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# Summer stratification and germination: A viable option for recovery of hybrid seedlings in low chill peach and nectarines

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## ABSTRACT

***In-vitro* embryo rescue; and summer stratification and germination under controlled conditions was tested for recovering hybrid seedlings in crosses involving low chilling peach and nectarines. The embryos from all the cross combinations showed very high *in vitro* embryo germination (>85%) on basal MS medium, which can be effectively performed at hard mature or full ripe stage. The stratification period of the hybrid seed varied from 36.3 days in Shan-i-Punjab × Florda Prince to 44.7 days in Tropic Beauty × Florda Grand. At the end of four weeks of transfering the cultures to culture room, maximum plant height (33.7 mm) was recorded in Shan-i-Punjab × Tropic Beauty, which did not differ significantly from the cross Florda Crest × Tropic Beauty. The fruit development period of the seed parent varied from 78.3 days in Shan-i-Punjab × Tropic Beauty and Shan-i-Punjab × Florda Prince to 104.7 days in Tropic Beauty × Florda Grand. Stratification media of cocopeat + vermiculite + perlite (2:1:1) resulted in the highest seed germination but, the actual germination percentage varied with cross combinations. Highest germination (81.5%) was recorded in the cross FlordaGlo × Tropic Sweet, which did not differ significantly from Tropic Beauty × Florda Grand. It was followed by seed germination (68.0%) in Florda Grand × Tropic Beauty. Under high density nursery system, the hybrid seedlings of FlordaGlo × Tropic Sweet showed highest growth (160 cm) and branches (13). The germination of hybrid seeds was positively correlated (0.86) with fruit development period of the seed parent (FDP) and negatively correlated (-0.85) with chilling requirement of seed parent. The proportion of rosseted seedlings was negatively correlated with FDP (-0.61) and positively correlated (0.39) with chilling requirement of the seed parent. The cross combinations with higher FDP of seed parent resulted in higher seed germination of hybrid seed. Hence, controlled climate stratification and germination after harvest can be a viable option for recovery of hybrid seedlings and reducing the breeding cycle in crosses involving seed parents with higher FDP.**

**Key words**: Hybridization, *in vitro* embryo rescue, *Prunus.*

## INTRODUCTION

In India, peach and nectarines are grown in Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim, Tamil Nadu and Mizoram over an area of 18,000 ha with an annual production of 1,07,000 tonnes (NHB, 9). The introduction of low chill, early maturing and better quality peach and nectarine cultivars from USA by Punjab Agricultural University, Ludhiana during 1968 to 2001 played a significant role in the spread of peach cultivation in subtropical regions of India (Singh *et al*., 11). The restriction on exchange of improved varieties due to patent laws and commercial interests of private players of the western countries is hampering the growth of peach industry in India. Local peach and nectarine breeding programme will help in creating a huge lot of locally adaptable genotypes for rigorous selection. Apart from low chilling requirement, better skin colour, flesh colour, firmness, consistency, sugar: acid ratio and storage life are the major breeding targets (Byrne *et al*., 3). The present challenge for peach industry is to increase the fruit consumption, which mainly relies on enhancing taste and nutritional quality (Desnoues *et al*., 5). Of late, higher sugar content is the primary objective of new peach breeding programmes in the western countries to revert the decreasing consumer demand for peach (Cirill *et al*., 4). Besides, low chilling and early ripening trait in peach is an important objective in peach fruit breeding programmes due to higher returns from these varieties (Anderson and Byrne, 1). Crosses involving early ripening parents can lead to higher proportion of progeny with early ripening trait. However, hybrid seeds from these parents germinated poorly due to their immature embryos or embryo abortion when fruits are ripe. Embryo culture technique has been used with great success with early ripening peach and nectarine (Byrne *et al*., 3). Peach embryos rescued at 75 days after hybridization resulted in maximum germination when stratified at 4ºC for 45 days (Sundouri *et al*., 12). Embryo rescue is a valuable tool for the development of early ripening cultivars, however, the use of traditional stratification-germination is preferable wherever possible (Bacon and Byrne, 2). Further,

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in subtropics, sometimes there is high mortality of embryo rescued seedlings as the hardening time coincides with the hot weather. Stratifying the hybrid seeds immediately after harvest during summers under controlled conditions can be viable option for higher recovery of hybrids and shortening the breeding cycle. Hence, the present studies were conducted to study the efficacy of embryo rescue; and summer stratification and germination under controlled conditions for recovery of peach and nectarine hybrid seedlings involving early maturity and low chill seed parents.

## MATERIALS AND METHODS

Controlled pollinations were performed among different peach and nectarine varieties in the Fruit Research Farm of Department of Fruit Science, Punjab Agricultural University, Ludhiana during February, 2014. The treatments consisted of the crosses as described in Table 1 and 2. The anthers from the male parents were collected at balloon stage and dehisced in a silica gel desiccator. The pollen was collected in 10 ml vials and stored at 5°C till use. The flower buds of the female parent were emasculated at balloon stage in the morning (9-11 am) and pollinated with camels hair brush during the day (11.30 am to 2.00 pm) on the same day. The *in vitro* embryo rescue was used to rescue the seedlings and the results are presented in Table 1. In the embryo rescue experiments, the fruits were harvested at firm mature stage and ripe stage when the fruit just start softening from the distal end. The fruits were surfaced sterilized with mercuric chloride (0.1%) for 15 min. The fruit were then cracked open under a laminar flow under aseptic conditions with a double blade secateur (Kuker Art 72). The seed coat was peeled off and the embryo (8-10 mm) was placed on basal MS medium (Murashige and Skoog, 8) in test tubes with 20 ml medium. The test tubes containing cultured embryos were stratified at 3-4°C in dark conditions till >75% radicle emergence and the stratification period for each cross were recorded. After this the test tubes were transferred to a culture room with 25 ± 2°C temperature and 16 hour photoperiod for four weeks. The experiment was laid out as completely randomized design and the data regarding the embryo germination (%), plant height, root length, number of root hairs, and rosseting were recorded after 4 week. The experiment was laid as completely randomized design with three replications.

In view of the higher cost and *ex vitro* mortality; and poor recovery of hybrid seedlings with *in vitro* embryo rescue during the past years, summer stratification and germination under controlled conditions was also tested. In case of summer stratification and germination

**Table 1.** Germination, growth and stratification period of *in vitro* embryos rescued seedlings of low chill peach and nectarine genotypes.

| Parentage | Embryo germination (%) | | Plant height (mm) | Internodal length (mm) | Root length (mm) | Root hairs (No.) | Rosseting (%) | Stratification period (days) | Fruit developmental period (days) | Chilling hours of seed parent (CU) | Seedling growth period (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fruit at ripe stage | Fruit at hard mature stage | | | | | | | | | |
| Florda Grand × Tropic Beauty | 91.67$^{a}$ (9.57) | 89.0$^{a}$ (9.43) | 24.7$^{c}$ | 6.2$^{c}$ | 25.0$^{a}$ | 20.3$^{a}$ | 7.7 | 40.7$^{ab}$ | 96.7$^{b}$ | 100 | 6.0 |
| Shan-i-Punjab × Florda Prince | 85.33$^{c}$ (9.24) | 87.3$^{ab}$ (9.35) | 29.0$^{b}$ | 8.8$^{abc}$ | 15.3$^{c}$ | 11.0$^{c}$ | 6.0 | 36.3$^{b}$ | 78.3$^{e}$ | 300 | 6.0 |
| Shan-i-Punjab × Tropic Beauty | 86.67$^{bc}$ (9.31) | 85.0$^{bc}$ (9.22) | 33.7$^{a}$ | 9.4$^{ab}$ | 27.0$^{a}$ | 20.3$^{a}$ | 6.4 | 40.3$^{ab}$ | 78.3$^{e}$ | 300 | 5.3 |
| Florda Crest × Tropic Beauty | 89.00$^{ab}$ (9.43) | 86.0$^{b}$ (9.11) | 32.3$^{a}$ | 9.9$^{a}$ | 19.7$^{b}$ | 18.7$^{a}$ | 7.3 | 40.3$^{ab}$ | 90.0$^{c}$ | 350 | 5.0 |
| Tropic Beauty × Florda Grand | 87.33$^{bc}$ (9.35) | 83.0$^{c}$ (9.11) | 24.3$^{c}$ | 6.8$^{bc}$ | 20.0$^{b}$ | 17.3$^{ab}$ | 7.3 | 44.7$^{a}$ | 104.7$^{a}$ | 150 | 5.3 |
| Suncoast × Sun Rise | 89.33$^{ab}$ (9.45) | 87.0$^{ab}$ (9.33) | 23.7$^{c}$ | 7.3$^{abc}$ | 18.0$^{bc}$ | 14.7$^{b}$ | 6.0 | 42.7$^{a}$ | 80.7$^{d}$ | 375 | 5.7 |
| $LSD_{0.05}$ | (0.17) | (0.13) | 2.87 | 2.71 | 3.46 | 3.45 | NS | 5.48 | 1.95 | - | NS |

Means within a column followed by similar letter do not differ significantly (p≤0.05).Values in parenthesis are square root transformed values.

**Table 2.** Seed germination, seedling growth and rosseting in hybrid seedlings of peach and nectarine genotypes recovered by summer stratification.

| Parentage | Germination (%) | Height (cm) | Branches (No.) | Rosseting (%) | Fruit developmental period | Chilling units of seed parent (cu) |
|---|---|---|---|---|---|---|
| Sun Coast × Punjab Nectarine | 37.7$^{c}$ (6.13) | 130.0$^{b}$ | 8.7$^{c}$ | 5.6 | 95.7bc | 375 |
| Tropic Beauty × Florda Grand | 80.3$^{a}$ (8.96) | 116.0$^{b}$ | 9.0$^{b}$ | 0.0 | 104.3a | 150 |
| Florda Grand × Tropic Beauty | 68.0$^{b}$ (8.23) | 115.6$^{b}$ | 8.7$^{b}$ | 0.0 | 96.3c | 100 |
| Punjab Nectarine × Suncoast | 43.1$^{c}$ (6.58) | 115.0$^{b}$ | 7.0$^{b}$ | 0.0 | 92.3 | 325 |
| FlordaGlo × Tropic Sweet | 81.5$^{a}$ (9.02) | 160.0$^{a}$ | 13.0$^{a}$ | 7.3 | 103.7a | 150 |
| $LSD_{0.05}$ | (0.52) | 16.41 | 2.37 | NS | 3.81 | - |

Means within a column followed by similar letter do not differ significantly (p≤0.05). Values in parenthesis are square root transformed values.

of seed under controlled conditions the fruits were collected at full maturity during second fortnight of May stored at 5°C up to two days till the seed is extracted. The fruit development period of the seed parent was also recorded from fruit set. The extracted seeds were washed properly and divided into lots of 30 seeds. The seeds were stratified in different media, *viz*. cocopeat + perlite (2:1), cocopeat + vermiculite (2:1), cocopeat + vermiculite + perlite (2:1:1) and sand. The stratification media were sprinkled with carbendazim (Bavistin®) suspension @ 1 g/l to the moist media. The seed lots were stored in the different stratification media at 4±2°C for stratification till 25-30% radicle emergence is seen in the seeds (10 weeks). After stratification the seeds were sown in solarized medium having soil, farm yard manure and cocopeat (2:1:1) in root trainers with 300 cc cells and maintained under a plant growth chamber with 25±2°C day and 21±2°C night temperature. The data regarding the germination percentage and rossetting percentage was recorded 45 days after sowing. The data regarding the hybrid seedling growth and branch number was recorded 11 months after transferring in the hybrid seedlings in field under high density nursery system (HDN) at 0.3 m × 1.0 m. The experiment was laid as completely randomized design with three replications whereas under HDN system the seedlings were planted following randomized block design. The data regarding the FDP was analysed as per randomized block design. The data was subjected to analysis using statistical software SAS (V 9.3 SAS Institute Inc, USA). When the interactions among treatments were significant ($p \leq 0.05$) mean separations were done by least significant difference (LSD).

## RESULTS AND DISCUSSION

In general, higher *in vitro* embryo germination was recorded in embryos rescued from fruits at ripe stage as compared to hard mature maturity stage of fruit (Table 1). The advancement in the development stage might have led to greater embryo maturity, which might have led to better embryo germination with embryos harvested at ripe stage. However, culturing the embryos at full ripe stage resulted in very high microbial contamination as the fruit become fully soft and juicy. Developmental stage for successful embryo culture varies with variety and climatic conditions where the trees are grown. Hence, successful embryo culture at one location may not conform in another place (Scorza and Sherman, 10). Infanter and González (6) concluded that the embryo survival was higher in peach when harvested from the most advanced mature fruits. Further, irrespective of the stage of the embryo germination more than 80 per cent embryo germination was recorded in all the cross combinations. At the end of four weeks of transferring the cultures to culture room, tallest plants (33.7 mm) were recorded in Shan-i-Punjab × Tropic Beauty, which did not differ significantly from plant height in cross Florda Crest × Tropic Beauty. The highest internodal length (9.9 mm) was recorded in Florda Crest × Tropic Beauty, which was at par with Shan-i-Punjab × Tropic Beauty. Similarly, the highest root length (27.0 mm) and root hairs (20.3) was recorded in Shan-i-Punjab × Tropic Beauty. In all the cross combinations, the plant height more than 20 mm, was ideal for handling of the plant material. Rosseting was also observed in the hybrid seedlings (Fig. 2) and it ranged from 6.0 per cent in seedlings from Suncoast × Sun Rise and Shan-i-Punjab × Florda Prince to 7.7 per cent in open-pollinated Florda Grand × Tropic Beauty. Jeengool and Boonprakob (7) studied the effect of plant growth regulators on *in vitro* embryo germination and most embryos germinated well (>85%) with or without plant growth regulators, *viz*. benzyl adenine and gibberellic acid. The stratification period of the hybrid seed varied from 36.3 days in Shan-i-Punjab × Florda Prince to 44.7 days in Tropic

Beauty × Florda Grand. The fruit development period of the seed parent varied from 78.3 days in Shan-i-Punjab × Tropic Beauty and Shan-i-Punjab × Florda Prince to 104.7 days in Tropic Beauty × Florda Grand. The *ex vitro* survival of *in vitro* embryo rescued hybrid seedlings was around 20 per cent due to very high atmospheric temperatures during their transplanting in July. Even under plant growth chamber the high humidity resulted in root rot and high mortality.

In summer stratification and germination experiment under controlled conditions, the seed germination varied with the genotype combination (Table 2). Highest germination (81.5%) was recorded in Florda Glo × Tropic Sweet, which did not differ significantly from seed germination in Tropic Beauty × Florda Grand. It was followed by seed germination (68.0%) in Florda Grand × Tropic Beauty. The lowest germination (37.7%) was recorded in Sun Coast × Punjab Nectarine, which did not differ significantly from seed germination in Punjab Nectarine × Suncoast. Higher seed germination and seedling growth was recorded from crosses in which the seed parent had lower chilling requirement and higher FDP. The highly significant positive correlation (0.86) between FDP and germination per cent indicate that crosses involving seed parent with higher FDP resulted in higher seed germination. Further, seed germination also showed a negative correlation with chilling period. Seed viability was poor in early ripening peach genotypes and *in vitro* embryo culture is needed to ensure germination (Byrne *et al.*, 3). In the present studies, higher germination was obtained in the hybrid seed from crosses involving. The seedlings from Florda Glo × Tropic Sweet showed highest plant height (160.0 cm) and number of branches (13) after 11 months of transplanting under high density nursery system at 0.3 × 1.0 m (Table 2), whereas, the seedling height in all the other cross combinations did not differ significantly. The stratification media significantly affected the hybrid seed germination in different cross combinations. Irrespective of the parentage, stratification medium cocopeat + vermiculite + perlite (2:1:1) resulted in highest seed germination during stratification, while, minimum seed germination was recorded with sand (Fig. 1). Highest water holding capacity (Fig. 1), better aeration and other physical properties of the media might have affected the seed germination. The correlation studies showed high positive correlation (0.86) between hybrid seed germination and fruit development period of the seed parent, which was also statistically significant (Table 3). The hybrid seed germination and chilling requirement of seed parent were negatively correlated (-0.85). Fruit developmental period of the seed parent was also negatively correlated (-0.61) with the proportion of rosseted seedlings. Hence, seed stratification and germination under controlled conditions after fruit harvest during summers is a viable option for higher recovery of hybrid seedlings over embryo rescue in low chilling peach and nectarine genotypes with longer developmental period. This can save around 7-8 months of a breeding cycle in a year.

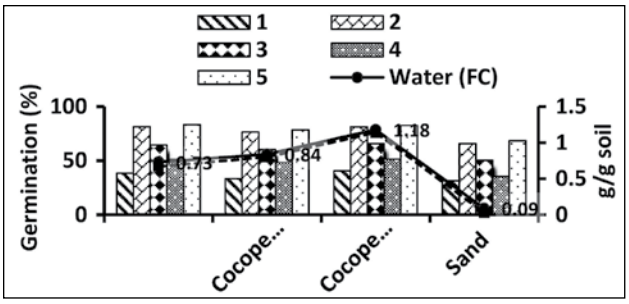


**Fig. 1.** Effect of stratification media on germination (%) of hybrid seeds and water holding capacity at field capacity (FC) and permanent wilting point (PWP). 1: Sun coast × Punjab Nectarine; 2: Tropic Beauty × Florda Grand; 3: Florda Grand × Tropic Beauty; 4: Punjab Nectarine × Suncoast; 5: FlordaGlo × Tropic Sweet.

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**Table 3.** Correlation between various variables.

| Parameter | Fruit developmental period | Chilling unit of seed parent | Rosseting |
|---|---|---|---|
| Seed germination | 0.86** | -0.85** | -0.19 |
| Fruit developmental period | 1.00 | -0.69 | -0.61** |
| Chilling of seed parent | -0.69 | 1.00 | 0.39* |

*Significant P≤0.05; **Significant P≤0.01

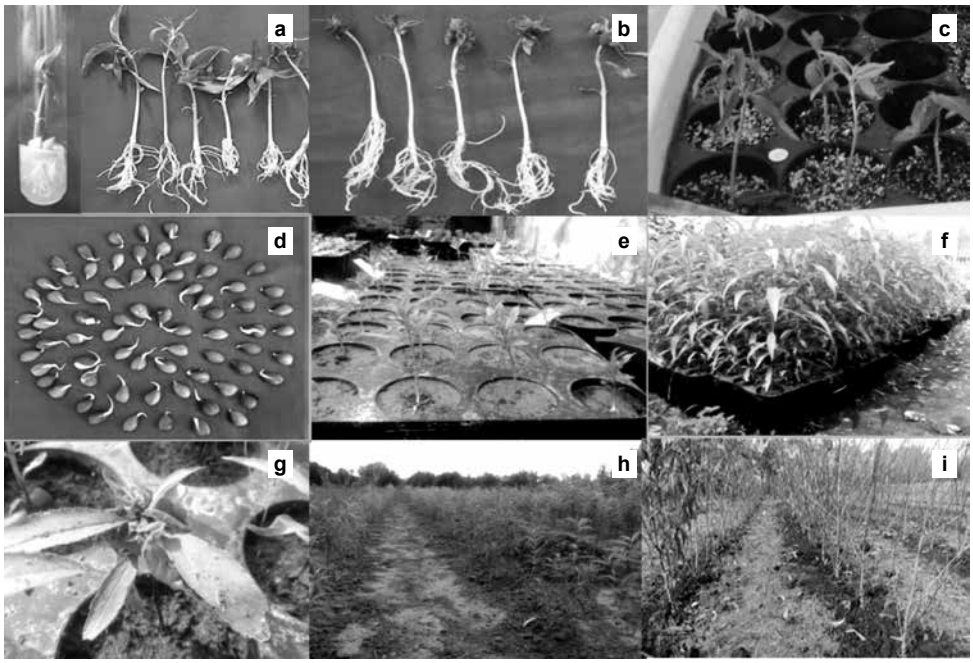


**Fig. 2.** Different stages in the development hybrid seedlings (A) *In vitro* embryo rescued seedlings, (B) *In vitro* rosseting, (C) Hardening *in vitro* seedlings, (D) Summer stratified seeds of Flordaglo × Tropic Sweet in cocopeat + vermiculite + pertile (2:1:1). (E, F) Seedlings of Flordaglo × Tropic Sweet at 30 and 90 DAS (G). *Ex vitro* rosseting. (H,I) HDN at planting (7-month-old seedlings) and after 17 months (1st winter).


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# Evaluation of different blueberry genotypes under mid-hill conditions of Himachal Pradesh

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## ABSTRACT

**Southern highbush blueberry genotypes, namely, Gulf Coast, Jewel, Misty, Sharpblue and two rabbiteye genotypes, *viz.*, Alapaha and Austin were evaluated for growth, flowering and fruiting behavior under mid hill conditions of Himachal Pradesh during 2014-16. The variability among genotypes was observed for their growth habit, foliar characteristics, flowering and fruit quality attributes. Genotype, Misty exhibited single stem trunk with spreading and upright growth habit, whereas, all other genotypes including rabbiteye exhibited multi-stem trunk. However, southern highbush genotype Jewel had more spreading growth habit with vigorous cane having wide crotch angle as compared with other genotypes. In Alapaha, the bush structure was more compact with upright branching having thin and week cane growth. The genotypes also exhibited great variation in leaf shape, colour and size. The variation in flowering and fruiting behavior was also observed among them, Gulf Coast and Misty were earliest to bloom, while Austin was last. Similarly, Gulf Coast and Misty were the earliest in berry maturity. First picking date in Gulf Coast and Misty was on April 25 and harvesting was continued upto first week of May, while Austin was last (last week of May to first week of June). There was also a great variation in the physico-chemical properties of berries among cultivars. The pooled data of three years showed maximum (1.83 g) berry weight in Jewel whereas; Austin produced smallest berries (0.98 g). Similarly, the total soluble solids and titratable acid contents also varied significantly. All genotypes including rabbiteye type had TSS content more than 10% which has been reported as minimum quality index in blueberries. Based on this study it can be concluded that all southern highbush blueberry genotypes as well as two rabiteye genotypes were found promisisg and thus can be grown in northern parts of India where soil is acidic and winter is cool enough to meet-out the chilling requirements.**

**Key words:** Blueberry, performance evaluation, mid hill conditions.

## INTRODUCTION

Blueberries (*Vaccinium* spp.) are native to the north-eastern United States, now becoming important commercial fruit worldwide (Jimenez *et al*., 8). Most of the cultivated highbush blueberries are northern highbush (*V. corrymbosum*), which are being grown in cooler regions of the world, however, with the development of low chilling new southern cultivars its production to the southern and warmer areas has increased. The southern highbush blueberries are hybrids and have greater heat tolerance with lower winter chilling requirement than northern highbush blueberries. Similarly, rabbiteye blueberries (*V. virgatum* syn. *V. ashei* Reade) are native to the southeastern United States were developed in regions with long, hot summers, and they behaved differently in the Pacific Northwest than in their home environments (Bernadine *et al.*, 2).

Nutritionally, blueberries are good source of carbohydrates, vitamins, anthocyanins and several minerals, besides they also contain high amount of iron. Blueberries also provide fair amounts of bioactive compounds with high antioxidant activities, such as flavonoids (flavonols, anthocyanins and others) and phenolic acids (Schotsmans *et al*., 12).

In India, its commercial cultivation has not been reported yet but could be a potential future crop for diversification and nutritional security (Jayant, 7; Negi *et al*., 10). In Himachal Pradesh, highbush and rabbiteye genotypes, *viz*., Jewel, Misty, Bluecrop, Gulfcoast, Primadonna, Sharpblue, Duke, Alapaha and Springwide were introduced at Palampur in 2006-07. Out of these, only Jewel, Misty, Gulf Coast, Sharpblue and Alapaha survived and adapted. Similarly, two rabbiteye genotypes, *viz*., Austin and Brightblue were transferred from National Plant Genetic Resource, Shimla, India to Palampur and were planted at the same location but only Austin survived and acclimatized well to prevailing conditions and now bearing fruits. Thus, an attempt was made to see the possibilities of blueberry production in Himachal Pradesh, where soil is slightly acidic and winter is cool enough to meet the chilling requirements for southern highbush and rabbiteye blueberries.

