb213	AAAGGACCGGCGAAGAAAGCGG	CCCAGCTCAAACCGATGCCCAA	$(AG)^6$	10	134-250	00.000	0.881	0.85	0.026089
GI_KVRb214	AAAGAGAGGTCATCTTAGTGAGGGGG	TGTTGGCTTGGTCGTAACGGCT	$(GT)^6$	6	150-251	0.148	0.792	0.742	0.062789
GI_KVRb219	TGTTGGGAAGTAAAAGGAGGGAGCA	TGACCTAGGCATCCATCTCCCCT	$(TGT)^5$	7	113-178	0.5	0.785	0.733	0.063197
GI_KVRb220	TGTGGGGATGGCAAATGAGGTGA	TGCCATTCGGTTGGGGCATACT	$(CAC)^5$	10	143-173	0.115	0.829	0.788	0.044338
GI_KVRb234	TGGCGTGCAGTTCTTCCTCCCA	GGGATCGCATCCAACATTCATTTCCA	$(CAA)^5$	3	173-215	0.154	0.335	0.303	0.304896
GI_KVRb242	TGCAACAACAGGCTCAGGCACA	TGGTGGAGGCACGGGTTGAACA	$(CCA)^5$	15	189-215	0.5	0.907	0.881	0.018089
GI_KVRb243	TGAGCGACCGTGCCTGATGTTG	AGGGCTCCCTCACCCTCTACCTTA	$(CAG)^5$	13	141-171	0.36	0.864	0.83	0.032098
GI_KVRb341	ACAAGCATGCCAAACGTAGCCGA	TGAAGAAGTGCCCAACCCCACT	$(TGG)^5$	12	136-170	0.517	0.78	0.741	0.071213
GI_KVRb352	AAGACGGGTGGCGGTGGAGAAA	AGAAGCGAACCCTCTCCTCCTGA	$(TCT)^8$	13	362-403	0.552	0.866	0.835	0.033609
GI_KVRb357	TGACAATACGTGGGGAGATCCGT	TGTTCAGGCTCAATCCCTTCGTGC	$(AATA)^7$	16	115-191	0.000	0.886	0.861	0.021333
GI_KVRb368	TCCGTGCCAATTCCCTGGCAAC	TGACCTGTCGCCTTAGCTACCCT	$(AAAAT)^5$	17	249-310	0.192	0.925	0.9	0.014054
GI_KVRb373	AGCTAGGGGGCAACCTGTACCA	TGCTATTGAATTCGTGTTGGTGGTGA	$(CAATAC)^5$	8	151-168	0.481	0.818	0.778	0.048049
GI_KVRa011	TCCGTCCATCCGTTCGTCCGTT	ACCGGATGGGATCCAGCGATGT	(CGTC) 6cgtt $(CGTC)^7$	12	100-136	0.172	0.75	0.722	0.074675

Table 2: Summary of Genetic Analysis

	Mean	Range
Polymorphic Information Content (PIC)	0.8416	0.303- 0.96
Observed Heterozygosity (Ho)	0.2813	0.000- 0.679
Expected Heterozygosity(He)	0.8701	0.335- 0.979
Allele per locus	16.395	3- 33
Probability of Identity (PI)	0.03506	0.00329- 0.304896

Total number of Alleles : 787 Total probability of Identity : 8.132729e-080

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