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## MATERIALS AND METHODS

The present study was carried out CSKHPKV, Palampur, during the year 2014-16 on four southern highbush blueberry genotypes, *viz*., Jewel, Misty, Gulf Coast, and Sharpblue along with two rabbiteye types Austin and Alapaha. The experimental field is situated at 1,240 m above mean sea level and 32°8' E latitude and 76°3' N longitude. The area represents sub-temperate sub-humid mid hill of the state, soil is clay loam with pH 6.0 and the climate is humid, temperature sometimes reaches up to 35°C during summer, winters are relatively cold with occasional snowfall and heavy rains during three months (June to August). The plants were planted on raised beds at 1.5 m × 1.5 m distance, to avoid waterlogging during rainy season. The plants were given only FYM and the basin area of each plant was incorporated with pine needles and shredded barks but the data on lowering of pH by pine needles and barks was not recorded.

Plant characteristics, *viz*., growth habit, branching density, branch angles, colour of juvenile growth, matured cane colour and defoliation pattern were observed visually and from each cultivars foliar characteristics, *viz*., leaf sprouting/ emergence date, leaf shape, colour of leaf blade, lamina and foliage pattern were recorded. Leaf size and area, leaf fresh and dry weight was determined. Fruit quality attributes (weight, size, TSS, acidity and TSS: acid ratio) were recorded as per standard methods for three years. The quality parameters were estimated following standard procedures (AOAC, 1). The experiment was laid on randomized block design with three replications and each replication constituted one plant. The data on fruit quality attributes were recorded for three years and pooled. The whole data thus obtained was analyzed by using DOS based statistical software Assex at 5% level of significance.

## RESULTS AND DISCUSSION

The data on vegetative growth and plant characters, *viz*., growth habit, branching density and pattern, juvenile growth cane colour, colour of mature cane and defoliation during winter were recorded and presented in Table 1. It is clear from the data that all genotypes irrespective of species had multi-stem, upright and spreading growth habit except Misty, had single stem with weak and lanky growth. However, growth was more spreading with canes having wide crotch angles in Jewel as compared to other cultivars. Alapaha produced thin new canes, more upright with small internodal length and Sharpblue a southern highbush blueberry exhibited less spreading with upright growth, however overall growth of new cane was more as compared to Alapaha and Austin (data not presented).

Branching density in rabbiteye cultivar Alapaha was more among other genotypes, followed by Jewel and sparsely dense in Misty (data not presented). The growth of new cane was stout and vigorous in Jewel and in Alapaha it was observed weak and thin. The crotch angle also varied among the genotypes, it was observed wide and spreading type in Jewel, Gulf Coast and narrow in genotypes Misty and Sharpblue. The colour of juvenile growth was observed light green to silvery green in different genotypes. Similarly, genotypes also exhibited varying cane/shoot colour at maturity and it was observed green in genotypes; Misty, Gulf Coast, Sharpblue and pinkish-grey in Alapaha. In Austin, the mature cane was slightly grayish and in Jewel it was green with slight pink ting on them. Tree morphology is a major characteristic

**Table 1.** Plant characteristics of southern highbush and rabbiteye blueberry genotypes.

| Genotype / Trait | Jewel | Misty | Gulf Coast | Sharpblue | Alapaha* | Austin* |
|---|---|---|---|---|---|---|
| Growth habit | Multistem and spreading | Single stem and Upright | Multistem and upright | Multistem and upright | Multistem and upright | Multistem and upright |
| Density of branches | Dense and stout | Sparse and weak branches | Dense | Dense | Highly dense thin canes | Sparse |
| Branch angle | Wide | Narrow | Wide | Narrow | Medium | Medium |
| Branching pattern | Spreading | Spreading and lanky growth | Spreading | Upright spreading | Spreading with small internodes | Upright and lanky growth |
| Juvenile growth | Light green | Pinkish green | Light green | Dark green | Pinkish green | Silvery green |
| Cane colour | Green with pink ting | Green | Green | Green | Pinkish | Grayish green |
| Defoliation during dormancy | Partially | Partially | Partially | Partially | Fully | Fully |

*Rabbiteye blueberry

feature of any genotype, which is governed by genetic makeup and prevailing climatic factors of that region. There was complete defoliation in both types of rabbiteye blueberry genotypes in dormancy, whereas, in all southern highbush defoliation varied from 60 to 90 per cent (data not presented).

After dormancy, leaf bud unfolding is a major event that determines the adaptability of particular genotypes to that particular region. In this study, it was observed that, all four southern highbush genotypes along with Alapaha (rabbiteye) started leaf bud bursting/unfolding in the month of February (Table 2a). Misty was earliest amongst the genotypes (February 7), while in Austin this event was late and leaf emergence was observed in second week of March. Most of the southern highbush blueberry genotypes required less chilling hours as compared to northern genotypes (Francis *et al.*, 5). The leaf lamina/ blade shape was simple and unifoliate in all genotypes but there was variation in leaf colour among different genotypes (Table 2a). The matured leaf lamina colour was dark green in Sharpblue, green in Jewel, light green in Misty and Gulf Coast, whereas, in Alapaha it was slight pinkish and in Austin silvery green. Similarly, variation was also observed in leaf lamina shape, which varied from elliptic to obovate elliptic. It was observed ovate elliptical in Jewel, obovate in Austin and rest of the genotypes (Misty, Gulf Coast, Sharpblue and Alapaha) had elliptical leaf shape. Foliage pattern also varied among the genotypes (Table 2a) and it was observed highly dense in rabbiteye genotypes Alapaha followed by highbush blueberry genotypes; Sharpblue, Jewel and Gulf Coast, however, foliage was sparse in Austin and Misty. The other quantitative foliar characteristics like; leaf lamina length, width, area and weight (fresh and dry) of the different blueberry genotypes also varied significantly (Table 2b). The maximum leaf lamina length and width (8.43 and 3.81 cm) was observed in rabbiteye genotypes Austin and minimum was recorded in Alapaha (3.74 and 1.80 cm). Similarly, leaf area and weight were also significantly higher in Austin as compared to other genotypes except Jewel in which the fresh and dry weights were maximum (0.40 and 0.20 g/ leaf) as compared to other genotypes.

All the southern highbush blueberry genotypes initiated opening of flowers in the month of January (Table 3), whereas, in both rabbiteye genotypes this event was late. Accordingly, the full bloom stage was observed earliest in Gulf Coast on February 15 followed by Misty (February 16), Jewel (February 20) and Sharpblue (February 22). However, in both rabbiteye genotypes this stage was observed late,

**Table 2a.** Leaf characteristics of southern highbush and rabbiteye blueberry genotypes.

| Genotype / Trait | Jewel | Misty | Gulf Coast | Sharpblue | Alapaha* | Austin* |
|---|---|---|---|---|---|---|
| Leaf shape | Simple | Simple | Simple | Simple | Simple | Simple |
| Leaf colour (blade) | Dark green | Light green | Light green | Medium green | Pinkish green | Silvery green |
| Leaf lamina shape | Ovate-elliptical | Elliptic | Elliptic | Elliptic | Elliptic | Obovate-elliptic |
| Leaf lamina margin | Entire | Entire | Entire | Entire | Entire | Entire |
| Leaf emergence | 10 February | 7 February | 10 February | 25 February | 2 March | 15 March |
| Foliage pattern | Dense | Sparse | Dense | Slightly dense | Highly dense | Sparse |

*Rabbiteye blueberry

**Table 2b.** Leaf characteristics of southern highbush and rabbiteye blueberry genotypes.

| Trait / Genotype | Leaf lamina length (cm) | Leaf lamina width (cm) | Leaf area ($cm^2$) | Leaf weight (g/leaf) | |
|---|---|---|---|---|---|
| | | | | Leaf fresh weight | Leaf Dry weight |
| Jewel | 6.03 | 3.60 | 17.75 | 0.41 | 0.20 |
| Misty | 5.32 | 2.83 | 16.94 | 0.37 | 0.14 |
| Gulf Coast | 5.26 | 2.99 | 15.36 | 0.25 | 0.12 |
| Sharpblue | 7.07 | 3.51 | 17.82 | 0.34 | 0.18 |
| Alapaha* | 3.74 | 1.80 | 8.76 | 0.15 | 0.08 |
| Austin* | 8.43 | 3.81 | 18.22 | 0.39 | 0.19 |
| CD (P=0.05) | 0.17 | 0.21 | 1.48 | 0.03 | 0.02 |

*Rabbiteye blueberry

**Table 3.** Flowering behavior of different blueberry genotypes.

| Trait \ Genotype | Jewel | Misty | Gulf Coast | Sharpblue | Alapaha* | Austin* |
|---|---|---|---|---|---|---|
| Start date of flowering | 21 January | 18 January | 16 January | 26 January | 20 February | 20 March |
| End date of flowering | 9 March | 2 March | 1 March | 11 March | 11 April | 8 May |
| Full bloom period | 20 February | 16 February | 15 February | 22 February | 10 March | 22 April |
| Bloom period (days) | 47 | 43 | 44 | 44 | 50 | 48 |
| Colour of opened flower | White | White | White | White | Pink | Slightly pinkish |

*Rabbiteye blueberry

in Alapaha full bloom stage was observed in March 10 and in Austin it was observed April 22. There was a less variation in respect of colour of opened flower or corolla. It was observed slight pinkish in Alapaha and pink in Austin and all the southern highbush types had white coloured flowers. Flowering is also another important event in any plants for determining the outcome and economy of those plants. Flower bud differentiation and flowering are governed by various factors, and in this study all genotypes under observation successfully adapted the prevailing climate and soil conditions and thus established well under sub-temperate sub-humid mid hills of Himachal Pradesh, India. However, in a study by Timothy *et al.* (13) reported that the process of flowering in *Vaccinium darrowi* and southern highbush blueberry was photo-periodically sensitive and promoted by short days, while flower bud development was enhanced under long days. The vegetative and reproductive development in both *V. darrowi* and *V. corymbossum* hybrids was profoundly influenced by photoperiod and increasing vegetative growth was positively correlated with increasing photoperiod in several species, including northern highbush and lowbush blueberry (Hall and Ludwig, 6). However, the role of photoperiod in flower bud initiation in southern highbush blueberry genotypes is unknown and observations of southern types on the Corindi Plateau of New South Wales, Australia indicated that flower bud initiation occur year round (Wright, 14).

In all four southern highbush blueberry genotypes, harvesting commenced from the last week of April and earliest amongst them were Misty and Gulf Coast (April 25 each) in the year 2015-16, followed by Jewel (Fig. 1, 2, 3). Among rabbiteye genotypes, Alapaha was early to mature as compared to Austin. The former genotype attained 25% maturity on May 21 in the year 2015 and May 18 in 2016. Overall harvesting duration, *i.e*. date of first harvesting to last picking date for all genotypes was 42 and 46 days in the year 2015-16, respectively (Fig. 1 & 2). In a study by NeSmith (11) concluded that rabbiteye cultivars Alapaha and Climax took 82 days from 50 percent bloom to attain 50% ripening stage at 10 sites in USA. However, Austin a rabbiteye blueberry reached 50% anthesis 10 days later than rabbiteye cultivar Climax in Alapaha region of Southern Regional Blueberry Evaluation trials (Georgia), therefore, it reached 50% ripe stage slightly later than Climax at

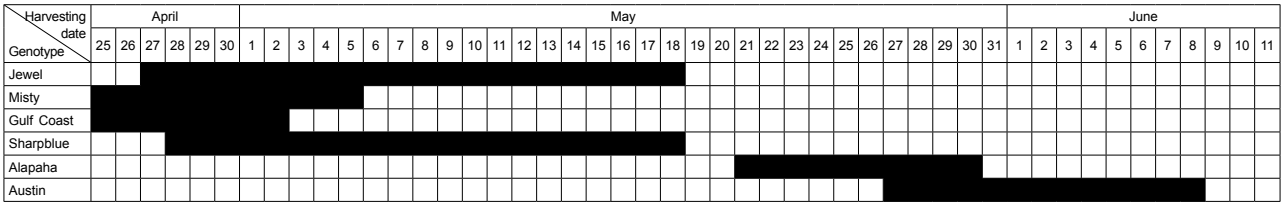


**Fig. 1.** Harvesting duration of southern highbush and rabbiteye blueberry genotypes (2015).

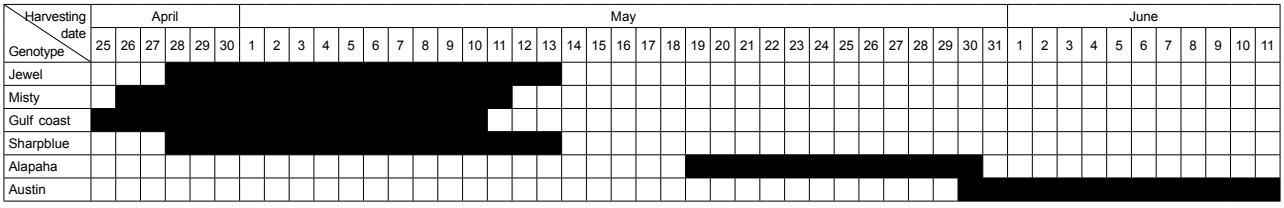


**Fig. 2.** Harvesting duration of southern highbush and rabbiteye blueberry genotypes (2016).


**Fig. 3.** Different blueberry genotypes. a; Jewel, b; Sharpblue, c; Misty, d; Gulf Coast (Southern high bush blueberry) and e; Austin; d; Alapaha (rabbiteye blueberry).

two other stations and slightly earlier at one location.

The various berry quality attributes like; length, breadth, weight, TSS, acidity and TSS/acid ratio with respect to genotypes were found significantly different (Tables 4 & 5). It is clear from the pooled data for three years (2014, 2015 and 2016) presented in Table 4 that fruit length was recorded maximum in Misty (1.49 cm), breadth and weight was observed maximum in Jewel (1.84 cm and 1.83 g, respectively). Whereas, minimum of all these quality attributes were observed in Alapaha, *i.e.* 1.15 cm, 1.40 cm and 0.99 g, respectively. The data on total soluble solids, acidity and TSS/acid ratio for three years were pooled and is presented in Table 5. It is clear from the table that maximum soluble solids content was recorded in Misty, followed by Gulf Coast and minimum (10.44%) in Alapaha. Similarly, the genotypes also showed significant variation in titratable acidity and maximum (0.83%) was recorded in Austin and lowest in Gulf Coast, *i.e.* 0.57%, along with Jewel and Alapaha having 0.67% each. Total soluble solids to acid ratio also varied significantly among the genotypes (Table 5) and was observed maximum (20.21) in Gulf Coast and minimum in Austin. All the genotypes had soluble solids concentration higher than 10%, which has been proposed as a minimum quality index for blueberries (Kazim *et al*., 9).

The predominant organic acid in most of the highbush blueberry cultivars was citric acid (83%), while other organic acids such as succinic, malic and quinic acids were approximately 11, 2 and 5 per cent, respectively, however, in rabbiteye blueberries (*V. ashei*) the predominant organic acids were succinic and malic acid with 50 and 34%, respectively (Ehlenfeldt *et al*., 4). During the three year period, all the genotypes yielded soluble solids concentration higher than 10 per cent. Similarly, soluble solids to titratable acid ratio ranged from 12.81 to 20.21, Gulf Coast had maximum value due to its low acid content.

Based on the above findings it can be concluded that all the four southern highbush as well as both

**Table 4.** Berry quality of southern highbush and rabbiteye blueberry genotypes.

| Trait / Genotype | Length (cm) | | | | Breadth (cm) | | | | Weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2016 | Pooled | 2014 | 2015 | 2016 | Pooled | 2014 | 2015 | 2016 | Pooled |
| Jewel | 1.40 | 1.36 | 1.41 | 1.39 | 1.78 | 1.88 | 1.86 | 1.84 | 1.65 | 1.98 | 1.86 | 1.83 |
| Misty | 1.37 | 1.42 | 1.44 | 1.41 | 1.63 | 1.57 | 1.64 | 1.61 | 1.41 | 1.64 | 1.62 | 1.56 |
| Gulf Coast | 1.22 | 1.19 | 1.18 | 1.20 | 1.58 | 1.57 | 1.58 | 1.58 | 1.44 | 1.63 | 1.66 | 1.58 |
| Sharpblue | 1.31 | 1.23 | 1.24 | 1.26 | 1.67 | 1.72 | 1.71 | 1.70 | 1.52 | 1.72 | 1.77 | 1.67 |
| Alapaha* | 1.18 | 1.19 | 1.21 | 1.19 | 1.68 | 1.62 | 1.74 | 1.68 | 1.53 | 1.12 | 1.42 | 1.46 |
| Austin* | 1.17 | 1.14 | 1.13 | 1.15 | 1.32 | 1.35 | 1.40 | 1.36 | 0.89 | 1.06 | 1.01 | 0.99 |
| $CD_{0.05}$ | 0.04 | 0.04 | 0.05 | 0.02 | 0.03 | 0.10 | 0.07 | 0.04 | 0.11 | 0.06 | 0.06 | 0.06 |

*Rabbiteye blueberry

**Table 5.** Berry quality of southern highbush and rabbiteye blueberry genotypes.

| Trait / Genotype | TSS (%) | | | | Titratable acidity (%) | | | | TSS/acid ratio | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2016 | Pooled | 2014 | 2015 | 2016 | Pooled | 2014 | 2015 | 2016 | Pooled |
| Jewel | 10.27 | 11.23 | 12.30 | 11.27 | 0.70 | 0.69 | 0.61 | 0.67 | 14.56 | 16.20 | 20.16 | 16.97 |
| Misty | 11.42 | 12.13 | 13.51 | 12.36 | 0.79 | 0.77 | 0.66 | 0.74 | 14.73 | 15.81 | 20.48 | 16.89 |
| Gulf Coast | 10.07 | 10.23 | 14.20 | 11.50 | 0.57 | 0.58 | 0.57 | 0.57 | 17.64 | 17.93 | 25.07 | 20.21 |
| Sharpblue | 10.05 | 10.01 | 14.12 | 11.39 | 0.70 | 0.71 | 0.60 | 0.65 | 14.23 | 14.17 | 23.48 | 17.12 |
| Alapaha* | 10.98 | 10.38 | 11.97 | 11.11 | 0.67 | 0.69 | 0.66 | 0.67 | 16.36 | 15.02 | 18.01 | 16.58 |
| Austin* | 10.16 | 10.09 | 11.13 | 10.46 | 0.88 | 0.89 | 0.71 | 0.82 | 11.52 | 11.30 | 15.61 | 12.75 |
| $CD_{0.05}$ | 0.314 | 0.35 | 1.20 | 0.45 | 0.069 | 0.072 | 0.064 | 0.050 | 1.92 | 2.12 | 3.61 | 2.22 |

*Rabbiteye blueberry

rabbiteye blueberry genotypes have shown good response to the prevailing agro-climatic conditions and hence, these genotypes can be grown in India.

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# Evaluation of apple cultivars under sub-temperate mid hill conditions of Himachal Pradesh

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## ABSTRACT

**The evaluation of 15 apple cultivars was carried out at Progeny cum Demonstration Orchard, Kwagdhar, district Sirmaur, under sub-temperate region of Himachal Pradesh during 2010 and 2011. The maximum tree height (3.29 cm), tree spread (1.82 m) were recorded in 'Fuji Kiku', whereas, tree volume (8.34 $m^3$), scion girth (30.25 cm), annual shoot growth (39.97 cm) and leaf area (38.77 $cm^2$) in 'Gala Mischala'. The highest fruit set, yield and productivity were recorded (69.07%, 9.68 kg/tree and 24.21 t/ha, respectively) in 'Oregon Spur-II'. The bigger size fruits in terms of length (59.67 cm), breadth (67.12 cm), and ascorbic acid content (56.37 mg/100 g) were found in 'Red Chief', while, maximum fruit weight (155.6 g) was observed in Silver Spur. The highest TSS (15.95%) was recorded in 'Gale Gala', whereas, more acidic (0.90%) fruits were found in 'Breaburn'. The maximum total sugars (11.94%) were found in 'Sansa' and highest reducing sugars (6.56%) were found in Oregon Spur II. The best fruit shape and surface colour was observed in 'Super Chief" and 'Camspur'. The longer duration of flowering (16 days) were recorded in cultivar Sun Fuji and Silver Spur. The cultivar 'Super Chief' registered maximum spur density (10.80/ $cm^2$), and lowest fruit drop (18.09%), whereas, 'Fuji Kiku' and 'Gale Gala' recorded the least spur density (2.56 and 3.19/ $cm^2$, respectively). The cultivar Pink Lady was earliest to flower whereas; 'Red Fuji' was last to harvest. From the present investigations it may be concluded that 'Red Chief', 'Super Chief', 'Oregon Spur II' and 'Camspur' in Delicious group, 'Gala Mischala' in Gala group, and 'Auvil Early Fuji' and 'Sun Fuji' in Fuji group have good yield potential, earliness and better fruit quality within their respective groups and may be recommended for cultivation in sub-temperate conditions of Himachal Pradesh.**

**Key words:** Apple cultivars, fruit quality, performance, vegetative growth.

## INTRODUCTION

Apple (*Malus domestica* Borkh.) has become number one commercial fruit crop in Himachal Pradesh, and is grown over an area of 1,01,485 ha with annual production of 8,92,112 MT (Anon, 2). The most widely grown commercial apple cultivars belong to Delicious group, which constitutes 90% of the apple plantations in H.P. (Jindal and Mankotia, 7). The area under apple cultivation in sub-temperate region is shrinking and the situation is likely to worsen further in times to come, because of non completion of chilling requirement, monoculture of non-spur Delicious group of varieties, erratic rainfall and prolonged drought period during critical stages of fruit growth and development.

The standard Delicious apple cultivars have comparatively higher chilling requirements, tendency towards irregular bearing, high sensitivity to temperature fluctuations, particularly during flowering, late maturity and comparatively low yield potential. It is evident from the past records that the apple growing pockets of low valley areas of state, *viz*., Rajgarh in Sirmour, Kumarsain in Shimla, parts of Sunder Nagar areas in Mandi and some parts of Kullu districts had huge plantations of apple, which contributed significantly towards apple production upto early 90's. With changing climatic conditions apple has shown declining trend in productivity in these areas. Besides quantitative loss, the quality of fruit being produced in these areas has also degraded and orchardists have to resort to chemical sprays for colour development, which in turn affect the tree physiology adversely. It is emphasized upon that the sub-temperate region/ areas of the state shall regain its status of contributing sizable apple produce if new cultivars with low chilling requirements, high spur density and profuse bearing potential are screened for such areas. Therefore, the present study was carried out to evaluate the performance of some new apple cultivars with respect to growth, flowering, fruiting, quality and productivity for their suitability for commercial cultivation under changing climate scenario.

## MATERIALS AND METHODS

The experiment was conducted during 2010 and 2011 on 7-8 year-old apple plants in the Progeny cum Demonstration Orchard (PCDO), Kwagdhar, District Sirmaur, under sub-temperate region of Himachal Pradesh. The orchard is situated at 1700 m amsl. The

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uniform plants of 15 apple cultivars, *viz*. Auvil Early Fuji, Braeburn, Camspur, Fuji Kiku, Gala Mischala, Gale Gala, Golden Smoothy, Oregon Spur-II, Pink Lady, Red Chief, Red Fuji, Sansa, Silver Spur, Sun Fuji and Super Chief planted during March 2004 at a spacing of 2 m × 2 m. The data on vegetative characters like scion girth (cm), annual shoot growth (cm) and tree volume were recorded in the month of December. The leaf area ($cm^2$) was recorded in the month of September with leaf area meter. The dates of bud swell, green tip, pink bud, initiation of flowering and date of petal fall were recorded visually by observing peculiar stage of respective parameter. The duration from date of initiation of flowering to the date of start of petal fall was calculated as duration of flowering. The full bloom period in each cultivar was noted when more than 75-80 per cent of the flowers had opened. The fruit set, fruit drop and spur density were recorded as per standard methods. Time of fruit harvest was recorded on the basis of total days after full bloom stage to harvest maturity for each replication of each cultivar. Mature fruits were harvested and the weight was recorded with the help of single pan electronic balance. Further, fruit yield per hectare was calculated by multiplying the fruit yield per plant to the number of plants per hectare. Physical characteristics of fruits like fruit length (mm), fruit diameter (mm), fruit shape, and fruit weight (g) were estimated as per standard methods (AOAC, 1). Fruit firmness was measured with the help of a fruit pressure tester (Magness-Taylor). Colour of fruits was observed visually after harvesting compared with colour chart of Royal Horticultural Society, London and assessed accordingly. Total soluble solid (TSS) was determined by Erma hand-held refractometer (0-32°B). The acidity (%), reducing, non-reducing and total sugars were estimated as per standard methods (AOAC, 1), while ascorbic acid was calculated as per procedure given by Ranganna (10). Data was analysed according to randomized block design.

## RESULTS AND DISCUSSION

Among all the cultivars under study, 'Gala Mischala' recorded maximum scion girth with highest pooled mean (30.25 cm). It also gave highest mean tree spread (2.22 m) and tree volume (8.34 $m^3$), whereas minimum tree vigour was observed in case of 'Super Chief' during both the years. The differences in the tree vigour may be attributed to the varietal characteristics and genetic makeup of the scion cultivar. The improved tree vigour of 'Gala Mischala' may be due to lower spur density, less fruit set and more fruit drop as evident from the (Table 1); as most of the metabolites were utilized for growth and *vice-versa* in case of 'Super Chief'. Maximum plant height (3.29 m), annual shoot growth (39.97 cm) and leaf area (38.77 $cm^2$) was recorded for 'Fuji Kiku'. The minimum tree height (1.65 m) and annual shoot growth (14.13 $cm^2$) were observed in 'Super Chief', which was probably due to high

**Table 1.** Performance of different apple cultivars with respect to plant growth characteristics (pooled mean).

| Cultivar | Scion girth (cm) | Tree height (m) | Tree spread (m) | Tree volume ($m^3$) | Annual shoot growth (cm) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|
| Auvil Early Fuji | 15.70 | 2.33 | 1.54 | 2.93 | 29.17 | 24.92 |
| Fuji Kiku | 28.67 | 3.29 | 1.82 | 5.74 | 39.97 | 38.77 |
| Red Fuji | 20.65 | 3.04 | 1.67 | 4.47 | 26.23 | 25.32 |
| Sun Fuji | 16.75 | 2.13 | 1.38 | 2.13 | 29.27 | 21.93 |
| Camspur | 20.55 | 2.93 | 1.27 | 2.47 | 18.95 | 26.37 |
| Oregon Spur II | 10.25 | 1.88 | 1.25 | 1.54 | 15.88 | 30.53 |
| Red Chief | 18.02 | 1.76 | 1.16 | 1.29 | 14.93 | 29.85 |
| Silver Spur | 20.23 | 2.66 | 1.23 | 2.17 | 15.46 | 32.37 |
| Super Chief | 14.95 | 1.65 | 1.05 | 0.76 | 14.13 | 32.66 |
| Golden Smoothy | 13.93 | 2.11 | 1.45 | 2.35 | 30.93 | 25.87 |
| Gala Mischala | 30.25 | 3.23 | 2.22 | 8.34 | 30.07 | 32.35 |
| Gale Gala | 14.20 | 2.23 | 1.29 | 1.95 | 25.23 | 28.43 |
| Sansa | 14.25 | 1.95 | 1.34 | 1.93 | 24.42 | 26.40 |
| Braeburn | 18.95 | 2.33 | 1.30 | 2.12 | 25.53 | 21.72 |
| Pink Lady | 12.75 | 1.91 | 1.26 | 1.60 | 18.46 | 27.98 |
| $CD_{0.05}$ | 2.40 | 0.59 | 0.18 | 0.99 | 2.27 | 2.21 |

productivity of this genotypes and *vice-versa* in case of 'Fuji Kiku'. Minimum leaf area (21.72 $cm^2$) was recorded in 'Breaburn' which was on par with 'Sun Fuji' (21.93 $cm^2$). Similar variations for various plant vigour characteristics have also been reported by Crassweller *et al.* (5) in a range of geographical and climatic areas within North-America (tree height); Bhat *et al.* (4) under high-hill conditions of Jammu and Kashmir (tree spread and scion girth); Sharma *et al.* (11) under mid-hill conditions of Himachal Pradesh and Wazbinska *et al.* (21) under temperate conditions of Poland (scion girth).

The 'Pink Lady' was found earliest among all the cultivars for many flowering (Table 2) and fruit set characteristics, *viz.*, date of bud swell (5th March ), date of green tip stage (9th March), date of pink bud stage (12th March), date of anthesis (15th March), date of full bloom (21st March) and date of petal fall (27th March). Moreover, 'Auvil Early Fuji', 'Braeburn', 'Sansa', 'Silver Spur' and 'Sun Fuji' were also found early in flowering and fruit set characteristics, whereas the cultivars, 'Gale Gala', 'Golden Smoothy', 'Oregon Spur II' and Red Fuji' were found late for most of the above flowering characteristics. The marked differences in time and duration of flowering in different cultivars may be attributed to inherent genetic characteristics of the cultivars (Sharma *et al*., 11; Biswajit *et al.*, 4). However, winter precipitation, temperature and ultimate accumulation of chilling hours are main factors for such drift in flowering (Jindal and Mankotia, 7).

The duration of flowering in different cultivars lasted for nearly two weeks under sub-temperate conditions of Kwagdhar, district Sirmaur, H.P. The maximum duration of flowering (16 days) was recorded in 'Sun Fuji' and 'Silver Spur', while minimum (12 days) in 'Pink Lady'. On the basis of duration of flowering, 'Sun Fuji', 'Auvil Early Fuji', 'Golden Smoothy' had longer blooming period, whereas, 'Pink Lady', 'Silver Spur', 'Red Chief', 'Gale Gala', 'Gala Mischala' and 'Fuji Kiku' had shorter duration of flowering ranging between 12-14 days. This may also be attributed to the varietal characteristics and prevailing climatic conditions at the time of flowering. These results are in line with Singh *et al.* (13) pink bud stage and time and duration of flowering; Sharma *et al.* (11) for duration of flowering; Biswajit *et al.* (4) for date of flowering and Mataa (9) for date of bud break.

The days from the full bloom to harvest was observed minimum (110.8 days) in 'Sansa' resulting into early fruit maturity, whereas the maximum period (186.5 days) from full bloom to harvesting were taken by 'Red Fuji' ensuing to the late crop maturity. On the

**Table 2.** Performance of different apple cultivars with respect to flowering traits.

| Cultivar | Date of bud swell | Date of green tip | Date of pink bud | Date of anthesis | Date of full bloom | Date of petal fall | Duration of flowering (days) | Days from full bloom to harvest | Date of fruit harvest |
|---|---|---|---|---|---|---|---|---|---|
| Auvil Early Fuji | 3rd March | 10th March | 14th March | 17th March | 24th March | 1st April | 15.00 | 146.5 | 16th, Aug |
| Fuji Kiku | 10th March | 13th March | 16th March | 20th March | 27th March | 2nd April | 13.00 | 144.8 | 18th, Sep |
| Red Fuji | 12th March | 14th March | 17th March | 20th March | 26th March | 3rd April | 14.00 | 186.5 | 27th, Sep |
| Sun Fuji | 6th March | 10th March | 13th March | 16th March | 23th March | 1st April | 16.00 | 146.0 | 16th, Aug |
| Camspur | 8th March | 11th March | 15th March | 19th March | 25th March | 2nd April | 14.00 | 115.5 | 18th, Aug |
| Oregon Spur II | 11th March | 14th March | 18th March | 21th March | 27th March | 4th April | 14.00 | 117.7 | 18th, Aug |
| Red Chief | 9th March | 13th March | 16th March | 20th March | 26th March | 2nd April | 13.00 | 117.7 | 18th, Aug |
| Silver Spur | 7th March | 11th March | 14th March | 17th March | 25th March | 2nd April | 16.00 | 115.2 | 18th, Aug |
| Super Chief | 10th March | 13th March | 16th March | 19th March | 26th March | 2nd April | 14.00 | 115.8 | 18th, Aug |
| Golden Smoothy | 10th March | 14th March | 17th March | 20th March | 28th March | 4th April | 15.00 | 142.5 | 16th, Aug |
| Gala Mischala | 10th March | 13th March | 16th March | 19th March | 27th March | 1st April | 13.00 | 113.5 | 27th, July |
| Gale Gala | 11th March | 14th March | 17th March | 20th March | 27th March | 2nd April | 13.00 | 116.7 | 27th, July |
| Sansa | 6th March | 9th March | 14th March | 17th March | 24th March | 29th March | 14.00 | 110.8 | 11th, July |
| Braeburn | 7th March | 10th March | 12th March | 16th March | 22th March | 30th March | 14.00 | 148.3 | 16th, Aug |
| Pink Lady | 5th March | 9th March | 12th March | 15th March | 21th March | 27th March | 12.00 | 185.5 | 18th, Sep |
| $CD_{0.05}$ | - | - | - | - | - | - | 1.40 | 2.47 | - |

basis of date of fruit harvesting all the cultivars can be grouped into early maturing ('Sansa', 'Camspur', 'Gala Mischala', 'Gale Gala', 'Oregon Spur-II', 'Red Chief', 'Silver Spur' and 'Super Chief'), mid season maturing ('Auvil Early Fuji', 'Braeburn', and 'Sun Fuji') and late maturing cultivars ('Fuji Kiku', 'Pink Lady', 'Golden Smoothy' and 'Red Fuji'). Time and duration of flowering are important traits in classification of apple with respect to their span of flowering under different regions and have been assessed by other workers (Biswajit *et al.*, 4).

The cultivar 'Super Chief' registered maximum spur density (10.80/ $cm^2$), and lowest fruit drop (18.09%), whereas, 'Fuji Kiku' and 'Gale Gala' recorded least spur (Table 3) density (2.56 and 3.19/ $cm^2$, respectively). Highest yield per plant (9.68 kg) and productivity (24.21 tonnes/ha) was recorded in 'Oregon Spur II', which was on par with 'Sun Fuji', 'Red Chief', 'Camspur', 'Red Fuji' and 'Super Chief' in terms of yield and productivity. The highest fruit set (69.07%) was recorded in 'Oregon Spur-II', which was closely followed by 'Red Chief', and 'Silver Spur'. The highest fruit drop (54.17%) was recorded in 'Gale Gala' but it was on par with 'Golden Smoothy', 'Gala Mischala', 'Sansa', 'Pink Lady' and 'Breaburn'. The higher productivity in Fuji and Spur type Delicious group may be due to the varietal characteristics and better adaptability of these cultivars under mid-hill conditions. Similar variations in different apple cultivars has also been observed for spur density, fruit set and fruit drop per cent, fruit harvesting date and yield characteristics (Biswajit *et al.*, 4).

The maximum fruit length (57.93 mm), and ascorbic acid content (56.37 mg/100 g) was observed for the cultivar 'Red Chief'. The minimum fruit length (42.73 mm) and breadth (41.32) was found in 'Gale Gala'. The cultivar 'Silver Spur' recorded the highest fruit weight (155.6 g), and TSS : acid ratio (92.7). Maximum fruit firmness (10.30 kg/$cm^2$) and acidity (0.90%) was observed in the cultivar 'Braeburn', whereas minimum fruit firmness (5.53 kg/$cm^2$ and acidity (0.15%) were recorded in 'Auvil Early Fuji and 'Silver Spur', respectively (Tables 4 & 5). Best shape and surface colour was observed in the cultivars 'Super Chief" and 'Camspur'. Among all the cultivars under study, the highest TSS (15.95°B) was recorded in 'Gale Gala', but had the poor fruit size. The cultivar 'Oregon Spur II' recorded highest reducing sugars (6.56%), whereas the highest total sugars (11.94%) and non-reducing sugars (5.47%) were recorded in the cultivar 'Sansa', which was at par with 'Silver Spur and 'Super Chief' in terms of non-reducing sugars. Similar variations for different traits were also been reported by Kumar and Verma (8) in mid-hill conditions of Kullu; Sumrah *et al.* (14) in Soan Valley of Pakistan; Dwivedi *et al.* (6) in Ladakh region of Jammu and Kashmir and Sharma *et al.* (14) under mid-hill conditions of Himachal Pradesh.

From the present investigation, it may be concluded that 'Red Chief', 'Super Chief', 'Oregon

**Table 3.** Performance of different apple cultivars with respect to fruiting parameters and productivity (pooled mean).

| Cultivar | Spur density (No./$cm^2$) | Fruit set (%) | Fruit drop (%) | Yield per tree (kg) | Productivity (t/ha) |
|---|---|---|---|---|---|
| Auvil Early Fuji | 6.99 | 47.67 (43.66) | 38.80 (38.51) | 7.08 | 17.71 |
| Fuji Kiku | 2.56 | 18.57 (25.39) | 28.58 (31.81) | 3.83 | 9.67 |
| Red Fuji | 3.87 | 41.32 (40.00) | 21.17 (27.14) | 9.08 | 22.71 |
| Sun Fuji | 6.50 | 41.36 (40.02) | 42.43 (40.57) | 9.67 | 24.10 |
| Camspur | 8.28 | 53.52 (47.02) | 33.11 (35.12) | 9.52 | 23.79 |
| Oregon Spur II | 8.90 | 69.07 (56.40) | 20.25 (26.64) | 9.68 | 24.21 |
| Red Chief | 8.83 | 65.22 (53.86) | 19.47 (26.16) | 9.58 | 23.96 |
| Silver Spur | 8.82 | 61.52 (51.67) | 19.52 (26.20) | 8.20 | 20.50 |
| Super Chief | 10.80 | 55.75 (48.30) | 18.09 (25.15) | 9.00 | 22.50 |
| Golden Smoothy | 6.67 | 48.09 (43.90) | 49.86 (44.91) | 3.50 | 8.75 |
| Gala Mischala | 6.32 | 46.61 (43.05) | 51.56 (45.90) | 6.72 | 16.79 |
| Gale Gala | 3.19 | 34.23 (35.78) | 54.17 (47.39) | 5.22 | 13.04 |
| Sansa | 6.36 | 55.94 (48.43) | 51.16 (45.67) | 5.08 | 12.71 |
| Braeburn | 6.93 | 53.08 (46.77) | 42.69 (40.79) | 3.67 | 9.17 |
| Pink Lady | 7.15 | 37.93 (37.95) | 49.03 (44.45) | 3.60 | 8.58 |
| $CD_{(0.05)}$ | 1.82 | 8.67 | 11.03 | 1.27 | 3.11 |

**Table 4.** Performance of different apple cultivars with respect to fruit physical characteristics.

| Cultivar | Fruit length (mm) | Fruit dia. (mm) | Fruit weight (g) | Fruit firmness (kg/ $cm^2$) | Fruit shape | Surface colour |
|---|---|---|---|---|---|---|
| Auvil Early Fuji | 54.37 | 73.28 | 142.7 | 5.53 | Globose | RED GROUP 42 A |
| Fuji Kiku | 43.90 | 58.16 | 114.8 | 7.28 | Round to obloid | RED GROUP 43 D |
| Red Fuji | 54.38 | 41.58 | 140.2 | 5.93 | Obloid | RED GROUP 42 B |
| Sun Fuji | 55.93 | 57.92 | 137.2 | 5.58 | Globose | RED GROUP 42 A |
| Camspur | 58.48 | 71.44 | 141.1 | 8.48 | Conical | RED GROUP 46 B |
| Oregon Spur II | 59.53 | 55.90 | 151.4 | 8.30 | Conical globose | RED GROUP 45 A |
| Red Chief | 59.67 | 67.12 | 154.7 | 8.58 | Oblong conical | RED GROUP 46 B |
| Silver Spur | 57.17 | 65.08 | 155.6 | 7.55 | Ovoid to conic | RED GROUP 43 B |
| Super Chief | 59.58 | 62.02 | 149.9 | 7.78 | Conic to cylendrical waisted | RED GROUP 46 A |
| Golden Smoothy | 51.78 | 49.83 | 104.3 | 7.76 | Oblong | YELLOW GREEN GROUP 150 C |
| Gala Mischala | 56.85 | 66.89 | 154.0 | 7.60 | Obloid to globose | RED GROUP 42 A |
| Gale Gala | 42.73 | 41.32 | 101.0 | 8.15 | Obloid to globose | RED GROUP 43 B |
| Sansa | 50.63 | 62.57 | 101.5 | 7.37 | Obloid | RED GROUP 41 B |
| Braeburn | 56.52 | 68.68 | 137.7 | 10.30 | Globose to oblong | RED GROUP 41 B |
| Pink Lady | 55.02 | 64.36 | 134.9 | 7.12 | Conical to obloid | RED GROUP 41 A |
| $CD_{0.05}$ | 2.82 | 3.83 | 5.86 | 0.37 | - | - |

**Table 5.** Performance of different apple cultivars with respect to fruit size and quality (pooled mean).

| Cultivar | TSS (%) | Acidity (%) | Reducing sugars (%) | Non-reducing sugar (%) | Total sugars (%) | Ascorbic acid (mg/100g) | TSS : acid |
|---|---|---|---|---|---|---|---|
| Auvil Early Fuji | 11.80 | 0.49 | 4.96 | 4.44 | 9.40 | 28.22 | 24.19 |
| Fuji Kiku | 11.67 | 0.44 | 4.98 | 4.13 | 9.11 | 22.20 | 26.25 |
| Red Fuji | 11.47 | 0.31 | 5.36 | 4.14 | 9.50 | 19.66 | 38.46 |
| Sun Fuji | 12.97 | 0.52 | 5.22 | 3.23 | 8.45 | 21.47 | 25.15 |
| Camspur | 10.98 | 0.41 | 5.58 | 2.53 | 8.11 | 50.42 | 26.74 |
| Oregon Spur II | 10.53 | 0.24 | 6.56 | 3.65 | 10.21 | 43.91 | 44.21 |
| Red Chief | 10.37 | 0.24 | 6.53 | 4.00 | 10.53 | 56.37 | 42.95 |
| Silver Spur | 12.32 | 0.15 | 5.26 | 5.46 | 10.72 | 50.47 | 92.70 |
| Super Chief | 11.25 | 0.29 | 5.30 | 4.93 | 10.23 | 33.83 | 38.32 |
| Golden Smoothy | 12.72 | 0.56 | 5.05 | 2.67 | 7.73 | 30.94 | 22.92 |
| Gala Mischala | 12.70 | 0.52 | 4.61 | 2.78 | 7.54 | 44.39 | 24.60 |
| Gale Gala | 15.95 | 0.57 | 4.23 | 3.36 | 7.54 | 44.21 | 28.16 |
| Sansa | 14.43 | 0.51 | 6.46 | 5.47 | 11.94 | 22.89 | 28.15 |
| Braeburn | 11.60 | 0.90 | 3.09 | 4.80 | 7.88 | 33.82 | 12.93 |
| Pink Lady | 11.07 | 0.55 | 5.73 | 5.19 | 10.92 | 28.04 | 20.26 |
| $CD_{0.05}$ | 0.61 | 0.04 | 0.33 | 0.55 | 0.53 | 3.36 | 12.66 |

Spur II' and 'Camspur' in Delicious group, 'Gala Mischala' in Gala group, and 'Auvil Early Fuji' and 'Sun Fuji' in 'Fuji' group have good yielding potentials, earliness and better fruit quality within their respective groups and can be grown commercially.

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# Comparative study on clonal and seedling progenies of selected cocoa (*Theobroma cacao* L.) genotypes

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## ABSTRACT

**Cocoa (*Theobroma cacao* L.) is grown as a component crop in palm-based cropping systems in South India. Systematic seed gardens were established for hybrid seed production in a limited scale and clonal propagation methods were standardized in cocoa. Since the area expansion programmes require more planting material in a short span with easy management, this attempt was made to assess and compare the performance of selective genotypes, *i.e.* clones and seedlings. The clones exhibited sufficient vigour, larger canopy, lower branching and early bearing nature. Yield compiled over six years showed that VTLC-5, VTLC-1 and VTLCC-1 had more pods both as clone and seedling. Eight clones recorded > 45 pods/ tree/ year, >40 beans/ pod and high dry bean yields ranged from 1.54 to 2.08 kg at the age of tenth year and seven seedlings recorded >40 pods/ tree/ year with >35 beans/ pod and dry bean yields ranged from 1.57 to 1.88 kg. It was suggested that, to get early, uniform and high yield, clonal plants are preferable, but it requires systematic training and pruning in the initial years of growth to maintain optimal canopy. From the average dry bean yield of clones and seedlings, it was suggested that the genotypes VTLCH-3, VTLCH-4, VTLCH-2 and VTLCH-1 may also be utilized for seedling production. All these high yielding genotypes had single dry bean weight of one gram and above; and favourable shelling percentage, nib recovery and fat contents making them suitable for chocolate industry as well.**

**Key words**: Cocoa, clones, performance, seedling population.

## INTRODUCTION

National Horticulture Mission identified cocoa as a potential plantation crop because of its demand both in domestic and international markets, which necessitated identification of productive genotypes for area expansion. Indian chocolate industry and confectionaries required 60,000 tonnes of dry beans for the year 2025 as against the current production of 45,000 tonnes (DCCD, 3). About 2,20,000 ha to be brought under cocoa to meet out this demand for which 150.7 million quality seedlings are required. It was also estimated that around 1,895 and 388 thousand ha area is available under coconut and arecanut, respectively (DES, 4) to be utilised for cocoa cultivation for which more planting materials are required in a short span with easy management. Only narrow range of clones is available now and work must continue both on selection and breeding to identify outstanding clones. Because of the existence of incompatibility in cocoa, systematic seed gardens with self incompatible but cross compatible parents are required for production of hybrid seeds (Wood and Lass, 14). Softwood grafting and budding methods were standardised with success rate of 90 per cent to get true-to-type and productive clones of known parentage. Though the genotype is fixed in the clone over environmental influence, the cost and time involved in production of clones is found to be higher than seedlings (Herklots and Murray, 6). Farmers also expressed difficulty in maintaining the crop in the intercropping system because of the typical growth habit of cocoa, which is branching in multiple tiers. Earlier in the cocoa breeding programmes individual trees were selected from local landraces/ seedling populations and used in hybridization programmes without assessing them in clonal trials which lead to confusing results. Later clonal selection programmes were initiated in the beginning of this century for early evaluation and to confirm the genetic gain combining with desirable traits (Adomako and Adu-Ampomah, 1). Further, open-pollinated pods were harvested from these clonal trees and used as 'clonal seeds' for large scale multiplication (Herklots and Murray, 6). With this background, this study was conducted to compare the growth and yield performance of clones and seedlings of selective genotypes and to identify potential genotypes to be utilised both as clones and seedlings.

## MATERIALS AND METHODS

Twelve cocoa genotypes including clones and hybrids were selected, multiplied as soft wood grafts

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and open-pollinated pods were collected from clonal trees and raised as seedlings. Both these clones and seedlings were planted at a spacing of 3 m × 3 m in the arecanut garden at CPCRI, Regional Station, Vittal, Karnataka in 2005, in a randomized block design with 6 trees/ plot in three replications. Normal recommended dosage of 100:40:140 NPK was applied and 20 l water/ day as drip during six months of rainless period was given. All genotypes were evaluated for their height (m), girth (cm), first branching height (m), number of branches and canopy area ($m^2$) and the data on ten-year-old trees are presented. Considering the canopy as cone shaped, the canopy area was measured using the formula $\pi rl$, whereas, r = EW + NS/ 4 and $l = \sqrt{r^2 + h^2}$, h = canopy height and expressed in $m^2$. Number of pods yielded by individual trees of each genotype during each harvest was compiled for six years from 5 to 10 years. Five pods per genotype were used to measure the pod characteristics and beans were processed through fermentation and drying and 100 beans were used to measure the bean traits. Fat was estimated by Soxhlet's apparatus with solvent extraction method. Data were analysed with MSTATC programme.

## RESULTS AND DISCUSSION

The growth habit of both clones and seedlings of cocoa genotypes showed significant difference in all the characters studied (Table 1). Among the clones, the plant height ranged from 3.22 to 4.01 m, whereas in seedlings it ranged from 3.25 to 3.88 m and seven clonal genotypes were taller than seedlings; however girth was more in eight seedling genotypes. The jorquetting or the first branching heights were lower invariably in all the clones irrespective of genotypes giving a bushy appearance, which is predicted, as the grafts are prepared from fan branches of mother trees (Wood, 13). Number of branches and canopy area was higher in clones compared to seedlings. Though systematic training and pruning measures were taken up to maintain the jorquetting heights, number of branches and canopy spread in the cropping system, significant genotypic difference was observed within clones and seedlings with regard to plant habit. For mixed cropping and for high density planting systems, it is suggested to select shorter plants with compact canopy to obtain high yield efficiency combining with other traits and productivity, which was proved with trials with Trinitarios at Costa Rica and Brazil (Monteverde *et al*., 11), which facilitated short jorquette both in parents and progenies. The optimal canopy area of 15-20 $m^2$ is recommended for optimal productivity especially in the grafted plants and under arecanut-based intercropping systems (Thomas and Balasimha, 12). In the clonal evaluation trials, the relationship between vigour of plant, pod yield and yield efficiency are important when planted at one density (Lachenaud, 8) and in our trial too the plants with high vigour showed high pod yield.

Pod yield per tree per year and the stability of yield over years was found reliable selection criterion in identifying potential cocoa genotypes and also exhibited positive correlation with total yield (Mallika *et al*., 10). Yield data was compiled over 6 years in

**Table 1.** Growth parameters of clones and seedlings of cocoa genotypes.

| Genotype | Height (m) | | Girth (cm) | | Jorquetting (m) | | No. of branches | | Canopy area ($m^2$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling |
| VTLCH-1 | 3.26 | 3.65 | 31.2 | 35.4 | 1.03 | 1.57 | 8.94 | 5.90 | 15.0 | 12.2 |
| VTLCH-2 | 3.50 | 3.39 | 33.5 | 36.4 | 0.95 | 1.49 | 11.0 | 6.53 | 16.1 | 11.2 |
| VTLCH-3 | 3.95 | 3.52 | 35.8 | 38.7 | 1.18 | 1.43 | 11.2 | 8.58 | 20.1 | 14.1 |
| VTLCH-4 | 3.31 | 3.54 | 31.8 | 35.5 | 1.10 | 1.67 | 10.8 | 5.33 | 16.7 | 10.6 |
| VTLCC-1 | 3.65 | 3.25 | 34.7 | 32.3 | 1.12 | 1.21 | 10.3 | 7.89 | 15.9 | 11.1 |
| VTLC-05 | 3.76 | 3.88 | 35.7 | 38.8 | 1.11 | 1.24 | 10.4 | 8.66 | 18.4 | 16.6 |
| VTLC-07 | 3.39 | 3.60 | 29.4 | 36.0 | 0.95 | 1.53 | 9.45 | 7.06 | 17.2 | 14.9 |
| VTLC-19 | 3.63 | 3.54 | 36.8 | 36.4 | 0.90 | 1.81 | 9.33 | 8.27 | 17.4 | 10.8 |
| VTLC-30 | 3.75 | 3.63 | 39.7 | 33.2 | 1.08 | 1.70 | 10.8 | 7.85 | 20.4 | 11.4 |
| VTLC-61 | 3.22 | 3.54 | 33.2 | 37.5 | 1.22 | 1.37 | 10.4 | 6.66 | 15.2 | 14.4 |
| VTLC-66 | 4.01 | 3.55 | 40.3 | 35.8 | 1.16 | 1.58 | 8.56 | 7.06 | 21.3 | 10.9 |
| VTLC-1 | 3.74 | 3.26 | 38.4 | 38.3 | 1.00 | 1.44 | 10.2 | 7.65 | 20.1 | 12.0 |
| CD at 5% | 0.75 | 0.60 | 10.1 | 5.90 | 0.43 | 0.50 | 3.15 | 3.62 | 8.14 | 5.22 |

case of clones since they had early bearing nature and over 5 years in seedlings (Table 2). Precocious bearing was observed in clonal plants irrespective of genotype, which is in agreement with earlier workers (Wood, 13). Herklots and Murray (6) when tested the clonal cuttings of ICS-45, they gave high yield in their fourth year than seedlings, which gave high yield only in the seventh year. They also identified that even in marginal lands, the clones performed better. In Trinidad, cuttings from three clones of ICS-1, 45 and 95 when compared with seedlings from open-pollinated pods of same three clones as clonal seeds, performance of clones excelled the seedlings when assessed over 13 years, while clones even started yielding from second year onwards. In present trial, both clones and seedlings showed gradual increase in pod yield with increasing tree ages. In the sixth year the pod yield ranged from 11.6 to 22.9 pods/ tree and 8.87 to 10.4 pods/ tree, which showed considerable increase in the tenth year, ranged from 39.3 to 50.4 and 32.7 to 45.8 in clones and seedlings, respectively. Eight genotypes had >45 pods and seven genotypes had >40 pods/ tree/ year as clone and seedling respectively at the age of tenth year. From our results, it was observed that the genotypes VTLC-5, VTLC-1 and VTLCC-1 yielded high both as clones and seedlings. From the mean of both clones and seedlings in tenth-year-old trees, it was observed that VTLCH-1, 2, 3, 4 also yielded high, since they are originally of hybrid progeny and their hybrid vigour is fixed in clones as well as the clonal seeds. It showed the possibility of harvesting these pods directly from clonal trees and can be used for planting material production. High yielding nature of these hybrids was earlier documented by Bhat *et al.* (2) in the progeny trials as well as in the clonal evaluation trial in the initial years of growth (Elain Apshara *et al*., 5). This will further reduce the need of establishing bi-clonal orchards to produce particular hybrid with its two parents.

Pod characteristics respect to weight, length, breadth, husk: bean ratio and number of beans per pod showed significant difference among the genotypes (Table 3). Pods from clonal plants were bigger than seedlings except in two genotypes. The cumulative factors, number of pods, total pod weight and average single pod weight contribute to the harvest efficiency of a hybrid in cocoa. Length and breadth of pods directly contributed to the size and weight of pods. The required bean number of 35 beans per pod was observed in all genotypes both as clones and seedlings. Number of bold beans represents the apparent fertility (Lachenaud *et al*., 9) and variation was observed with number of beans per pod in Ivory Coast (Lachenaud, 7). Other traits related to bean are given in Table 4. The wet to dry bean ratio ranged from 2.56 to 3.76 in clones and from 2.25 to 3.32 in seedlings. The highest ratio in few clones may be because of the big pods which constituted more husk.

Beans of one g and above are preferred by the processing units. Fermented and dried beans were measured for their single bean weight, which ranged from 0.88 to 1.15 in clones and 0.87 to 1.16 in

**Table 2.** Pod yield performance of clones and seedlings of cocoa genotypes.

| Year | 5 | 6 | | 7 | | 8 | | 9 | | 10 | | Mean (clone + seedling) 10th year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | Clone | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling | |
| VTLCH-1 | 12.7 | 17.7 | 11.7 | 19.4 | 18.1 | 21.6 | 18.2 | 32.3 | 28.2 | 45.2 | 40.2 | 42.7 |
| VTLCH-2 | 14.8 | 14.1 | 11.9 | 24.1 | 21.8 | 32.7 | 22.9 | 33.2 | 23.9 | 45.1 | 40.4 | 42.8 |
| VTLCH-3 | 14.0 | 14.3 | 13.4 | 24.6 | 29.8 | 26.5 | 29.1 | 41.8 | 32.0 | 45.5 | 41.3 | 43.4 |
| VTLCH-4 | 11.8 | 12.7 | 14.5 | 20.8 | 20.0 | 40.5 | 20.5 | 38.7 | 27.7 | 47.6 | 40.0 | 43.8 |
| VTLCC-1 | 19.0 | 22.8 | 10.6 | 29.9 | 20.1 | 37.4 | 21.1 | 46.3 | 21.7 | 45.9 | 41.0 | 43.5 |
| VTLC-05 | 22.7 | 22.9 | 12.7 | 31.4 | 31.8 | 44.2 | 31.5 | 52.0 | 39.0 | 50.4 | 45.8 | 48.1 |
| VTLC-07 | 16.5 | 19.4 | 12.2 | 28.1 | 17.6 | 27.9 | 17.1 | 30.2 | 28.0 | 40.9 | 33.3 | 37.1 |
| VTLC-19 | 16.0 | 20.0 | 8.87 | 26.3 | 15.1 | 25.6 | 15.9 | 28.4 | 28.7 | 39.3 | 38.3 | 38.8 |
| VTLC-30 | 19.8 | 19.2 | 11.9 | 30.7 | 24.1 | 39.8 | 24.3 | 42.1 | 25.7 | 44.8 | 35.9 | 40.4 |
| VTLC-61 | 11.7 | 12.3 | 10.9 | 24.5 | 27.6 | 35.4 | 28.6 | 36.1 | 42.1 | 46.5 | 32.7 | 39.6 |
| VTLC-66 | 11.2 | 11.6 | 11.1 | 24.4 | 15.7 | 28.2 | 15.1 | 31.2 | 33.9 | 44.5 | 35.8 | 40.2 |
| VTLC-1 | 15.9 | 19.8 | 10.4 | 26.2 | 27.7 | 28.1 | 27.1 | 39.7 | 31.3 | 47.8 | 43.6 | 45.7 |
| CD at 5% | 1.57 | 6.82 | 6.26 | NS | NS | 5.64 | 6.12 | NS | NS | 6.85 | 1.06 | |

**Table 3.** Pod characters of cocoa clone and seedling genotypes.

| Genotype | Pod wt. (g) | | Pod length (cm) | | Pod breadth (cm) | | Husk: bean ratio | | No. of beans/ per fruit | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling | Clone | Seedling | Mean |
| VTLCH-1 | 432 | 347 | 15.7 | 14.3 | 7.0 | 6.3 | 3.05 | 3.00 | 40.5 | 40.2 | 40.4 |
| VTLCH-2 | 505 | 350 | 17.0 | 15.9 | 7.5 | 7.8 | 3.06 | 2.26 | 41.1 | 39.6 | 40.4 |
| VTLCH-3 | 432 | 370 | 16.5 | 14.8 | 7.3 | 6.9 | 2.69 | 3.30 | 44.1 | 38.9 | 41.5 |
| VTLCH-4 | 445 | 441 | 16.8 | 18.6 | 7.1 | 7.9 | 2.96 | 2.53 | 40.7 | 40.3 | 40.5 |
| VTLCC-1 | 434 | 366 | 17.4 | 16.5 | 6.6 | 8.0 | 2.57 | 2.79 | 43.5 | 38.6 | 41.1 |
| VTLC-05 | 403 | 351 | 14.3 | 14.2 | 7.2 | 7.4 | 2.51 | 3.08 | 40.8 | 41.1 | 41.0 |
| VTLC-07 | 501 | 484 | 16.9 | 16.1 | 7.5 | 7.5 | 2.81 | 3.41 | 41.1 | 36.9 | 39.0 |
| VTLC-19 | 428 | 357 | 16.4 | 14.7 | 6.9 | 7.3 | 2.24 | 2.43 | 40.1 | 39.1 | 39.6 |
| VTLC-30 | 408 | 453 | 15.8 | 15.9 | 7.1 | 7.3 | 2.68 | 2.95 | 35.2 | 38.5 | 36.9 |
| VTLC-61 | 416 | 427 | 16.3 | 17.1 | 6.9 | 7.7 | 2.62 | 3.32 | 39.4 | 35.9 | 37.7 |
| VTLC-66 | 504 | 423 | 16.2 | 18.1 | 7.5 | 7.1 | 2.56 | 3.81 | 40.3 | 38.5 | 39.4 |
| VTLC-1 | 473 | 420 | 16.4 | 16.9 | 7.5 | 7.4 | 2.72 | 2.84 | 40.9 | 41.5 | 41.2 |
| CD at 5% | 190 | 182 | 2.78 | 2.67 | 0.99 | 1.49 | 0.75 | 1.43 | 7.95 | 7.76 | |

**Table 4.** Bean traits of clones and seedlings of cocoa genotypes.

| Genotype | Wet: Dry | | SBW (g) | | DBY (kg) | | | Shell (%) | | Nib recovery (%) | | Fat (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Clone | Seedling | Clone | Seedling | Clone | Seedling | Mean | Clone | Seedling | Clone | Seedling | Clone | Seedling |
| VTLCH-1 | 3.01 | 2.50 | 1.00 | 1.00 | 1.83 | 1.62 | 1.73 | 16.9 | 16.4 | 83.1 | 83.6 | 50.0 | 50.5 |
| VTLCH-2 | 3.02 | 2.25 | 1.04 | 1.00 | 1.93 | 1.60 | 1.77 | 16.7 | 15.5 | 83.3 | 84.5 | 51.0 | 50.0 |
| VTLCH-3 | 3.15 | 3.32 | 1.00 | 1.00 | 2.01 | 1.61 | 1.81 | 14.3 | 13.2 | 85.7 | 86.8 | 50.0 | 50.0 |
| VTLCH-4 | 3.18 | 3.25 | 1.00 | 1.00 | 1.94 | 1.61 | 1.78 | 16.6 | 16.4 | 83.4 | 83.6 | 50.0 | 50.0 |
| VTLCC-1 | 3.16 | 3.27 | 0.99 | 0.99 | 1.98 | 1.57 | 1.78 | 16.1 | 16.5 | 83.9 | 83.5 | 49.9 | 49.8 |
| VTLC-05 | 3.17 | 2.59 | 1.01 | 1.00 | 2.08 | 1.88 | 1.98 | 15.6 | 15.5 | 84.4 | 84.5 | 52.0 | 50.0 |
| VTLC-07 | 3.34 | 2.81 | 1.07 | 1.16 | 1.80 | 1.43 | 1.62 | 12.1 | 10.0 | 87.9 | 90.0 | 54.0 | 53.0 |
| VTLC-19 | 3.27 | 3.10 | 0.98 | 0.92 | 1.54 | 1.38 | 1.46 | 17.2 | 16.0 | 82.8 | 84.0 | 48.1 | 42.5 |
| VTLC-30 | 2.56 | 2.53 | 1.15 | 1.12 | 1.81 | 1.55 | 1.68 | 13.2 | 16.5 | 86.8 | 83.5 | 55.0 | 52.0 |
| VTLC-61 | 3.38 | 2.85 | 0.90 | 0.91 | 1.65 | 1.07 | 1.36 | 18.8 | 13.4 | 81.2 | 86.6 | 45.0 | 46.4 |
| VTLC-66 | 3.03 | 3.22 | 1.04 | 0.97 | 1.87 | 1.34 | 1.61 | 14.7 | 16.2 | 85.3 | 83.8 | 53.0 | 45.2 |
| VTLC-1 | 2.94 | 3.17 | 1.06 | 1.02 | 2.07 | 1.85 | 1.96 | 12.6 | 14.5 | 87.4 | 85.5 | 52.5 | 51.0 |

SBW = Single dry bean weight; DBY = Dry bean yield

seedlings and nine as clones and eight as seedlings recorded more than one g single dry bean weight. Among the clones, it ranged from 1.54 to 2.08 and 1.07 to 1.88 kg in seedling genotypes. Shelling and nib recovery percentages, which are important with the confectioner's point of view, were calculated. They ranged from 12.1 to 19.1% in clones and 13.2 to 20.6% in seedlings and 80.9 to 87.9% in clones and 79.4 to 90% among the seedlings, respectively. Fat content ranged from 40 to 55% in clones and 40.2 to 53% in seedlings. Beans of more than 1 g showed more than 50% fat contents. Based on vigour, optimal canopy, mean pod yield over years, single dry bean weight, number of beans, dry bean yield, shelling, nib recovery and fat contents the best performers were selected.

From this trial, it was suggested that in order to get early, uniform and high yield, clonal plants are preferable, but it requires systematic training and pruning in the initial years of growth to maintain optimal canopy. Ten-year-old trees of seven genotypes both as clones and seedlings recorded high pod and

dry bean yields. VTLC-5, VTLC-1, VTLCC-1 and seedlings of four hybrids VTLCH-1, 2, 3, 4 can be utilized for seedling production for the immediate requirement of area expansion. All these high yielding genotypes had single dry bean weight of > 1 g, 13-17% shell, 84-86% nib recovery and 50% fat and thus suitable for chocolate industry as well. Further continuous evaluation is required in the later years of crop life for confirmation on both quantitative and qualitative parameters.

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# Performance of coconut hybrid MYD × WCT in the Brahmaputra valley region of Assam

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## ABSTRACT

**A long term evaluation of four coconut hybrids with a local check was conducted under rainfed conditions at the Horticultural Research Station, Kahikuchi of Assam Agricultural University. The experimental material consisted of four hybrids with different cross combinations, *viz*., WCT × COD, MYD × WCT, WCT × GBGD, COD × WCT along with AGT (Assam Green Tall) as local control planted during 1985 and evaluated for yield performance till 2003. The palms of the hybrid MYD × WCT were semi-tall, took 60 months for 50% of the palms to exhibit flowering. The results revealed that the hybrid, MYD × WCT was superior to other hybrids and local check with respect to nut yield (114.2 nuts/ palm/ year), copra out turn (3.72 tonnes/ ha/ year) and estimated oil out turn (2.53 tonnes/ ha) under rainfed conditions of Assam. Hybrid possessed higher quantity of organoleptically 'good' tender nut water (325 ml) with TSS of 6.2°Brix, 36.5 ppm of Na and 2350 ppm of K.**

**Key words:** Coconut, hybrid, rainfed, nut yield, copra, tender nut.

## INTRODUCTION

Coconut (*Cocos nucifera* L.) is one of the important plantation crops in Assam, which provides food, drink, beverage, medicine, fibre and a variety of raw materials for production of an array of products of commercial importance. Though coconut is considered to be a non-traditional crop of this region, the cultivation prevailing since time immemorial has resulted in certain well adapted ecotypes. There are two major forms available in coconut, *viz*., Tall and Dwarf. The Tall type is primarily out-crossing, while the Dwarf type is mainly self pollinating (with a few exceptions). The Tall genotypes are mostly common, commercially cultivated in all coconut growing regions of the world, while the Dwarf types are usually grown for ornamental and breeding purpose. The Tall and Dwarf types have been utilized for development of hybrids, combining the early flowering traits of dwarfs with the hardiness and high yielding characters of Tall parents and also exploitation of hybrid vigour. Genetic improvement of coconut has been effective through selection and varietal cross hybrids. Success of varietal cross hybrids in coconut is due to the advantages of early bearing and high yield. With the discovery of hybrid vigour in coconut by Patel (7) in a cross between West Coast Tall and Chowghat Green Dwarf paved the way for the successful breeding programme in coconut all over the world. Most of the hybrid evaluations conducted involved inter-varietal crosses of Dwarf × Tall and Tall × Dwarf types and the superiority of hybrids over local tall cultivars in terms of precocity, number of nuts per ha and copra/ nut were established (Satyabalan and Vijayakumar, 9; de Taffin *et al*., 13). In order to improve the coconut yield, newer varieties and hybrids are being introduced for testing its performance under Assam conditions. The present investigation was carried out for identifying a better performing coconut hybrid for cultivation in the Brahmaputra valley region.

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## MATERIALS AND METHODS

The experiment was carried out at the Horticultural Research Station, Kahikuchi, Guwahati, Assam under All India Coordinated Research Project on Palms, which is situated at 26°.3' N latitude and 91°.7' E longitude with an altitude of 64.0 m above mean sea level (MSL). The average maximum temperature is 32°C in summer and 26°C in winter, while the average minimum temperature is 23°C in summer and 16°C in winter. The station enjoys a sub-tropical climate, with an annual rainfall of about 1500 mm. The soil of the experimental site was Alluvial clay-loam with a pH of 4.9, low in available nitrogen (236.0 kg/ha), medium in available phosphorus (26.0 kg/ha), medium in available potassium (162.0 kg/ha) with an organic carbon of 0.45 per cent. The experimental material consisted of four cross combinations, *viz*., West coast tall (WCT) × Chowghat orange dwarf (COD), Malayan Yellow Dwarf (MYD) × WCT, WCT × Gangabondum Dwarf (GBGD), COD × WCT which were received from ICAR-CPCRI,

Kasaragod along with Assam Green Tall (AGT) as local check planted during 1985 and evaluated for yield performance till 2003. The details of parents are presented in Table 1. The hybrids along with control were planted under rainfed conditions in a replicated trial in a completely randomized complete block design with four replications and six palms per treatment. Spacing adopted was 7.5 m × 7.5 m with density of 175 palms per hectare. Regular manuring of NPK fertilizers @ 690:400:1050 g/ palm was provided along with organic manures in two split doses every year. Data pertaining to nut production, estimated copra out turn, tender nut traits recorded from 1994 to 2003 (ten years) was used for assessing the performance of the hybrids for nut yield. Morphological characters related to leaf, inflorescence, fruit and fruit components were also recorded in the adult palms. Data was subjected to statistical analysis using analysis of variance.

## RESULTS AND DISCUSSION

In the present investigation, significant differences were observed on the number of functional leaves, length of petiole and length of leaflet bearing portion among the various cross combinations and variety (Table 2). However, annual leaf production did not differ significantly among the hybrids and variety. The hybrid MYD × WCT recorded the highest number of functional leaves (32.0) with the shortest petiole (128.6 cm) and length of leaflet bearing portion (368.2 cm). The lowest number of functional leaves (28.0) was found in WCT × GBGD, whereas maximum length of petiole (144.9 cm) and length of leaflet bearing portion (387.8 cm) were observed in Assam Green Tall. Among the hybrids, MYD × WCT significantly took 60 months for 50% of palms exhibit flowering, while AGT took 84 months for 50% flowering. Based on the flowering data recorded, the hybrid MYD × WCT was a regular bearer and commenced flowering in 46 months after planting under rain-fed conditions (Table 3). Similar results were also recorded by Jerard *et al*. (2) at ICAR-CPCRI, Kasaragod. With regard to number of inflorescences per palm and number of female flowers, the hybrid MYD × WCT showed significantly the highest values for these characters compared to other hybrids (Table 3) and was on par with Assam Green Tall. On the other hand, the lowest number of inflorescences, female flowers and bunches harvested were observed in WCT × COD.

It can be seen that fruit set percentage of various hybrids and a variety was within the range of 24.6 to 29.6. Significantly the maximum fruit set (29.6%) was obtained in MYD × WCT and the lowest (24.6%) in WCT × GBGD. The variation in fruit set percentage among the coconut hybrids was also recorded by some workers (Nair *et al*., 4; Thomas *et al*., 15). In coconut, inter-spadix overlapping of female and male

**Table 1.** Details of parental coconut palms in the crosses.

| Genotype | Parental details |
|---|---|
| MYD × WCT | Selection from Malayan Yellow Dwarf as female parent and selection from West Coast Tall as male parent. |
| COD × WCT | Selection from Chowghat Orange Dwarf as female parent and selection from West Coast Tall as male parent. |
| WCT × GBGD | Selection from West Coast Tall as female parent and selection from Gangabondam as male parent. |
| WCT × COD | Selection from West Coast Tall as female parent and selection from Chowghat Orange Dwarf as male parent. |
| Assam Green Tall | Selection from Assam Green Tall. |

**Table 2.** Performance of coconut hybrids under rainfed conditions.

| Genotype | No. of functional leaves | Annual leaf production | Petiole length (cm) | Length of leaflet bearing portion (cm) |
|---|---|---|---|---|
| MYD × WCT | 32.0 | 12.0 | 128.6 | 368.2 |
| COD × WCT | 29.6 | 11.2 | 142.9 | 376.4 |
| WCT × GBGD | 28.0 | 11.0 | 134.4 | 384.2 |
| WCT × COD | 28.8 | 11.6 | 135.1 | 374.7 |
| Assam Green Tall | 30.3 | 11.9 | 144.9 | 387.8 |
| CD (P = 0.05) | 1.12 | NS | 3.98 | 10.62 |

**Table 3.** Yield attributing characters of coconut hybrids and variety under rainfed conditions.

| Genotype | No. of inflorescences/ palm | No. of female flowers/ palm | No. of bunches harvested | Fruit set (%) | Months to 50% flowering |
|---|---|---|---|---|---|
| MYD × WCT | 11.9 | 386.0 | 8.7 | 29.6 | 60 |
| COD × WCT | 11.1 | 341.2 | 7.9 | 26.0 | 68 |
| WCT × GBGD | 11.0 | 327.6 | 7.6 | 24.6 | 72 |
| WCT × COD | 10.6 | 326.4 | 7.1 | 25.8 | 71 |
| Assam Green Tall | 11.8 | 378.0 | 8.2 | 27.8 | 84 |
| CD (P = 0.05) | 0.41 | 19.7 | NS | 1.67 | 7.33 |

phases are an important factor in fruit set along with cross-pollination from nearby palms carried by agents like wind, insects etc. (Henderson, 1). The highest values for all the morphological and yield attributing characters observed in the hybrid MYD × WCT can be attributed due to the heterotic effect of the hybrid (Rao and Koyamu, 8; Swaminathan and Nambiar, 12; Kumaran *et al*., 3; Jerard *et al*., 2).

In the present study, the average nut yield recorded over ten years from 1994 to 2003 and the estimated mean copra yield of hybrids and AGT (check) are given in Table 4. The average nut yield over ten years among the hybrids ranged from 80.6 nuts per palm per year (WCT × GBGD) to 114.2 nuts per palm per year (MYD × WCT). The local control (AGT) recorded 105 nuts per palm per year. The hybrid MYD × WCT performed significantly better than other hybrids and AGT in respect of nut yield and this hybrid also recorded the highest values for copra content (186.0 g), copra yield (21.24 kg/palm), copra out turn (3.72 tonnes/ha), oil content (68.0%) and oil yield (2.53 tonnes /ha) compared to other hybrids and local control (Table 4). The high yielding potential of MYD × WCT has also been reported by Jerard *et al.* (2). The oil extracted from the copra of this hybrid was reported to have 45.4 per cent lauric acid (Naresh Kumar *et al*., 6). The better performance of the hybrid MYD × WCT might be attributed to the WCT's performance as a pollen parent or specific combining ability of MYD × WCT. The hybrid MYD × WCT was reported to exhibit higher level of drought tolerance owing to the different physiological and biochemical traits, epicuticular wax content and also VAM association (Voleti *et al*., 16, Thomas *et al*., 14; Shivasankar and Kasturi Bai, 11; Shivasankar and Chempakam, 10). It was reported that this hybrid exhibited six to seven-fold increase in stomatal resistance during severe stress as compared to pre-stress, thus checking the transpirational loss of water. Maintenance of water balance through effective stomatal regulation and wax content coupled with the activities of the stress response enzymes indicate the drought tolerance nature of the hybrid MYD × WCT. A positive relationship of vesicular arbuscular mycorrhiza (VAM) colonization was reported with stomatal resistance and leaf water potential, the two characters directly associated with drought tolerance (Thomas *et al*., 14). Comparison of VAM colonization pattern during and after stress revealed the superiority of MYD × WCT over the hybrid COD × WCT (Chandra Sankara) in harbouring highest level of VAM colonization in roots during stress period. The relative drought tolerant nature of WCT × COD hybrid was reported earlier, in comparison with COD × WCT by Kumaran *et al.* (3). An earlier study has indicated Gangabondam Green Dwarf (GBGD) to be a good general combiner and LCT × GBGD as good specific combiner (Nampoothiri *et al*., 5). The

**Table 4.** Mean yield performance of coconut hybrids and a variety over ten years (1994 to 2003) under rainfed conditions.

| Genotype | Nut yield (No. of nuts/palm/yr) | Copra content (g /fruit) | Copra yield (kg /palm) | Copra out turn (tonnes/ ha) | Oil content in copra (%) | Oil yield (tonnes/ ha) |
|---|---|---|---|---|---|---|
| MYD × WCT | 114.2 | 186 | 21.24 | 3.72 | 68.0 | 2.53 |
| COD × WCT | 88.7 | 178 | 15.79 | 2.76 | 66.7 | 1.84 |
| WCT × GBGD | 80.6 | 182.5 | 14.71 | 2.57 | 66.0 | 1.70 |
| WCT × COD | 84.2 | 175.6 | 14.79 | 2.59 | 67.2 | 1.74 |
| Assam Green Tall | 105.0 | 164.5 | 17.27 | 3.02 | 65.0 | 1.96 |
| CD (P = 0.05) | 9.07 | 8.01 | 3.10 | 0.41 | NS | 0.24 |

tender nut traits of the hybrid MYD × WCT (Table 5) showed its potential for tender nut purpose. The average quantity of tender nut water was 325 ml. Based on the organoleptic test; the tender nut water was classified as 'good' in taste with a TSS of 6.2°Brix. The tender nut water had 36.5 ppm of Na and 2350 ppm of K content.

The morphological traits of the parental and hybrid palms are shown in Table 6. The palms of this hybrid are semi-tall without prominent bole and attain an average height of 6.15 m at 18 years after planting. The colour of the petiole was green and bears green

**Table 5.** Tender nut traits of hybrid MYD × WCT.

| Parameter | MYD × WCT |
|---|---|
| Tender nut weight (g) | 1798.6 |
| Volume of water (ml) | 325.0 |
| TSS (°Brix) | 6.20 |
| Total sugars (g/100 ml) | 6.50 |
| Amino acids (mg/100 ml) | 1.90 |
| Sodium (ppm) | 36.5 |
| Potassium (ppm) | 2350.0 |

**Table 6.** Morphological and fruit component traits of coconut hybrid MYD × WCT compared with parental palms.

| Parameter | Female parent MYD | Male parent WCT | Hybrid MYD × WCT |
|---|---|---|---|
| Age of palm (years) | 22 | 26 | 18 |
| Category | Dwarf | Tall | Semi Tall |
| Crown shape | Circular | Circular | Circular |
| Presence of ball | Absent | Present | Absent |
| Plant height (cm) at 18 years | 376.8 | 750.3 | 615.0 |
| Girth of trunk (cm) | 62.9 | 79.3 | 69.7 |
| Total No. of leaves | 31 | 36 | 32 |
| Petiole length (cm) | 109.9 | 136.9 | 128.6 |
| Length of leaflet bearing portion (cm) | 326.1 | 421.1 | 368.2 |
| No. of leaflets | 90 | 122 | 96 |
| Leaflet length (cm) | 105 | 122 | 117.0 |
| Leaflet breadth (cm) | 4.68 | 5.9 | 5.30 |
| No. of leaf scars on 1 m length | 33.5 | 15.2 | 22.0 |
| Length of 10 internodes (cm) | 31.0 | 51.5 | 41.5 |
| Age at 50% flowering (months) | 66 | 98 | 60 |
| Inflorescence length (cm) | 81.1 | 105 | 89.6 |
| Length of spikelet bearing portion (cm) | 42.8 | 47.4 | 44.0 |
| Stalk length (cm) | 38.5 | 57.6 | 51.7 |
| Spikelet length (cm) | 30.9 | 40.1 | 41.0 |
| No. of spikelets in the inflorescence | 39.5 | 37.67 | 42.5 |
| No. of female flowers | 22.5 | 18.8 | 32.4 |
| No. of inflorescences on the crown | 10.0 | 12.0 | 11.9 |
| Fruit colour and shape | Yellow, Oval | Green, Oval | Green, Oval |
| Fruit length (cm) | 18.3 | 25.9 | 22.5 |
| Fruit breadth (cm) | 14.5 | 14.5 | 15.2 |
| Fruit weight (g) | 565.0 | 1196.12 | 980.0 |
| Thickness of husk (cm) | 2.0 | 3.0 | 2.1 |
| Shape of husked fruit | Round | Round | Round |
| Weight of husked fruit (g) | 382.11 | 566.38 | 641.5 |
| Per cent of husk to whole fruit | 32.37 | 52.31 | 34.5 |
| Kernel thickness (cm) | 1.2 | 1.2 | 1.5 |
| Kernel weight per fruit (g) | 244.9 | 283.0 | 278.0 |
| Copra weight per fruit (g) | 129.0 | 178.0 | 186.0 |

coloured, oval fruits and the husked fruits are round in shape. The palms of the female parent are dwarf with yellow colour petiole take 66 months for 50% of the palms to bear flower under rainfed conditions, fruits are bright yellow. The palms of the male parent were tall, bear green fruits and take 98 months for 50% of the palms to bear flower under rainfed conditions. The hybrid palms exhibited desirable values for weight of fruit, fruit yield, kernel weight and copra content besides many morphological traits such as earliness of flowering, shorter petiole length and higher number of female flowers.

Thus, from this study it can be concluded that, the hybrid MYD × WCT is suitable for cultivation in Assam as well as adjoining states. The hybrid is precocious in bearing, comes to bear at the age of 46 months, semi-tall type, high yielder (114.2 nuts/ palm/ year) also offers an excellent potential for tender nut use. Potential of the hybrid under rainfed situation due to its drought tolerance character suggests its suitability to Brahmaputra valley region of Assam where coconut is predominantly being cultivated under rainfed conditions.

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# Studies on influence of preharvest bagging of fruits on quality of mango cv. Ratna

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## ABSTRACT

**An investigation was undertaken to study the effect of different types of bag for pre-harvest bagging on physico-chemical properties of mango cv. Ratna during 2014-2016. The experiment was conducted in randomized block design with three replications. The fruits were bagged at marble stage (45 days from fruit set) with different types of bag, which constituted the various treatments, *viz*., $T_1$ = News paper bag; $T_2$ = Brown paper bag; $T_3$ = Scurting bag; $T_4$ = Transparent PP bag; $T_5$ = Butter paper bag; $T_6$ = Muslin cloth bag; $T_7$ = Brown paper bag with polythene coating; $T_8$ = Black polythene bag; $T_9$ = Opaque white polythene bag and $T_{10}$ = control (no bag). Bagging with newspaper bag, scruting bag and muslin cloth bag improved fruit retention, fruit and pulp weight, fruit diameter, total and reducing sugars, ascorbic acid and shelf-life of fruits. Pre-harvest bagging with different types of bag did not change the sensory qualities of ripe fruits. The per cent spotted fruits; incidence of diseases and pests was significantly reduced by pre-harvest bagging. The newspaper bag, scurting bag and muslin cloth bag were found to be meritorious among the various bags tried.**

**Key words**: Mango, pre-harvest bagging, physico-chemical composition, pests and diseases.

## INTRODUCTION

Mango (*Mangifera indica* L.) is the 'National Fruit' of India. The Konkan region of Maharashtra is one of the major mango growing belts in India. In Konkan, 1.82 lakh ha area is under mango cultivation with annual production of 1.28 lakh MT (Anon, 3). Among the various mango varieties in the region, Ratna (Neelum × Alphonso) is a prominent hybrid under cultivation. It is a regular bearer, semi-dwarf and high yielding variety. The fruits are large (320 g) with firm and fibreless deep orange coloured pulp. The demand for this variety is increasing day by day due to its good keeping quality. It is free from spongy tissue, which is a prominent physiological disorder in Alphonso. In recent years, the climatic aberrations such as sudden rise in the temperature and humidity, abnormal rains especially during fruit development are often experienced in Konkan region. Such adverse climate not only affects the external appearance of the fruit but also aggravate the pests incidence such as mealy bug. Bagging provides physical barrier over fruit, which prevents mechanical damage and bruises to fruit. It also protects the fruit from pests and diseases and also helps for ideal fruit development (Sharma *et al*., 14). Pre-harvest bagging of fruits is done to prevent damage occurring due to bruises, wounds, scars and to produce cleaner fruit peel with attractive colour (Bayogan *et al*., 4). Several types of locally available materials can be used for bagging. Though pre-harvest bagging possess prospects in mango it was seldom attempted and standardized. Hence, an experiment was undertaken to study the influence of pre-harvest bagging of fruits at egg stage on mango cv. Ratna.

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## MATERIALS AND METHODS

The trial was conducted at Department of Horticulture, College of Agriculture, Dr BSKKV, Dapoli, Maharashtra during 2014-2015 and 2015-2016 on mango cv. Ratna. The soil of experimental plot was red lateritic with uniform depth and good drainage conditions. Uniformly grown 20-year-old grafted Ratna mango trees were selected. The experiment was conducted in randomized block design with ten treatments replicated three times with a unit of 25 fruits per treatment per replication. Different types of bags constituted the treatments, *viz*. $T_1$ = News paper bag; $T_2$ = Brown paper bag; $T_3$ = Scurting bag; $T_4$ = Transparent PP bag; $T_5$ = Butter paper bag; $T_6$ = Muslin cloth bag; $T_7$ = Brown paper bag with polythene coating; $T_8$ = Black polythene bag; $T_9$ = Opaque white polythene bag and $T_{10}$ = control (no bag). Uniformly grown fruits at egg stage (45 days after fruit set) were selected for bagging. The size of bags was 25 cm × 20 cm. Before bagging six perforations (≤ 4 mm dia.) were made for proper ventilation at the

bottom of all bags except for scurting and muslin cloth bags. The bags were stapled properly at the stalk of each fruit of respective treatments so that it would not fall down and there would not be any open space. The scurting and muslin cloth bags were tied with the help of thread to the fruit stalk. The observations, *viz.* fruit retention (%) and days required for harvesting after bagging were recorded. Five fruits were randomly selected per treatment per replication and observations on fruit length (cm), fruit diameter (cm), fruit weight (g), pulp weight (g), TSS (°Brix), acidity (%), reducing sugars (%), total sugars (%), ascorbic acid (mg/100 g of fruit pulp), β-carotene (µg/100 g of pulp) and shelf-life of fruits (days) were recorded. The length and diameter of the fruit were measured with the help of digital Vernier calipers. Total soiluble solids (°Brix) of the fruits were estimated using standard procedure (AOAC, 1). Titrable acidity was estimated by titrating known amount of pulp against 0.1 N NaOH using phenolphthalein as indicator (Ranganna, 12). Reducing sugar and total sugars were determined by method suggested by Lane and Eynon (9) as described by Ranganna (12). Ascorbic acid content of fruit was estimated using standardized 2,6-dichlorophenol indophenol dye. The total carotenoids of the pulp were calculated as per method suggested by Ranganna (12). The end of shelf-life was noted when the fruits were spoiled. The statistical analysis was performed as per the ANOVA suggested by Panse and Sukhatme (11) and standard deviation was computed as per the procedure advocated by Rangaswamy (13).

## RESULTS AND DISCUSSION

The highest fruit retention was found in the treatment $T_1$ (89.33%), which was at par with $T_3$ (86.33%) (Table 1). It was followed by $T_2$ (82.4%) and $T_6$ (81.67%). The minimum fruit retention in mango was observed in $T_4$ (68.67%). The results indicated that newspaper, scurting, brown paper and muslin cloth bags were superior to unbagged control and other treatments. The average number of days required for harvesting of fruit after bagging was 53.42 days. Earliest harvesting was recorded in $T_{10}$ (52 days), $T_4$ (52.17 days) and $T_8$ (52.33 days), which were significantly superior over rest of the treatments. Late harvest was noticed in $T_1$ (55.00 days) and $T_3$ (54.50 days). The fruits in butter paper bag, brown paper bag with polythene coating and opaque white polythene bag took similar days for harvesting, whereas, fruits in polythene and black polythene bags were harvested earlier. The abiotic factors, *viz.* temperature and humidity play critical role in fruit growth and development. Bagging on fruits alters the microenvironment (Sharma *et al*., 14). The favourable microclimate surrounding the fruit leads to more fruit retention. The less retention in $T_4$ (68.67%) and $T_9$ (72.67%) might be due to development of high temperature inside the bag as in both these treatments, the base material used was polythene. The delay in maturity due to fruit bagging was also reported earlier in tomato (Leite *et al*., 10).

**Table 1.** Effect of types of bag on fruit retention and days required for harvesting after bagging in mango fruit cv. Ratna.

| Treatment | Fruit retention (%) | Days required for harvesting after bagging |
|---|---|---|
| $T_1$ (Newspaper bag) | 89.33 ± 1.89 | 55.00 ± 0.94 |
| $T_2$ (Brown paper bag) | 82.42 ± 0.35 | 53.83 ± 0.71 |
| $T_3$ (Scurting bag) | 86.33 ± 1.41 | 54.50 ± 0.24 |
| $T_4$ (Transparent PP bag) | 68.67 ± 0.94 | 52.17 ± 0.24 |
| $T_5$ (Butter paper bag) | 79.33 ± 0.94 | 53.50 ± 0.71 |
| $T_6$ (Muslin cloth bag) | 81.67 ± 0.48 | 54.00 ± 0.47 |
| $T_7$ (Brown paper bag with polythene coating) | 77.14 ± 1.61 | 53.50 ± 0.24 |
| $T_8$ (Black polythene bags) | 76.67 ± 0.94 | 52.33 ± 0.47 |
| $T_9$ (Opaque white polythene bag) | 72.67 ± 0.94 | 53.50 ± 0.24 |
| $T_{10}$ Control (No bagging) | 76.84 ± 1.18 | 52.00 ±0.94 |
| Range | 68.67-89.33 | 52.00-55.00 |
| Mean | 79.11 | 53.42 |
| CD at 5% | 5.75 | 0.87 |

Pre-harvest bagging with newspaper bag, scurting bag and muslin cloth bag significantly improved physical parameters, *viz*., weight of fruit, length of fruit, diameter of fruit, pulp weight and stone weight over unbagged control fruits (Table 2). The fruits of $T_1$ recorded the highest weight (477.28 g), which was at par with $T_3$ (470.65 g) followed by $T_6$ (468.35 g). The lowest fruit weight was seen in $T_{10}$ (415.64 g). The longest fruit was observed in $T_1$ (11.45 cm), which was at par with $T_6$ (11.39 cm), $T_3$ (11.38 cm), $T_2$ (11.33 cm) and $T_5$ (11.32 cm). The highest diameter was found in $T_3$ (9.22 cm), which was at par with $T_1$ (9.20 cm), $T_6$ (9.19 cm) and $T_2$ (9.07 cm). The highest pulp weight was observed in $T_1$ (382.73 g), which was at par with $T_3$ (381.30 g) and $T_6$ (379.07 g). The highest stone weight was noted in $T_1$ (52.61 g), which was at par with $T_3$ (52.45 g), $T_6$ (52.28 g) and $T_2$ (50.45 g). The deviation observed for pulp to stone ratio at harvest was non-significant. Covering the fruit with a bag at a particular developmental stage influence their growth and size. Reports on effects of fruit bagging on fruit size and weight opined that it may be due to differences in the type of bag used, fruit and cultivar responses (Sharma

**Table 2.** Effect of types of bag on physical parameters of fruits of mango cv. Ratna at harvest stage.

| Treatment | Fruit wt. (g) | Fruit length (cm) | Fruit dia. (cm) | Pulp wt. (g) | Stone wt. (g) | Pulp: stone ratio |
|---|---|---|---|---|---|---|
| $T_1$ (News paper bag) | 477.28 ± 9.95 | 11.45 ± 0.01 | 9.20 ± 0.07 | 382.73 ± 2.16 | 52.61 ± 1.62 | 7.32 ± 0.22 |
| $T_2$ (Brown paper bag) | 450.66 ± 5.38 | 11.33 ± 0.17 | 9.07 ± 0.15 | 366.18 ± 1.03 | 50.45 ± 1.86 | 7.30 ± 0.29 |
| $T_3$ (Scurting bag) | 470.65 ± 8.53 | 11.38 ± 0.08 | 9.22 ± 0.07 | 381.30 ± 3.97 | 52.45 ± 1.24 | 7.28 ± 0.25 |
| $T_4$ (Transparent PP bag) | 423.09 ± 9.31 | 10.93 ± 0.14 | 8.84 ± 0.12 | 351.44 ± 2.57 | 45.15 ± 1.50 | 7.81 ± 0.19 |
| $T_5$ (Butter paper bag) | 448.16 ± 8.16 | 11.32 ± 0.03 | 9.04 ± 0.02 | 359.29 ± 0.21 | 47.50 ± 0.97 | 7.63 ± 0.12 |
| $T_6$ (Muslin cloth bag) | 468.35 ± 8.53 | 11.39 ± 0.04 | 9.19 ± 0.04 | 379.07 ± 3.54 | 52.28 ± 1.06 | 7.27 ± 0.21 |
| $T_7$ (Brown paper bag with polythene coating) | 426.45 ± 7.81 | 11.13 ± 0.13 | 8.83 ± 0.24 | 352.46 ± 7.31 | 47.36 ± 2.06 | 7.46 ± 0.48 |
| $T_8$ (Black polythene bags) | 424.59 ± 7.29 | 10.91 ± 0.37 | 8.86 ± 0.01 | 352.76 ± 1.08 | 46.82 ± 2.50 | 7.56 ± 0.42 |
| $T_9$ (Opaque white polythene bag) | 430.65 ± 9.55 | 11.11 ± 0.04 | 8.89 ± 0.05 | 358.39 ± 7.41 | 46.03 ± 1.30 | 7.80 ± 0.06 |
| $T_{10}$ Control (no bagging) | 415.64 ± 8.14 | 11.06 ± 0.009 | 8.76 ± 0.10 | 347.33 ± 3.26 | 43.43 ± 1.34 | 8.01 ± 0.32 |
| Range | 415.64 - 477.28 | 10.91 - 11.45 | 8.76 - 9.22 | 347.33- 382.73 | 43.43 - 52.61 | 7.27 - 8.01 |
| Mean | 443.55 | 11.20 | 8.99 | 363.09 | 48.41 | 7.54 |
| CD at 5% | 8.46 | 0.18 | 0.17 | 11.84 | 3.48 | NS |

*et al*., 14). Bagging of 'Nam Dok Mai 4' mango fruits with two-layer paper bags, newspaper and golden paper bags increased fruit weight (Watanawan *et al*., 15). Microenvironment created by newspaper bag, scurting bag and muslin cloth bag had congenial effect on fruit growth. All these three treatments recorded more duration for harvesting than that of unbagged control fruits. The fruits bagged in polythene bag were harvested earlier than the unbagged fruits.

The pre-harvest bagging had significant effect on acidity, TSS, reducing sugars, total sugars, ascorbic acid and β-carotene content of fruits at harvest (Table 3). At harvest, the unbagged control fruits recorded minimum TSS (8.45°Brix), total sugars (3.21%) and ascorbic acid (58.40 mg/ 100 g). The fruits of $T_4$ had the highest reducing sugar (1.92%) and β-carotene (331.17 µg/ 100 g). The fruit of $T_1$ had the second highest performance for reducing

**Table 3.** Effect of types of bag on chemical composition of mango cv. Ratna fruits at harvest stage.

| Treatment | Titratable acidity (%) | TSS (°Brix) | Reducing sugar (%) | Total sugars (%) | Ascorbic acid (mg/100 g) | β-carotene (µg /100 g) |
|---|---|---|---|---|---|---|
| $T_1$ (Newspaper bag) | 1.75 ± 0.04 | 8.75 ± 0.07 | 1.91 ± 0.05 | 3.51 ± 0.01 | 69.60 ± 6.79 | 330.67 ± 1.24 |
| $T_2$ (Brown paper bag) | 1.78 ± 0.04 | 8.65 ± 0.07 | 1.88 ± 0.01 | 3.36 ± 0.01 | 67.20 ± 1.13 | 318.56 ± 2.59 |
| $T_3$ (Scurting bag) | 1.77 ± 0.05 | 8.80 ± 0.14 | 1.86 ± 0.01 | 3.50 ± 0.02 | 68.40 ± 5.09 | 325.37±2.42 |
| $T_4$ (Transparent PP bag) | 1.91 ± 0.06 | 8.62 ± 0.16 | 1.92 ± 0.01 | 3.46 ± 0.08 | 58.80 ± 3.96 | 331.17 ± 3.14 |
| $T_5$ (Butter paper bag) | 1.75 ± 0.01 | 8.93 ± 0.05 | 1.80 ± 0.01 | 3.28 ± 0.04 | 62.80 ± 3.96 | 323.76 ± 2.59 |
| $T_6$ (Muslin cloth bag) | 1.76 ± 0.02 | 8.88 ± 0.02 | 1.89 ± 0.05 | 3.47 ± 0.12 | 65.20 ± 5.09 | 324.97 ± 2.27 |
| $T_7$ (Brown paper bag with polythene coating) | 1.90 ± 0.01 | 8.63 ± 0.14 | 1.78 ± 0.02 | 3.24 ± 0.01 | 59.20 ± 5.66 | 326.09 ± 2.32 |
| $T_8$ (Black polythene bags) | 1.91 ± 0.03 | 8.73 ± 0.19 | 1.73 ± 0.01 | 3.28 ± 0.00 | 60.80 ± 4.53 | 318.40 ± 3.86 |
| $T_9$ (Opaque white polythene bag) | 1.88 ± 0.04 | 8.48 ± 0.02 | 1.77 ± 0.01 | 3.27 ± 0.08 | 60.40 ± 2.83 | 317.93 ± 0.94 |
| $T_{10}$ Control (no bagging) | 1.92 ± 0.03 | 8.45 ± 0.07 | 1.74 ± 0.02 | 3.21 ± 0.05 | 58.40 ± 3.39 | 328.40 ± 2.37 |
| Range | 1.75 - 1.92 | 8.45 - 8.93 | 1.73 - 1.92 | 3.21 - 3.51 | 58.40 - 69.60 | 317.93 - 331.17 |
| Mean | 1.83 | 8.69 | 1.83 | 3.36 | 63.08 | 324.54 |
| CD at 5% | 0.05 | 0.19 | 0.04 | 0.08 | 3.95 | 1.41 |

sugar and β-carotene. The total sugars (3.51%) and ascorbic acid (69.60 mg/ 100 g) recorded in $T_1$ was the best. The fruits of $T_5$ (8.93°B) had the maximum TSS and those of $T_{10}$ (1.92%) had the maximum acidity. The variation observed in chemical composition of mango fruits can be attributed to the changed microenvironment around fruit during its growth and development. The bagged fruits recorded the highest content of vitamin C, sucrose, glucose and fructose over control in Zill mango (Hongxia *et al*., 7). The bagging of date palm fruits improved the total sugars (Harhash and Al-Obeed, 6).

At ripe stage, the fruits of $T_1$ exhibited the maximum TSS (22.48°Brix), total sugars (13.96%) and ascorbic acid (58.40 mg/ 100 g) (Table 4). Fruits of $T_8$ (0.30%) had maximum acidity and $T_6$ had maximum reducing sugar (4.16%) and β-carotene (11575.59 µg/ 100 g). The fruits of $T_1$ and $T_3$ had recorded the minimum acidity (0.22%) and $T_8$ had minimum TSS (21.38°B), reducing sugars (3.89%) and ascorbic acid (49.60 mg/ 100 g). The fruits of $T_9$ had the minimum total sugars (9.74%) and $T_{10}$ had minimum β-carotene (10617.19 µg/ 100 g). Sensory evaluation with respect to colour, flavour, texture was non-significant among various treatments under study. It indicated that the organoleptic qualities of fruit were not affected by pre-harvest bagging in mango cv. Ratna. The data show that the difference for fruit shelf-life was significant. The highest shelf-life was noticed in treatments $T_1$, $T_3$ and $T_6$ (17.83 days) and was significantly superior over other treatments. The lowest shelf-life was found in $T_4$ (14.17 days). The longer shelf-life of bagged fruits indicated that the effect of bagging persisted even after ripening. The bagging led to higher contents of chemical components such as TSS, total sugars, reducing sugar, acidity and ascorbic acid in guava fruit (Abbasi *et al*., 2).

The percentage of spotted fruits was significantly differed due to various bagging treatments (Table 5). The minimum spotted fruits in mango was observed in treatment $T_1$ (10.00%), which was at par with $T_3$ (13.33%), $T_6$ (16.67%) and $T_2$ (23.33%). maximum spotted fruits were recorded in $T_{10}$ (76.67%). Similarly, the fruits of $T_1$, $T_2$, $T_3$, $T_5$ and $T_6$ were free from stem end rot and anthracnose. Whereas, in unbagged fruits the incidence was 6.67 and 7.33 per cent, respectively. The unbagged fruits had more infestation of mealy bug (8.67%) and fruit fly (11.33%). The fruits of $T_1$, $T_3$, $T_5$ and $T_6$ did not show incidence of mealy bug. Whereas, the treatments $T_1$, $T_2$, $T_3$, $T_5$ and $T_6$ produced fruits free from fruit fly infestation. Bagging provided physical barrier between fruit and pests. The bagging of mango fruits in cv. Amrapali was found superior to increase the quality of fruits in respect of minimum black spotted

**Table 4.** Effect of types of bag on chemical composition, sensory evaluation and shelf-life of mango cv. Ratna fruits at ripe stage.

| Treatment | Titratable acidity (%) | TSS (°Brix) | Reducing sugars (%) | Total sugars (%) | Ascorbic acid (mg/100 g) | β-carotene (µg /100 g) | Sensory evaluation | | | Shelf-life (days) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Colour | Flavour | Texture | |
| $T_1$ (Newspaper bag) | 0.22 ± 0.02 | 22.48 ± 0.07 | 4.13 ± 0.04 | 13.96 ± 0.59 | 58.40 ± 3.39 | 11067.92 ± 134.82 | 7.67 ± 0.24 | 7.69 ± 0.03 | 7.71 ± 0.06 | 17.83 ± 0.71 |
| $T_2$ (Brown paper bag) | 0.23 ± 0.01 | 22.03 ± 0.57 | 4.09 ± 0.05 | 13.54 ± 0.68 | 55.20 ± 3.39 | 11416.26 ± 145.19 | 7.58 ± 0.24 | 7.64 ± 0.04 | 7.60 ± 0.26 | 16.50 ± 0.71 |
| $T_3$ (Scurting bag) | 0.22 ± 0.00 | 22.47 ± 0.28 | 4.14 ± 0.07 | 13.68 ± 0.72 | 57.60 ± 4.53 | 10952.66 ± 147.79 | 7.79 ± 0.06 | 7.58 ± 0.05 | 7.56 ± 0.27 | 17.83 ± 0.24 |
| $T_4$ (Transparent PP bag) | 0.29 ± 0.01 | 21.75 ± 0.12 | 3.98 ± 0.04 | 11.78 ± 0.64 | 56.80 ± 1.13 | 11497.76 ± 333.05 | 7.25 ± 0.12 | 7.17 ± 0.06 | 7.27 ± 0.27 | 14.17 ± 0.24 |
| $T_5$ (Butter paper bag) | 0.25 ± 0.02 | 22.10 ± 0.33 | 4.01 ± 0.05 | 10.68 ± 0.56 | 54.00 ± 2.83 | 10965.87 ± 129.64 | 7.62 ± 0.07 | 7.70 ± 0.06 | 7.61 ± 0.19 | 17.00 ± 0.47 |
| $T_6$ (Muslin cloth bag) | 0.23 ± 0.01 | 22.35 ± 0.21 | 4.16 ± 0.01 | 13.78 ± 0.71 | 58.00 ± 5.09 | 11575.59 ± 140.01 | 7.65 ± 0.21 | 7.61 ± 0.28 | 7.58 ± 0.35 | 17.83 ± 0.24 |
| $T_7$ (Brown paper bag with polythene coating) | 0.28 ± 0.00 | 21.85 ± 0.78 | 3.91 ± 0.15 | 9.80 ± 0.39 | 52.00 ± 3.39 | 10801.93 ± 44.79 | 7.75 ± 0.47 | 7.33 ± 0.70 | 7.30 ± 0.31 | 16.17 ± 0.24 |
| $T_8$ (Black polythene bags) | 0.30 ± 0.03 | 21.38 ± 0.07 | 3.89 ± 0.01 | 10.16±0.98 | 49.60 ± 2.26 | 10650.90 ± 82.93 | 7.78 ± 0.04 | 7.56 ± 0.32 | 7.25 ± 0.59 | 14.83 ± 0.24 |
| $T_9$ (Opaque white polythene bag) | 0.27 ± 0.00 | 21.52 ± 0.02 | 3.93 ± 0.09 | 9.74 ± 0.34 | 50.00 ± 2.83 | 10801.93 ± 304.06 | 7.61 ± 0.28 | 7.54 ± 0.29 | 7.25 ± 0.59 | 16.50 ± 1.18 |
| $T_{10}$ Control (No bagging) | 0.26 ± 0.01 | 21.50 ± 0.33 | 3.90 ± 0.12 | 10.32 ± 1.03 | 53.20 ± 5.09 | 10617.79 ± 98.41 | 6.88 ± 0.29 | 7.36 ± 0.84 | 6.79 ± 0.88 | 15.33 ± 0.47 |
| Range | 0.22 - 0.30 | 21.38 - 22.48 | 3.89 - 4.16 | 9.74 - 13.96 | 49.60 - 58.40 | 10617.19 - 11575.59 | 6.88 - 7.79 | 7.17 - 7.70 | 6.79 - 7.71 | 14.17 - 17.83 |
| Mean | 0.25 | 21.94 | 4.01 | 11.74 | 54.48 | 11034.86 | 7.56 | 7.52 | 7.39 | 16.40 |
| CD at 5% | 0.02 | 0.42 | 0.11 | 0.77 | 3.57 | 170.70 | NS | NS | NS | 0.67 |

**Table 5.** Effect of types of bag on percent spotted fruits, and infestation of pest and disease on fruits at harvest in mango cv. Ratna.

| Treatment | Spotted fruits (%) | Disease (%) | | Pest (%) | |
|---|---|---|---|---|---|
| | | Stem end rot | Anthracnose | Mealy bug | Fruit fly |
| $T_1$ (Newspaper bag) | 10.00 ± 4.71 | 0.00 | 0.00 | 0.00 | 0.00 |
| $T_2$ (Brown paper bag) | 23.33 ± 4.71 | 0.00 | 0.00 | 3.33 ± 0.94 | 0.00 |
| $T_3$ (Scurting bag) | 13.33 ± 9.43 | 0.00 | 0.00 | 0. 00 | 0.00 |
| $T_4$ (Transparent PP bag) | 73.33 ± 9.43 | 4.00 ± 1.89 | 4.67 ± 0.94 | 2.00 ± 0.94 | 6.00 ± 0.94 |
| $T_5$ (Butter paper bag) | 40.00 ± 9.43 | 0.00 | 0.00 | 0.00 | 0.00 |
| $T_6$ (Muslin cloth bag) | 16.67 ± 4.71 | 0.00 | 0.00 | 0.00 | 0.00 |
| $T_7$ (Brown paper bag with polythene coating) | 53.33 ± 9.43 | 3.33 ± 0.94 | 1.33 ± 0.00 | 4.67 ± 0.94 | 3.33 ± 0.94 |
| $T_8$ (Black polythene bags) | 66.67 ± 9.43 | 3.33 ± 0.94 | 4.67 ± 0.94 | 7.33 ± 0.94 | 4.67 ±0.94 |
| $T_9$ (Opaque white polythene bag) | 60.00 ± 9.43 | 2.00 ± 0.94 | 4.00 ± 0.00 | 6.00 ± 0.94 | 4.00 ± 0.00 |
| $T_{10}$ Control (no bagging) | 76.67 ± 4.71 | 6.67 ± 1.89 | 7.33 ± 0.94 | 8.67 ± 0.94 | 11.33 ± 0.94 |
| Range | 10.00 - 76.67 | 0.00-6.67 | 0.00 - 7.33 | 0.00-8.67 | 0.00-11.33 |
| Mean | 43.33 | 1.93 | 2.20 | 3.20 | 2.93 |
| CD at 5% | 15.02 | 2.75 | 1.75 | 1.90 | 1.37 |

fruits per cent among all treatments (Jakhar and Pathak, 8). In mango cv. Carabao, the incidence of fruit fly was reduced considerably by pre-harvest bagging (Buganic, 5).

Thus, the present study indicated that pre-harvest bagging (newspaper, scruting and muslin cloth) of mango cv. Ratna at egg stage by different types of bag proved to be beneficial for disease and pest-free fruit production with desirable fruit quality.

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# Estimation of mango growing areas using remote sensing

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## ABSTRACT

**An attempt was made to estimate acreage and to depict the spatial distribution of mango growing areas using linear imaging self-scanning image of remote sensing data for Saharanpur district of Uttar Pradesh. The image was processed using ENVI software. Unsupervised, supervised and decision tree classification were used for the acreage estimation. The study has clearly demonstrated the usefulness of remote sensing image for identifying and acreage estimation of mango orchards. The results also indicated that mango acreage estimation was more accurate in decision tree approach (92.92%) as compared to other two methods (81.73 and 80.43%, respectively). The overall result revealed that the finer resolution the time series data, better the accuracy of area estimation. The above study proves that the remote sensing data could be effectively used for other perennial horticultural crops with finer resolution time series data.**

**Key words**: Mango, acreage estimation, remote sensing, decision tree.

## INTRODUCTION

Remote sensing in general term, is described as the act of gathering data from a distance, which involves sensing and recording reflected or emitted energy and processing, analyzing and applying that information. Remote sensing technology has many attributes that would be beneficial for detecting, mapping and monitoring of vegetation. It is a tool offering well-documented advantages including a synoptic view, multispectral data, multi-temporal coverage and cost effectiveness. It has received considerable importance in the field of biological sciences in the recent years. There was wide consensus that remotely sensed data can provide an accurate and repeatable means of land cover mapping and monitoring, especially with respect to areas with rapidly changing land use and land management activities (Townshend *et al*., 13). In particular, remote sensing based approaches make use of the distinct spectral reflectance from different land cover types in association with the temporal variation of reflected radiation caused by the phenological dynamics in vegetation (Justice *et al*., 4; Loveland *et al*., 5).

The use of remotely sensed data in crop acreage estimation has been demonstrated by various researchers in different parts of the world (Saha and Jonna,10; Nualchawee, 8). Atkinson and Lewis (1) reported that this process primarily uses the spectral information provided in the remotely sensed data to discriminate between perceived groupings of vegetative cover on the ground by using the spatial and temporal information included in single date data and time series data, respectively. Gordon *et al*. (2) explored the usefulness of Landsat thematic mapper (TM) data for inventorying trees. They developed a unique signature from the orchard reflectance and subsequently used in area estimation. Gupta and Sharma (3) visually interpreted to delineate the various mango orchards in Malihabad tehsil of Lucknow, Uttar Pradesh using Landsat-5 TM satellite data. Yadav *et al.* (14) estimated acreage and mango production using Linear Imaging Self-Scanning System (LISS) LISS-II and LISS-III data. Temporal Indian Remote Sensing (IRS) Advanced Wide Field Sensor (AwiFS) (spatial resolution was 55 m) data were used to select optimum dates for its identification of apple orchards. IRS LISS III data (with spatial resolution 23 m) was used for area estimation of fruits like apple (Sharma and Shusma, 12), citrus, grapes and plantation crops (Palaniswami *et al.*, 9). Sahoo *et al*. (11) have worked out the composition of forest trees from multispectral high-resolution IKONOS data through spectral angle mapper classification technique. Nicolas and Chin (7) reported that supervised classification was done on SAR/ INSAR data set, using training areas selected in the TM image of swamp forest to derive four classes such as forest, rice, coconut and rubber.

Mango is one of the most widely grown fruits and till date very little or no spatial mapping of its growing areas has been done. Remote sensing is a powerful tool for mapping, monitoring and management of agricultural and horticultural resources. The application of remote sensing technology in combination with Geographic Information System (GIS) in area estimation of fruits like mango, banana,

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apple, citrus, grapes and plantation crops are meagre. Therefore, the present study was undertaken to estimate the acreage of mango orchards using multispectral remote sensing data.

## MATERIALS AND METHODS

Saharanpur district of Uttar Pradesh lies between 29° 34'45'' to 30° 21'30'' N latitude, 77° 09' to 78° 14'45'' E longitude and has a total area of 3,860 km$^2$. The major horticultural crops grown are mango and guava, followed by agricultural crops like wheat, rice, maize, *jowar, bajara*, sugarcane, oilseeds, cotton and jute. The data used in the study include satellite and collateral data of Saharanpur district. The later was collected from Department of Horticulture and National Horticulture Database (6). The multispectral satellite data of LISS-III of IRS -P6 was used for the study. The date of satellite pass was November 22, 2007. The path and row of satellite pass was 54 and 96, respectively. The detail technical specifications of the data product are given in Table 1.

Field survey was conducted for collection of detail ground truths about mango growing areas. Location coordinates in terms of latitude and longitude were recorded with the help of hand held GPS (Leica GS 5) under WGS 84 (Lat-Lon) coordinate system. Ground truth information was further used for classification of mango areas from satellite images. The vector map of Saharanpur district was prepared from the hard copies of district map, which were scanned and saved in JPG format. The JPG file was opened in GIS software, ARCGIS (ver 8.3) and digitization was done. The vector boundaries of Saharanpur district were obtained. The projection of the maps was defined as Geographic Latitude Longitude with WGS 84 as datum.

**Table 1.** Characteristic of data set of LISS III used in the study.

| Parameter/ Sensor | Data set characteristics |
|---|---|
| Spectral bands | |
| Band 2 (Visible) | 0.52 - 0.59 microns |
| Band 3 (Visible) | 0.62 - 0.68 microns |
| Band 4 (NIR) | 0.77 - 0.86 microns |
| Band 5 (SWIR) | 1.55 - 1.70 microns |
| Resolution | 23.5 m (Visible and near IR region) |
| Revisit | 24 days |
| Swath | 141 km (Visible and near IR region) |
| Path-row | 54 and 96 |
| Saturation radiance (mw/ cm$^2$/ sr/ micron) | B2 28-31<br>B3 25-38<br>B4 27-30<br>B5 6.9 |

The digital image processing software ENVI (ver. 4.3) was used. The pre-processing involves importing images and geometric corrections, which were done following the routine procedure and using Ground Control Points (GCPs) collected during ground trothing. The image was resized to cover only Saharanpur district using vector map of the study area. Three classification approaches were carried out, namely, unsupervised iso-data classification, supervised maximum likelihood classification and decision tree based classification to retrieve mango-growing areas. In case of isodata classification approach, the image was classified to 10 clusters. The mean spectral profile of each cluster was prepared and drawn together. Based on ground truth information, the spectral profiles were identified and mango-growing areas were classified. The training areas of mango growing and non-growing areas along with urban, river bed sand, agricultural land, fallow land and water bodies was done based on training sites using maximum likelihood classification approaches and the statistics for mango growing areas were generated.

The third approach was the decision tree based classifier, which performs multistage classifications by using a series of binary decisions to place pixels into classes. The spectral ranges of mango growing areas were defined based on training sites. The decision rule was made accordingly to separate mango growing areas from the image. The acreage estimated by remote sensing method was assessed for its accuracy and reliability using the National Horticultural Board (NHB) database (6).

## RESULTS AND DISCUSSION

The LISS-III image of Saharanpur is shown in both grey scale and false colour composite (FCC) with district vector boundary in Fig. 1 and zoomed closer view of the mango growing area in red colour is shown in Fig. 2b-c. The iso-data unsupervised classification of the image having 10 classes is depicted in Fig. 3. All these classes were identified based on their spectral profile and ground truth information. The mango growing areas are shown in green colour and its statistics was computed (Table 2). For supervised classification, 8 training sites were identified on the image based on ground truth. Maximum likelihood classification was done and classified image is shown in Fig. 4. The green colour shown in the classified image is the mango growing area. The area statistics was calculated and data is shown in Table 2.

The image was classification based on the decision tree approach and the classified image shown in yellow colour is the mango growing areas extracted from the image and the flow of decision

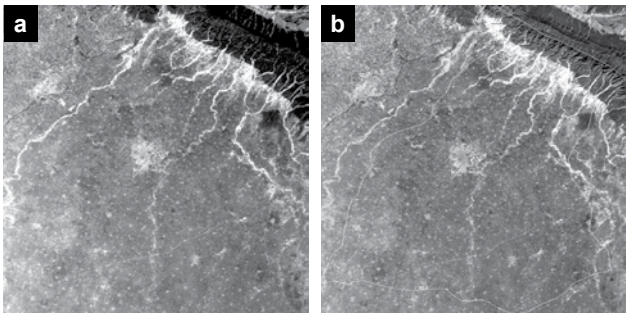


**Fig. 1.** a) Grey scale image and b) FCC of LISS-III data of Saharanpur district with district vector boundary.

**Table 2.** Classification accuracy of mango area estimated for LISS-III.

| Area (ha) | Actual area (ha) | Estimated area (ha) UNSU | Estimated area (ha) SU | Estimated area (ha) DT |
|---|---|---|---|---|
| Saharanpur LISS | 25,946 | 32,256 (24) | 31,743 (15) | 27,922 (8) |

used are depicted in decision tree, which is shown in Fig. 5. The total mango growing area calculated is depicted in Table 2. The mango area estimated through different approaches was compared with the statistics of the mango area obtained from the Department of Horticulture, Saharanpur as shown in Table 2.

Different classification approaches were evaluated for getting reliable statistics of mango growing area. While classifying LISS-III image of Saharanpur district and comparing with the ground truth and the statistics obtained from Government agency, it revealed that decision-tree based classification yielded better accuracy followed by maximum likelihood and unsupervised classification approaches. The unsupervised isodata classification approach is mainly based on statistical rules, and ground information has not been considered, thereby accuracy level of the results was very low. In case of supervised classification, *i.e.* maximum likelihood classification, the training sites were selected based on ground

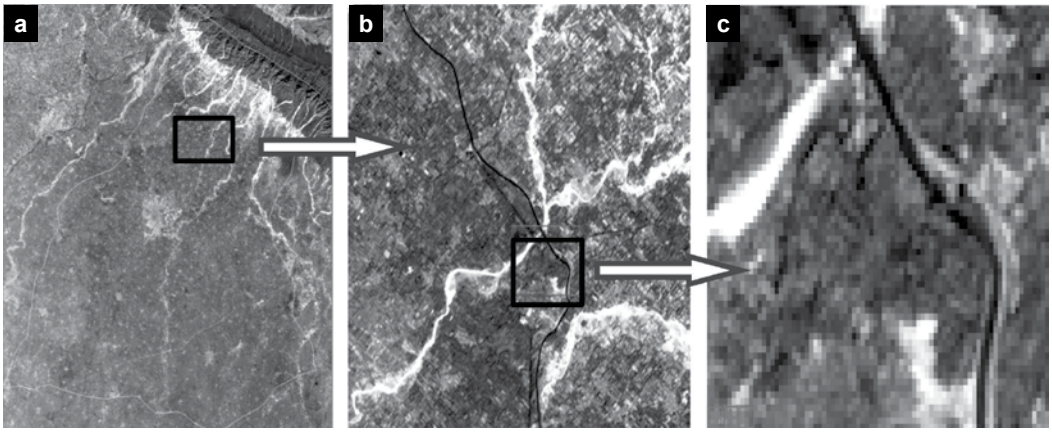


**Fig. 2.** a) FCC of LISS-III image as seen in zoomed windows (b, c) for an area having mango orchards.

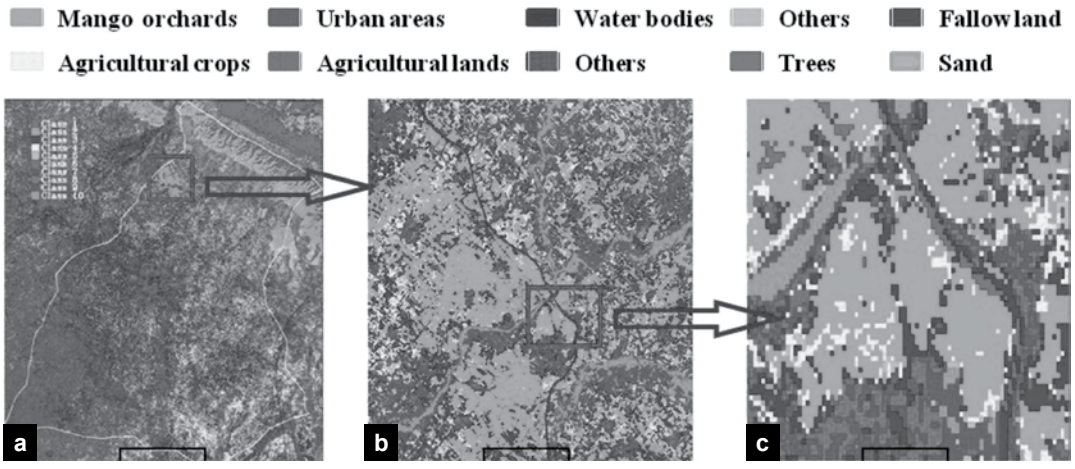


**Fig. 3.** a) Unsupervised classification of LISS-III image showing 10 different classes in (b) closer view and (c) a zoom of 4X.

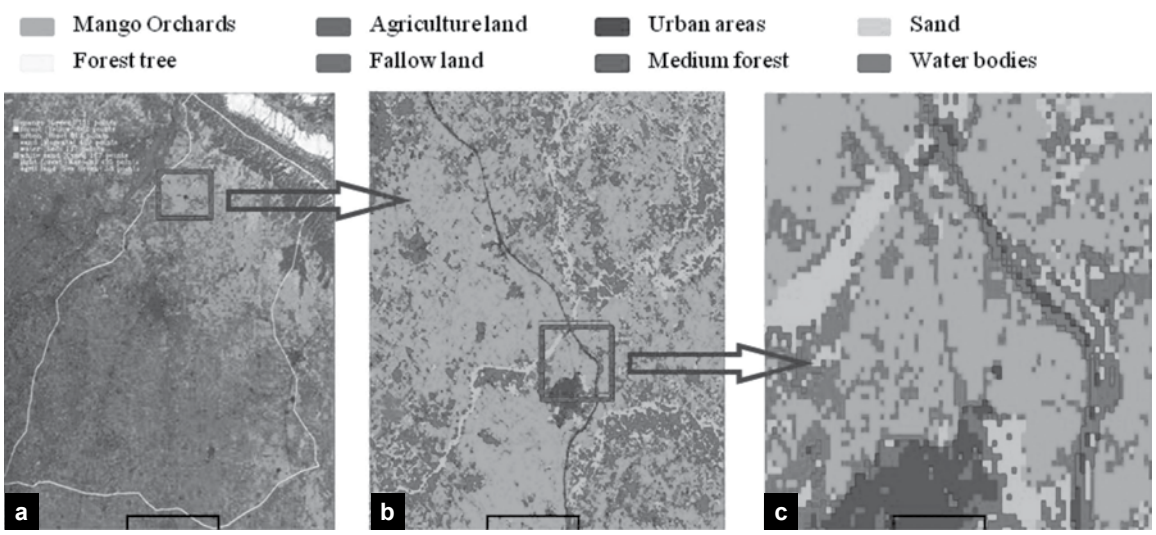


**Fig. 4.** a) Supervised classification of LISS-III image showing different classes (b) in closer view and (c) a zoom view 4X.

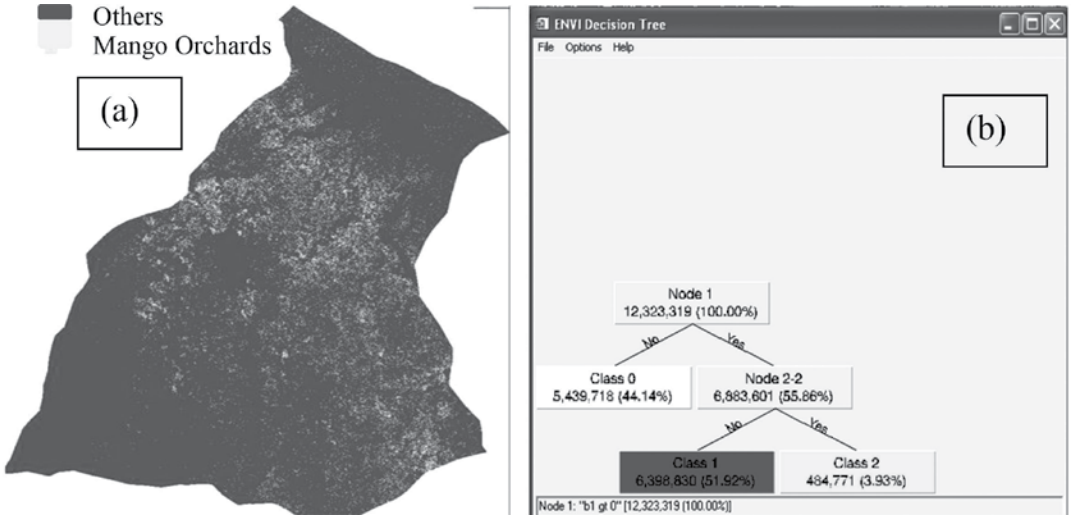


**Fig. 5.** Decision tree based classification of LISS-III image (a) yellow colours showing mango growing areas and maroon colour non mango growing areas (b) showing the decision tree with each node.

information and classification was done based on the training sites. This improved the accuracy over unsupervised classification. In case of decision tree based classification, the decision rules were made based on the spectral values of the training sites. This classification performs multistage classifications by using a series of binary decisions to place pixels into classes. Each decision divides the pixels in a set of images into two classes based on an expression.

The study clearly demonstrated the usefulness of Indian remote sensing data for identifying the mango orchards and acreage estimation. The decision tree approach was found to be more reliable, *i.e.*, 11.19-12.49% (Table 2) more accurate when compared to supervised and unsupervised classification for estimation of mango acreage. In future, remote sensing data of higher resolution and hyper-spectral data can be used to monitor, estimate acreage, spatial mapping of fruits crops distribution, to monitor the stress of fruit crops and diseases like malformation in mango orchards and other fruit crops.

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# Effect of consortia of potassium solubilizing bacteria and fungi on growth, nutrient uptake and yield of banana

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## ABSTRACT

**In the present study, three potassium solubilizing bacteria (KSB) *Pseudomonas* sp. (KSB 49, 239.75 mgl$^{-1}$; KSB 43, 228.25 mgl$^{-1}$ and KSB 47, 205.75 mgl$^{-1}$) and three potassium solubilizing fungi (KSF) *Aspergillus* sp. (KSF 3, 334.66 mgl$^{-1}$; KSF 13, 310.16 mgl$^{-1}$ and KSF 31, 297.66 mgl$^{-1}$) were found to be efficient to release potassium from mica as compared with the commercial potassium solubilizing bacterial (CKSB) strain *Frateuria aurantia* (CKSB1, 221.25 mgl$^{-1}$). Based on the compatibility test, the consortia of efficient potassium solubilizers were designed separately. The liquid bio-formulation for KSB consortium and talc powder for KSF consortium was found to be effective delivery system in banana field. For their influence on growth, nutrient uptake and yield of banana under field conditions with 13 treatments including KSB and KSF consortia with the application of graded levels of $K_2O$ (50, 75 and 100% RDF). The fungal consortium + 75% $K_2O$ treatment showed higher potential followed by bacterial consortium + 75% $K_2O$ treatment. The application of KSF consortium along with 75% $K_2O$ ($T_6$) recorded the highest marketable number of hands per bunch, fingers per hand, bunch weight and total yield (9.22, 16.22, 28.55 kg and 126.87 t/ha, respectively). The maximum total potassium uptake of 1198.17 kg/ha was recorded by KSF consortium + 75% $K_2O$ ($T_6$), which was at par with KSB consortium + 75% $K_2O$ ($T_3$) (1177.21 kg/ha). These findings clearly indicated that addition of KSF and KSB in the nutrient schedule, 25% savings of the potassium fertilizers, *i.e.*, 222.22 $K_2O$ kg/ha was possible.**

**Key words:** Potassium solubilization, *Pseudomonas* sp., *Aspergillus* sp., banana.

## INTRODUCTION

Banana is globally fourth most important fruit crop and India is the largest producer. Potassium ($K^+$) plays an important role in growth and development of plant and most abundant cations in the tissues of banana often up to 3 to 4 per cent of dry weight. One tonne of banana is estimated to remove 17 to 20 kg potassium (Bhalerao, 5). Potassium is one of the major nutrients limiting plant growth and nutrient balance sheets in most of the Indian soils have been negative. In India, most of the farmers have mainly focused on the application of nitrogen (N) and phosphorus (P) for crop production. The bio-intervention of waste mica with potassium solubilizing microorganisms could be another alternative to solubilize insoluble K in mica into plant available pool and used efficiently as a source of K fertilizer for sustaining crop production and maintaining soil potassium. A wide range of bacteria, namely, *Pseudomonas*, *Burkholderia*, *Acidothiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, *B. circulans* and *Paenibacillus* sp. have been reported to release potassium in accessible form from potassium bearing minerals in soils (Liu *et al.*, 22). *Aspergillus niger* (KF 1) has showed highest potassium solubilization and acid production by utilizing feldspar and potassium aluminium silicate as an insoluble source of potassium (Prajapati *et al.*, 15). In India, there is no reserve of K-bearing minerals for manufacturing of K fertilizers and the whole consumption of K fertilizers are imported, which leads to a huge amount of foreign exchange. Therefore, the application of potassium solubilizing microorganism is a promising approach for increasing K availability in soils. In the present study, efficient potassium solubilizers were subjected to release potassium from potassic mineral (mica). The three most efficient potassium solubilizing isolates of bacteria (*Pseudomonas* sp.) and fungi (*Aspergillus* sp.) were selected for development of consortium as delivery in the form of liquid /carrier bio-formulations in the banana.

## MATERIALS AND METHODS

A total of 42 potassium solubilizing bacterial (KSB) and 30 potassium solubilizing fungal (KSF) isolates were isolated from rhizosphere soil samples of banana, sugarcane, cabbage, potato, guava collected from 10 districts of western and northern Maharashtra, *viz*., Pune, Kolhapur, Sangali, Satara, Solapur, Ahmednagar, Nasik, Jalgaon, Nandurbar and Dhule in the year 2014-15. All the bacteria and

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fungi were isolated on Aleksandrov's agar medium Hu *et al.* (9) and Glucose-yeast extract-$CaCO_3$ agar medium Lisdiyanti *et al.* (11). From that, three efficient *Pseudomonas* sp. and three efficient *Aspergillus* sp. from rhizosphere soils of banana were selected for formation of consortia and field study. These isolates were identified using standard cultural, morphological and biochemical methodology. The bacterial and fungal isolates were examined for their ability to release K from Aleksandrov's broth (supplemented with 1% Muscovite mica). One ml of overnight culture of each of isolate was inoculated in to 25 ml of Aleksandrov's broth Hu *et al.* (9) and available K content was determined by flame photometry.

The tested microorganism, *viz.*, bacteria with bacteria, fungi with fungi and bacteria with fungi were inoculated in 25 ml glucose-yeast extract-$CaCO_3$ broth (dual culture) as per 21 combinations and incubated at 28 ± 2°C for 3-4 days. The compatibility test was carried out for the development of consortium of three selected efficient isolates of KSB (*Pseudomonas* sp.) and KSF (*Aspergillus* sp.). On the basis of compatibility test, the bioformulations of bacterial and fungal consortia were prepared separately. The three most efficient *Pseudomonas* sp. were grown on five test media for finding out the most suitable medium for mass multiplication of KSB consortium. For the preparation of suitable medium and to increase the shelf-life of *Pseudomonas* sp. the base material which contains emulsifier, dispersant, cell protectant, moisturizer and humectants *etc*. were used in different concentrations (Chandra, 7). Sterilized glucose-yeast extract-$CaCO_3$ broth was inoculated with a loopful of efficient isolates of KSB cultures and kept for incubation at 28 ± 2°C for 48 h in a shaker BOD at 130 rpm/ min. After attending the full growth ($10^9$ cfu/ ml), the bioinoculant was transferred separately into each of the sterilized test media at 1:3 ratio and kept for incubation at 28 ± 2°C for 72 h in shaker BOD at 130 rpm/ min. The formulations were stored in sterile plastic bottles after taking initial count.

The most efficient three *Aspergillus* sp. were multiplied on potato dextrose broth culture. The inocula thus obtained, were harvested and used for preparation of talc (adding with carboxymethyl cellulose) based and lignite based formulations Singh *et al.* (18). Initial population count was taken and packed in polypropylene bag and it was used for field experiment. The liquid (KSB)/ carrier (KSF) based inoculum was further tested for its shelf-life by recording cfu count at monthly interval for six months by serial dilution technique Warcup (33) on Glucose-yeast extract-$CaCO_3$ agar medium plates. The colonies were counted with the help of the colony counter after 48 and 72 h of incubation and expressed in terms of cfu/g or cfu/ml of bio-formulation. The survival percentage of potassium solubilizers in different bio-formulations were calculated as survival (%) is equal to population count at 180 days/ maximum population count × 100.

A field experiment was conducted at the Research Farm of Deptt. of Plant Pathology and Agricultural Microbiology in the year 2012-13 using bacterial (three efficient *Pseudomonas* sp.) and fungal (three efficient *Aspergillus* sp.) consortia bio-formulations in comparison with commercial formulation, KEMOFER *i.e.* potassium mobilizing potassium bio-fertilizer, Coimbatore, Tamil Nadu, to study their performance in enhancing the growth, K uptake and yield of banana cv. Grand Naine. The soil type was sandy loam in texture. The soil pH was 7.9, EC (0.15 dS $m^{-1}$), available nitrogen (209.05 kg $ha^{-1}$), available phosphorus (18 kg $ha^{-1}$), available potassium (408.66 kg $ha^{-1}$) and population of KSB ($6.66 \times 10^3$ cfu /g) and KSF ($2.33 \times 10^2$ cfu /g). The field was uniformly levelled and pits were dug at 1.5 m × 1.5 m spacing. The experiment was laid out in Randomized Block Design with three replications and 13 treatments including recommended dose of fertilizers (200 g N + 40 g $P_2O_5$ + 200 g $K_2O$ + 10 Kg FYM $plant^{-1}$). Bacterial consortium with 50, 75 and 100% $K_2O$, fungal consortium with 50, 75 and 100% $K_2O$, bacterial + fungal consortia with 50, 75 and 100% $K_2O$, commercial formulation with 50, 75 and 100% $K_2O$. The experiment was conducted under irrigated conditions. Bacterial consortium and fungal consortium were applied through seedling dip treatment and through soil at monthly interval four times as per the application of $K_2O$. Nitrogen and potassium were applied through the soil at monthly interval in seven and four splits, respectively (Bhalerao, 5). Phosphorus was applied as basal dose with FYM and bio-formulations through soil at the time of planting. Both consortia (3 ml or 3 g) were mixed with 3 kg FYM and then applied to soil.

Representative surface and subsurface soil samples were collected from each plot before planting, shooting and at harvest of the main banana plot for microbial population dynamics study and soil available NPK analysis. A known quantity (0.2 g) of the fine powdered plant and fruit samples were digested with 1:1 mixture of concentrated $H_2SO_4$ and $H_2O_2$ (for 30 min.) and this acid extract was used for the determination of N (microKjeldhal), P (vanado-molybdate yellow colour in nitric acid system) and K (flame photometry).

The statistical analysis of the data was carried out by employing completely randomized design (CRD) and randomized block design (RBD). Wherever

F tests was significant and interpretation of the results was carried out in accordance with Panse and Sukhatme (14).

## RESULTS AND DISCUSSION

The amount of potassium released from Muscovite mica by the potassium solubilizing bacterial isolates in Aleksandrov's broth was estimated at 8 days after incubation (DAI) and for KSF isolates 10 days after incubation. The isolates KSB 49 released maximum amount of potassium from mica 239.75 $mgl^{-1}$ followed by KSB 43 (228.25 $mgl^{-1}$), KSB 47 (205.75 $mgl^{-1}$) and control showed 42.81 $mgl^{-1}$. However, isolate KSB 49 recorded the highest potassium solubilisation than commercially used KSB as reference strain (CKSB1) (221.25 $mgl^{-1}$). They were further selected for development of bacterial consortium. The isolate KSF 3 recorded the highest potassium solubilisation (334.66 $mgl^{-1}$) than all other isolates, followed by KSF 13 (310.16 $mgl^{-1}$), KSF 31 (297.66 $mgl^{-1}$) and control showed 42.80 $mgl^{-1}$. They were selected for the development of fungal consortium. The decrease in pH of Aleksandrov's broth from initially adjusted pH of 7.0 was also noted at 8 days after incubation for KSB isolates and 10 days after incubation for KSF isolates. A reduction in pH of the medium, *i.e.* pH 5.65 was recorded by KSB 49 isolate followed by KSB 43, CKSB 1 (*F. aurantia*), and KSB 47, which reduced the pH of the medium to 5.71, 5.85 and 5.94, respectively. A reduction in pH of medium was recorded by KSF 3 (3.80) followed by KSF 13 (3.81) and KSF 31 (3.76). The positive relation of decrease in pH of the medium with increasing amounts of potassium solubilization was observed. These findings are in agreement with the findings of Girgis *et al.* (8) who showed that inoculation with selected strains of *Bacillus* sp. UBFBc1 and UBFBa7 released high concentrations of soluble K (236.2 and 195.3 $mgl^{-1}$, respectively) in MA-f (Feldspar) and MA-m (Mica) culture media. In support of the current study, Lopes-Assad (12) also studied two strains (CCT4355 and CCT911) of *Aspergillus niger.* The soluble K, titratable acidity and pH were analyzed and the solubilisation rate (SR) was calculated relative to the total K in the rock powder (2.921 mmolc/l). K-solubilizing capacities of strains KNP413, KNP414 and AS1.153 (*Bacillus mucilaginosus*) were monitored up to 5 days in Aleksandrov medium at 30°C. This lowered the medium pH from 7.5 to 5.12 at 4 days post-incubation; thereafter, pH of the medium remained stable. *Aspergillus terreus* solubilized (KF 2) insoluble potassium well in a liquid medium supplemented with Feldspar and caused a remarkable drop in pH (2.2 at 7 days of incubation) of culture medium. *Aspergillus niger* (KF 1) also showed remarkable drop in pH 2.7 at 7 days of incubation (Prajapati *et al.*, 15).

Three potassium solubilizing bacterial isolates (*Pseudomonas* sp.) were compatible with each other and three potassium solubilizing fungal isolates (*Aspergillus* sp.) were compatible with each other, but KSB and KSF were not compatible with each other. As per the results, a consortium of *Pseudomonas* sp. and a consortium of *Aspergillus* sp. were developed separately in the form of bio-formulations. Compatibility of the inoculants *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* tested through cross streak plate assay. The inoculants were found to be compatible with each other and were able to grow simultaneously without any inhibition in growth Anandaraj (2).

At 60 days, the test Medium 3 showed highest population $24.6 \times 10^8$ cfu/ ml of formulation. After the second month, the population of test media 3, 4 and 5 decreased, but test Medium 3 showed maximum population ($8.3 \times 10^8$ cfu/ ml) at 180 days (Fig. 1). Out of all five test media, the survival percentage in test Medium 3 was highest (33%) among all the tested media. Hence, the test Medium 3 was selected as the excellent liquid bio-formulation for KSB consortium for efficient delivery in the field. Further, the population in test medium 3 up to 360 days period was carried out. At 210 to 360 days, the population decreased from 7.1 to $0.1 \times 10^8$ cfu/ ml of formulation. The survival percentage (at cfu count, $\times 10^8$ cfu/ ml) for 0 to 360 days was 0.40%. Similar results were also obtained by Chandra (7) who studied the shelf-life of liquid inoculum *vs* carrier based inoculum of potassium mobilizing bacteria (KMB). KMB solid medium maintained the shelf-life up to 6 months but in suggested case of liquid formulations in KMB survived up to two years. This suggested the superiority of liquid formulation over carrier base formulations. The PMB and KMB bacteria retained their population up to $10^8$/ ml up to 12 months. The survivability of KSF 3, KSF 13 and KSF 31 was studied in two carriers, *i.e.*, talc and lignite. The shelf-life of different test carriers was carried out for 0-180 days (Fig. 2). In case of carrier based bio-formulations, the test medium talc powder showed highest population of $7.3 \times 10^7$ cfu/ ml at 8 days period and decreased later up to six months $2.0 \times 10^7$ cfu/ ml of formulation. The survival percentage was 27% in talc powder test medium as compared to the lignite (8.0%) (Fig. 2). Therefore, the talc powder was found to be an excellent bio-formulation of KSF consortium for efficient delivery in the field. In support of these findings, talc-based bio-formulation was found to be the best material to retain maximum number of viable propagules, *i.e.*, $29.7 \times 10^6$ cfu/g at 180 days of storage. It has also been found that the isolates can

retain their viability up to 120 days in all the cases (Shahid *et al.*, 16). The strains of *A. niger* fungus have high potential of K solubilization and could be used as alternative to chemical fertilizers (Lopes-Assad, 12). However, work available on the application of potassium solubilizing fungi (*Aspergillus* sp.) under field conditions is very scanty.

Based on the highest efficiency of K solubilization of selected three *Pseudomonas* sp. and three *Aspergillus* sp., developed as liquid bio-formulation of KSB consortium and talc formulation of KSF consortium were further examined for their performance to enhance growth, nutrient uptake and yield of banana. The number of leaves was more at the shooting stage. Among the treatments highest number of leaves were recorded with treatment having fungal consortium (KSF) with 75% $K_2O$ ($T_6$) (16.00 and 13.33 leaves/ plant, at shooting and harvesting stages, respectively) followed by treatment with bacterial (KSB) consortium with 75% $K_2O$ ($T_3$) (14.33 and 12.66 leaves/ plant) at both the stages as compared to commercial formulation and RDF ($T_1$). The application of 75% $K_2O$ showed higher No. of leaves than application of 100 and 50% $K_2O$ at both the stages. The pseudostem height and girth increased rapidly up to shooting stage and later on marginally up to harvest. The application of KSB consortium + 75% $K_2O$ ($T_3$) recorded the highest pseudostem height of 197.77 and 226.55 cm at both stages respectively, which was at par with the treatment of KSF consortium + 75% $K_2O$ ($T_6$) (197.33 and 224.55 cm, respectively). The pseudostem girth was significantly influenced by the application of potash level. The application of KSF consortium with 75% $K_2O$ ($T_6$) recorded maximum pseudostem girth of 59.33 and 68.77 cm at shooting and at harvest stage respectively, and it was at par with the treatment of KSB consortium with 75% $K_2O$ ($T_3$) (57.77 and 64.66 cm, respectively). The pseudostem height and girth of both the treatments were highest as compared with the application of commercial formulation and RDF ($T_1$). The lowest pseudostem height and girth were recorded in the treatment with combined application of KSB consortium and KSF consortium with 50% $K_2O$ ($T_8$) at both the stages, but in case of pseudostem, the height was lowest in treatment with commercial formulation and 50% $K_2O$ ($T_{11}$) at the

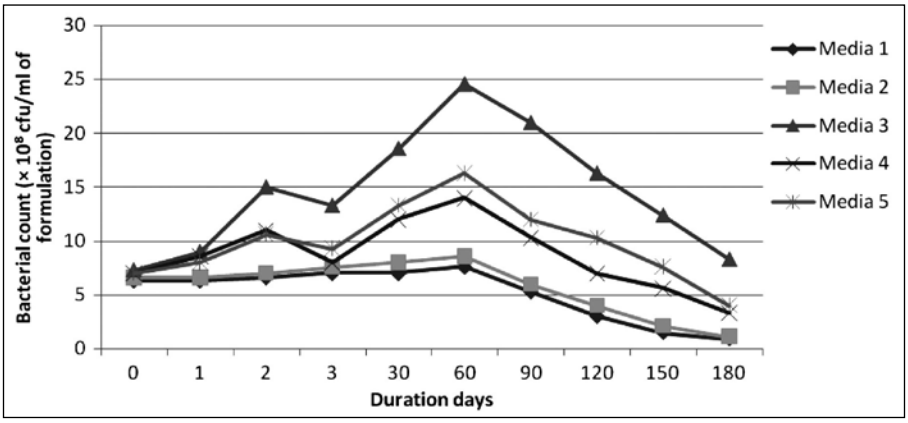


**Fig. 1.** Population of potassium solubilising bacteria (KSB) in liquid bio-formulations.

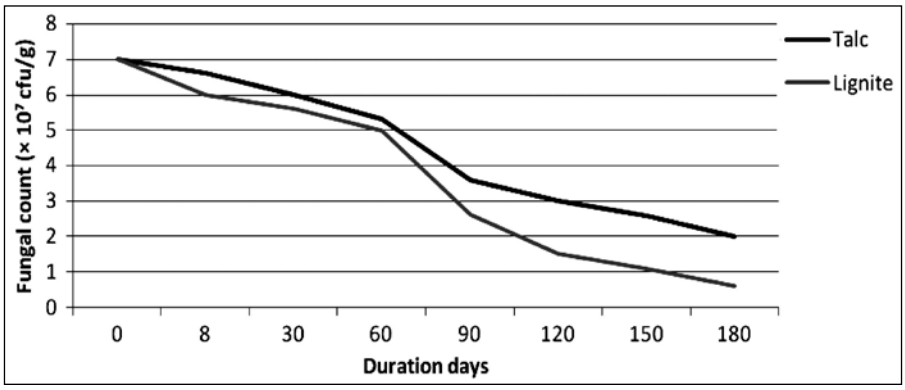


**Fig. 2.** Population of potassium solubilising bacteria (KSB) in carrier based bio-formulations.

shooting stage (175.11 cm) (Table 1). In the present study, the results obtained related to plant growth (Table 2) are comparable with the results of Sheng (17) who reported that silicate dissolving bacteria could improve soil P, K, Si reserves and promote plant growth. Similar observations on growth by KSB have been reported by several workers (Abou-el-Seoud and Abdel-Megeed,1; Badr, 4). Potassium solubilizing bacteria (*Bacillus* and *Pseudomonas*) were examined for the production of IAA and GA. All the isolates produced IAA and GA in the range of 1.10 to 16.50 and 0.60 to 3.29 µg/ 25 ml broth, respectively Archana *et al.* (3). Evidences exist which indicate that some *A. niger* isolates also produce IAA and other phytohormones Mostafa and Yovssef (13), which significantly increased the growth and yield.

At shooting stage, the rhizosphere population of potassium solubilizing bacteria (KSB) (21.66 × $10^5$ cfu/ g soil) was significantly higher when KSB consortium + 50% $K_2O$ ($T_2$) was applied, followed by KSB consortium + 75% $K_2O$ ($T_3$) (18.00 × $10^5$ cfu/ g soil), which were at par with each other. The KSB population increased rapidly up to shooting stage and later on marginally up to harvest (Table 2). At shooting, the rhizosphere population of potassium solubilising fungi (8.33 × $10^4$ cfu/g soil) was significantly higher with application of KSF consortium + 50% $K_2O$ ($T_5$), followed by fungal consortium + 75% potassium ($T_6$) (7.33 × $10^4$ cfu/g soil), which was at par with each other. At harvest, the rhizosphere population of potassium solubilising fungi (5.33 × $10^3$ cfu/g soil) was significantly higher when fungal consortium + 50% $K_2O$ ($T_5$) given, followed by KSF consortium + 75% $K_2O$ ($T_6$) and 100% $K_2O$ ($T_7$). The application of bacterial consortium, commercial consortium and combination of bacterial and fungal consortium and RDF did not differ with each other (Table 2). These results could be mainly due to production of growth hormones and may be due to release of organic acids. The total number of bacteria increased due to inoculation of *B. mucilaginosus* (KSB) from 8.4×$10^3$ to 9.6 ×$10^6$ cfu/ g, respectively compared to uninoculated control in the experiment of groundnut plant. *Pseudomonas* has ability to colonize rhizosphere of a wide variety of crops including cereals, pulses, oilseeds and vegetables (Chambel *et al.*, 6).

At shooting, the available N, P and K in soil (312.17, 25.80 and 593.60 kg/ha, respectively) were significantly higher when KSF consortium + 75% $K_2O$ ($T_6$) were given, followed by KSB consortium + 75% $K_2O$ ($T_3$) (310.85, 25.40 and 582.40 kg/ha, respectively), which was at par with each other for

**Table 1.** Effect of soil application of microbial (KSB and KSF) consortia under graded levels of potassic fertilizer on growth and duration of banana.

| Treatment | No. of leaves/ plant | | Height (cm) | | Pseudostem girth (cm) | | No. of days for shooting | No. of days for maturation |
|---|---|---|---|---|---|---|---|---|
| | Shooting | Harvest | Shooting | Harvest | Shooting | Harvest | | |
| $T_1$ | 11.11 | 11.00 | 179.89 | 197.33 | 49.00 | 57.77 | 242.55 | 347.33 |
| $T_2$ | 12.67 | 10.33 | 184.67 | 210.00 | 50.77 | 58.55 | 237.00 | 346.11 |
| $T_3$ | 14.33 | 12.66 | 197.77 | 226.55 | 57.77 | 64.66 | 230.67 | 333.33 |
| $T_4$ | 13.55 | 11.78 | 190.33 | 217.67 | 55.99 | 63.66 | 234.11 | 339.77 |
| $T_5$ | 12.22 | 10.89 | 187.33 | 208.55 | 52.44 | 59.99 | 236.67 | 347.22 |
| $T_6$ | 16.00 | 13.33 | 197.33 | 224.55 | 59.33 | 68.77 | 230.00 | 330.44 |
| $T_7$ | 13.33 | 11.89 | 193.44 | 219.22 | 56.81 | 63.44 | 233.67 | 337.88 |
| $T_8$ | 11.00 | 10.55 | 177.77 | 192.89 | 45.00 | 53.11 | 239.78 | 346.44 |
| $T_9$ | 11.00 | 10.66 | 176.88 | 195.77 | 48.22 | 56.77 | 241.00 | 347.11 |
| $T_{10}$ | 12.11 | 11.44 | 186.22 | 205.33 | 49.90 | 59.88 | 234.89 | 346.89 |
| $T_{11}$ | 11.33 | 10.67 | 175.11 | 194.44 | 46.00 | 54.77 | 238.33 | 347.44 |
| $T_{12}$ | 12.66 | 10.89 | 181.44 | 200.40 | 49.44 | 56.44 | 238.22 | 346.66 |
| $T_{13}$ | 13.45 | 11.33 | 187.88 | 212.77 | 54.77 | 61.67 | 234.00 | 344.77 |
| CD at 5% | 2.77 | 1.65 | 2.15 | 2.92 | 1.41 | 1.22 | 2.26 | 5.11 |

$T_1$ = Recommended dose of fertilizer (RDF) (200 g N + 40 g $P_2O_5$ + 200 g $K_2O$ g $plant^{-1}$); $T_2$ = Bacterial (KSB) consortium + 50% $K_2O$; $T_3$ = Bacterial (KSB) consortium + 75% $K_2O$; $T_4$ = Bacterial (KSB) consortium + 100% $K_2O$; $T_5$ = Fungal (KSF) consortium + 50% $K_2O$; $T_6$ = Fungal (KSF) consortium + 75% $K_2O$; $T_7$ = Fungal (KSF) consortium + 100% $K_2O$; $T_8$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 50% $K_2O$; $T_9$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 75% $K_2O$; $T_{10}$- Bacterial (KSB) consortium + Fungal (KSF) consortium + 100% K2O; $T_{11}$ = Commercial formulation + 50% $K_2O$; $T_{12}$ = Commercial formulation + 75% $K_2O$; T13 = Commercial formulation + 100% $K_2O$.

**Table 2.** Effect of soil applications of microbial (KSB and KSF) consortia under graded levels of potassic fertilizers on available N, P and K in soil and population dynamics of KSB and KSF in the rhizosphere.

| Treatment | Available N (kg/ha) | | Available P (kg/ha) | | Available K (kg/ha) | | KSB Population ($10^5$ cfu/g soil) | | KSF Population ($10^4$ cfu/g soil) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shooting | Harvest | Shooting | Harvest | Shooting | Harvest | Shooting | Harvest | Shooting | Harvest |
| $T_1$ | 284.30 | 183.33 | 22.80 | 18.64 | 526.46 | 294.96 | 6.66 | 1.66 | 0.73 | 0.53 |
| $T_2$ | 294.06 | 192.32 | 23.46 | 19.00 | 548.80 | 306.18 | 21.66 | 14.00 | 1.33 | 1.10 |
| $T_3$ | 310.85 | 206.25 | 25.40 | 20.48 | 582.40 | 332.27 | 18.00 | 11.33 | 0.90 | 1.66 |
| $T_4$ | 307.99 | 200.68 | 24.60 | 19.62 | 578.66 | 321.07 | 15.00 | 9.33 | 0.86 | 0.73 |
| $T_5$ | 296.85 | 196.50 | 23.80 | 19.22 | 560.00 | 309.89 | 3.33 | 2.33 | 8.33 | 5.33 |
| $T_6$ | 312.17 | 207.65 | 25.80 | 20.64 | 593.60 | 339.46 | 3.00 | 3.33 | 7.33 | 3.66 |
| $T_7$ | 303.73 | 204.86 | 25.00 | 20.03 | 574.93 | 328.53 | 2.66 | 2.66 | 5.66 | 2.33 |
| $T_8$ | 275.94 | 181.17 | 21.60 | 18.10 | 522.66 | 291.20 | 2.33 | 3.33 | 4.33 | 1.66 |
| $T_9$ | 278.72 | 186.74 | 22.33 | 18.24 | 526.40 | 302.47 | 4.66 | 5.66 | 2.60 | 1.33 |
| $T_{10}$ | 291.19 | 193.71 | 23.00 | 19.24 | 556.26 | 309.89 | 3.33 | 4.66 | 2.33 | 0.96 |
| $T_{11}$ | 281.51 | 182.56 | 22.53 | 18.48 | 537.60 | 294.96 | 16.33 | 11.00 | 0.10 | 0.80 |
| $T_{12}$ | 288.48 | 188.14 | 22.71 | 18.70 | 545.06 | 305.18 | 13.33 | 12.66 | 1.33 | 0.63 |
| $T_{13}$ | 301.03 | 199.00 | 24.10 | 19.42 | 569.77 | 313.63 | 10.33 | 7.33 | 0.86 | 0.60 |
| CD at 5% | 5.00 | 2.61 | 0.40 | 0.35 | 10.80 | 12.30 | 4.60 | 2.40 | 1.82 | 1.22 |

$T_1$ = Recommended dose of fertilizer (RDF) (200 g N + 40 g $P_2O_5$ + 200 g $K_2O$ g $plant^{-1}$); $T_2$ = Bacterial (KSB) consortium + 50% $K_2O$; $T_3$ = Bacterial (KSB) consortium + 75% $K_2O$; $T_4$ = Bacterial (KSB) consortium + 100% $K_2O$; $T_5$ = Fungal (KSF) consortium + 50% $K_2O$; $T_6$ = Fungal (KSF) consortium + 75% $K_2O$; $T_7$ = Fungal (KSF) consortium + 100% $K_2O$; $T_8$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 50% $K_2O$; $T_9$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 75% $K_2O$; $T_{10}$- Bacterial (KSB) consortium + Fungal (KSF) consortium + 100% K2O; $T_{11}$ = Commercial formulation + 50% $K_2O$; $T_{12}$ = Commercial formulation + 75% $K_2O$; T13 = Commercial formulation + 100% $K_2O$.

available N and P but on par for available $K_2O$. Among 100% $K_2O$ application treatments, the application of KSF consortium + 100% $K_2O$ ($T_4$) for available N and K (307.99 and 578.66 kg/ha, respectively) had a better effect as compared to others and RDF ($T_1$). The lowest soil available N, P and K (181.17, 18.10 and 291.20 kg/ha, respectively) were recorded in the treatment of combined application of bacterial and fungal consortia + 50% $K_2O$ ($T_8$) (Table 2).

In support of these findings Badr (4) reported inoculation of KSB and PSB in conjunction with amendment of its respective rock P or K materials that increased the availability of P and K in soil. Applied together, mixed inoculation and rock P and K materials, resulted in the highest availability of P and K in the soil increased about 25% of P and 15% of K as compared to the untreated control. Increasing the bioavailability of P and K in the soils with the inoculation of PGPR or with combined inoculation and rock materials have been reported by Lin *et al.* (10). Total N, P and K uptake were influenced by the application of 75% $K_2O$ along with a KSF consortium ($T_6$) (Table 3). The uptake of nitrogen increased linearly with all the treatments at harvest stage. Plant nitrogen uptake was the highest as compared with fruit nitrogen uptake. The application of KSB consortium + 75% $K_2O$ ($T_3$) (651.83 kg/ ha) was recorded second highest treatment after $T_6$ (661.47 kg/ ha) for total nitrogen uptake, which was at par with each other. In case of total nitrogen uptake, among the 100% $K_2O$ and 50% $K_2O$ application treatments, the application of 100% $K_2O$ had a better result than 50% $K_2O$. A similar trend was observed in case of plant and fruit nitrogen uptake (Table 3). Plant P uptake was more as compared with the fruit P uptake. Total uptake of P was influenced significantly (80.52 kg/ ha) by the application of 75% $K_2O$ + KSF consortium ($T_6$) among all the treatments followed by KSB consortium + 75% $K_2O$ ($T_3$) (75.73 kg /ha), which were at par with each other (Table 3). The total uptake of potassium increased constantly till harvest in all the treatments. The uptake by banana at harvest was significantly influenced by different potash levels. The maximum total potassium uptake of 1198.17 kg/ha was recorded by KSF consortium + 75% $K_2O$ ($T_6$), which was at par with KSB consortium + 75% $K_2O$ ($T_3$) (1177.21 kg/ha). Among the 50 and 100% $K_2O$ application treatments for total potassium uptake of banana, the application of 100% $K_2O$ had better result than 50% $K_2O$. In case of 100% $K_2O$ application treatments, KSF

**Table 3.** Effect of soil applications of microbial (KSB and KSF) consortia under graded levels of potassic fertilizers on N, P and K uptake by banana plant.

| Treatment | Plant N uptake (kg/ha) | Fruit N uptake (kg/ha) | Total plant N uptake (kg/ha) | Plant P uptake (kg/ha) | Fruit P uptake (kg/ha) | Total plant P uptake (kg/ha) | Plant K uptake (kg/ha) | Fruit K uptake (kg/ha) | Total plant K uptake (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 305.41 | 261.35 | 566.76 | 45.51 | 16.57 | 62.08 | 482.88 | 462.15 | 945.03 |
| $T_2$ | 319.99 | 280.48 | 600.47 | 46.86 | 17.84 | 64.70 | 509.16 | 466.93 | 976.09 |
| $T_3$ | 345.85 | 305.98 | 651.83 | 53.33 | 22.40 | 75.73 | 689.55 | 487.66 | 1177.21 |
| $T_4$ | 336.96 | 296.42 | 633.38 | 50.90 | 20.71 | 71.61 | 678.78 | 476.50 | 1155.28 |
| $T_5$ | 327.26 | 286.86 | 614.12 | 48.48 | 16.57 | 65.05 | 590.45 | 469.81 | 1060.26 |
| $T_6$ | 352.31 | 309.16 | 661.47 | 55.75 | 24.77 | 80.52 | 700.32 | 497.85 | 1198.17 |
| $T_7$ | 340.19 | 299.60 | 639.80 | 51.71 | 19.12 | 70.83 | 680.16 | 479.69 | 1159.85 |
| $T_8$ | 298.17 | 254.98 | 553.15 | 43.63 | 14.97 | 58.60 | 427.37 | 457.37 | 884.74 |
| $T_9$ | 308.67 | 267.70 | 576.37 | 43.63 | 15.60 | 59.23 | 441.70 | 457.37 | 899.07 |
| $T_{10}$ | 321.60 | 286.86 | 608.46 | 48.48 | 16.25 | 64.73 | 529.53 | 465.23 | 994.76 |
| $T_{11}$ | 307.80 | 264.54 | 572.34 | 44.57 | 15.93 | 60.50 | 453.20 | 458.97 | 912.17 |
| $T_{12}$ | 315.00 | 277.26 | 592.26 | 46.46 | 16.25 | 62.71 | 497.13 | 462.10 | 959.23 |
| $T_{13}$ | 334.45 | 293.23 | 627.68 | 50.09 | 18.40 | 68.49 | 661.87 | 474.90 | 1136.77 |
| CD at 5% | 9.05 | 6.65 | 11.70 | 2.17 | 1.81 | 5.01 | 18.50 | 7.13 | 27.16 |

$T_1$ = Recommended dose of fertilizer (RDF) (200 g N + 40 g $P_2O_5$ + 200 g $K_2O$ g $plant^{-1}$); $T_2$ = Bacterial (KSB) consortium + 50% $K_2O$; $T_3$ = Bacterial (KSB) consortium + 75% $K_2O$; $T_4$ = Bacterial (KSB) consortium + 100% $K_2O$; $T_5$ = Fungal (KSF) consortium + 50% $K_2O$; $T_6$ = Fungal (KSF) consortium + 75% $K_2O$; $T_7$ = Fungal (KSF) consortium + 100% $K_2O$; $T_8$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 50% $K_2O$; $T_9$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 75% $K_2O$; $T_{10}$- Bacterial (KSB) consortium + Fungal (KSF) consortium + 100% K2O; $T_{11}$ = Commercial formulation + 50% $K_2O$; $T_{12}$ = Commercial formulation + 75% $K_2O$; T13 = Commercial formulation + 100% $K_2O$.

consortium + 100% K ($T_7$) (1159.85 kg/ ha) was higher than others, followed by $T_4$ (1155.28 kg/ ha) and $T_{13}$ (1136.77 kg/ ha), which were at par with each other. The lowest total uptake of potassium of 884.74 kg/ ha was recorded due to the combined application of KSB and KSF consortium + 50% $K_2O$ ($T_8$), which was at par with the combined application of KSB and KSF consortium + 75% $K_2O$ ($T_9$) (899.07 kg /ha). A similar trend was observed in case of plant and fruit uptake (Table 3). The results are comparable with the findings of Badr (4) who reported that inoculation of KSB and PSB in conjunction with amendment of its respective rock P or the minerals increased the availability of P and K in soil, enhanced N, P and K uptake and promoted the growth of egg plant. Similarly, the dynamics of K release from waste mica inoculated with potassium solubilizing microorganism (*Bacillus mucilaginosus*) and to investigate its effectiveness as potassic-fertilizer using Sudan grass (*Sorghum vulgare* Pers.) var. *sudanensis* as test crop grown under two Alfisols.

The maximum length and girth of banana fruit (22.88 and 13.96 cm) was recorded by the application of fungal consortium + 75% $K_2O$ ($T_6$). It was followed by the bacterial consortium + 75% $K_2O$ ($T_3$) (22.22 and 13.90 cm), which were at par with each other. The treatment of commercial formulation ($T_{11}$, $T_{12}$ and $T_{13}$) and RDF ($T_1$) were significantly lower than the above treatment ($T_6$) (Table 4). The application of KSF consortium along with 75% $K_2O$ ($T_6$) recorded the maximum marketable number of hands per bunch, fingers per hand, bunch weight and total yield (9.22, 16.22, 28.55 kg and 126.87 t/ha, respectively) followed by KSB consortium + 75% $K_2O$ ($T_3$) (9.00, 16.11, 27.88 kg and 123.89 t/ha, respectively). These treatments were significantly superior to treatments with commercial formulation and RDF ($T_1$). The lowest number of hands per bunch (7.44) and fingers per hand (13.67) were recorded by the combined application of bacterial and fungal consortia along with 50% $K_2O$ ($T_8$), which was at par with treatments, $T_9$ (7.55 and 14.00), $T_{11}$ (7.33 and 13.90), $T_{12}$ (7.88 and 14.99) and RDF ($T_1$) (7.67 and 14.77) (Table 4). Similar findings were also noticed by Mostafa and Yovssef (13) using K solubilizing bacteria (*Bacillus* sp.) as compared to the reference strain (*Frateuria aurantia*) for their influence on growth, K uptake and yield of maize plants under glass house conditions. All the inoculated treatments with bacteria (*Bacillus* sp. KSB 11 recorded the highest yield 51.33 g/ plant)

**Table 4.** Effect of soil applications of microbial (KSB and KSF) consortia under graded levels of potassic fertilizers on grade of the fruit, bunch characteristics and yield of banana.

| Treatment | Fruit length (cm) | Fruit girth (cm) | No. of hands/ bunch | No. of fingers/ hand | Bunch wt. (kg) | Yield (t/ha) |
|---|---|---|---|---|---|---|
| $T_1$ | 20.88 | 12.44 | 7.67 | 14.77 | 21.55 | 95.76 |
| $T_2$ | 21.00 | 13.11 | 8.22 | 15.67 | 24.11 | 107.14 |
| $T_3$ | 22.22 | 13.90 | 9.00 | 16.11 | 27.88 | 123.89 |
| $T_4$ | 22.00 | 13.55 | 8.66 | 16.11 | 26.44 | 117.49 |
| $T_5$ | 21.77 | 13.22 | 8.26 | 15.81 | 24.67 | 109.63 |
| $T_6$ | 22.88 | 13.96 | 9.22 | 16.22 | 28.55 | 126.87 |
| $T_7$ | 22.11 | 13.77 | 8.88 | 16.11 | 26.77 | 118.96 |
| $T_8$ | 20.22 | 12.00 | 7.44 | 13.67 | 20.00 | 88.88 |
| $T_9$ | 20.22 | 12.11 | 7.55 | 14.00 | 20.00 | 88.88 |
| $T_{10}$ | 21.00 | 13.00 | 8.00 | 15.66 | 23.77 | 105.63 |
| $T_{11}$ | 20.55 | 12.22 | 7.33 | 13.90 | 20.11 | 89.36 |
| $T_{12}$ | 20.77 | 12.55 | 7.88 | 14.99 | 22.00 | 97.76 |
| $T_{13}$ | 22.00 | 13.44 | 8.55 | 16.00 | 25.77 | 114.52 |
| CD at 5% | 0.73 | 0.41 | 0.58 | 0.70 | 2.71 | 12.28 |

$T_1$ = Recommended dose of fertilizer (RDF) (200 g N + 40 g $P_2O_5$ + 200 g $K_2O$ g $plant^{-1}$); $T_2$ = Bacterial (KSB) consortium + 50% $K_2O$; $T_3$ = Bacterial (KSB) consortium + 75% $K_2O$; $T_4$ = Bacterial (KSB) consortium + 100% $K_2O$; $T_5$ = Fungal (KSF) consortium + 50% $K_2O$; $T_6$ = Fungal (KSF) consortium + 75% $K_2O$; $T_7$ = Fungal (KSF) consortium + 100% $K_2O$; $T_8$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 50% $K_2O$; $T_9$ = Bacterial (KSB) consortium + Fungal (KSF) consortium + 75% $K_2O$; $T_{10}$- Bacterial (KSB) consortium + Fungal (KSF) consortium + 100% K2O; $T_{11}$ = Commercial formulation + 50% $K_2O$; $T_{12}$ = Commercial formulation + 75% $K_2O$; T13 = Commercial formulation + 100% $K_2O$.

and was found to increase growth parameters and yield components compared to absolute control and 25 per cent of RDK control.

In conclusion, the present study resulted in potassium solubilizing bacteria *Pseudomonas* species and fungi *Aspergillus* species, which proved better than the reference strain (*Frateuria aurantia*). The best delivery system for banana is an excellent potassium solubilizing ability of *Aspergillus* consortium with talc formulation and *Pseudomonas* consortium with liquid bio-formulation. These findings clearly indicated 25% savings of potassic fertilizers, *i.e.*, 222.22 $K_2O$ kg/ ha.

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# Impact of split application of fertilizer at various growth stages on Kinnow productivity under semi-arid irrigated ecosystem

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## ABSTRACT

**A study was conducted at the experimental orchard of the Department of Horticulture, CCS Haryana Agricultural University, Hisar to study the impact of split application of recommended dose of N @ 800 g; $P_2O_5$ @ 320 g and $K_2O$ @ 105 g/plant/year at various growth stages in Kinnow orchard for consecutive three years. The soil of the experimental site was sandy loam, free from $CaCO_3$, poor in organic carbon, medium in phosphorus and high in potash content. The results of pooled data showed that application of nitrogen three times, 40% in February and 30% each in April and July; phosphorus two times, 50% each in February and April; and potash three times, 40% each in February and April and 20% in September reduced pre-harvest fruit drop (21.16%), increased number of fruits (10.19%) and increase in fruit yield about 10.29% with good quality in terms of TSS and peel thickness over control (recommended practice). Split application of N & K increased uptake, whereas, reverse was observed in phosphorus. The leaf N and K contents were highest when 30% of total N and 20% of total K/plant/year were applied in July (pre-autumn flush stage). Hence, there is a need to revise the current recommendation in response of K dose and frequency of N & K application on phenological stages to get optimum productivity of Kinnow orchard on sandy loam soils poor in organic carbon under semi-arid irrigated ecosystem of north western zone of India.**

**Keywords**: Fertilizer, growth stage, Kinnow mandarin, productivity, semi-arid irrigated conditions.

## INTRODUCTION

The introduction of Kinnow, a mandarin hybrid, gained popularity within a short span of time by replacing sweet oranges cultivation due to its high yielding potential. It showed a good promise and commercially success in North Indian states like Punjab, Haryana and Rajasthan falling under subtropical climate with distinct winter season. Mandarins constitute the bulk of citrus exports from India. Recently, Kinnow was exported from Punjab and Rajasthan to Sri lanka, UK, and Gulf countries. Unfortunately, the production from Kinnow orchards is very low than standard. One of the main reasons for low citrus orchard productivity in South-Western zone of Haryana is poor nutrition because of improper use of fertilizers input in respect of time, combination/ different NPK ratio. Fertilizers are being used by the growers at the start of spring flush and just after fruit set in April, thereafter, no fertilizer is applied up to harvesting in January. Kinnow is a heavy feeder of nutrients and water because of shallow root system and prolific bearing. After initial few years of good productive periods, the productivity and plant health starts declining and by then it becomes near impossible to eradicate multi-nutrient deficiency which eventually leads to citrus decline and reduction in yield and heavy fruit drop due to nutrient deficiency.

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Since, citrus is a tree and has growth in cycles, therefore until or unless, fertilizer application program is properly designed according to growth cycle, it is not possible to improve the plant health and fruit production substantially (Alva *et al.*, 1; Zaman and Schumann, 21). Hence, efficient use of fertilizer has become a first-order concern in modern citrus production system. The use of fertilizers by plant involves several steps, including uptake, assimilation, translocation, recycling and remobilization (Masclaux-Daubresse *et al.*, 9). Several factors affect fertilizer N uptake efficiency, including plant demand for N (Weinbaum *et al*., 19), the rate, timing of application, the form of N applied and soil type. Other factors inherent to plant as size and depth of root system, that determine the plant ability to intercept N before leached below the root zone (Scholberg *et al.*, 16) can also influence NUE. According to Stassen *et al.* (17) the seasonal pattern of N uptake can be used for scheduling the timing and rate of application. In this way, most efficient application is, therefore achieved, when synchronized with tree N demand. Saleem *et al.* (14) observed that split application of compound fertilizers was more effective than single application of the same or simple fertilizer in Kinnow. Seasonal variation in N uptake occurs, with the uptake appearing to be highest during period of active shoot growth (Maust and Williamson, 10; Menino *et al.,* 11).

Nevertheless, other phenological important stages may be identified and used as selection criteria to evaluate different fertilizer management strategies. Research efforts during the past decade have focused on the identification of several management practices like type of fertilizers, rate of application and method of application that improve the fertilizer use efficiency. Current citrus best management practices recommend fertilizer dose based on leaf tissue and soil analysis, instead of routine application (Boman *et al*., 3). Moreover, an appropriate timing, which maintain the supply of nutrients over the growth cycle constitute one of the most relevant strategies in improving fertilizer use efficiency (Martinez-Alcantara *et al*., 8) as well as sustainable productivity. Keeping in view the above facts, the present investigation was carried out with the objective to sustain the Kinnow productivity by applying different ratio of NPK at various growth and phenological stages.

## MATERIALS AND METHODS

The field experiment was conducted at the experimental orchard of the Department of Horticulture, CCS HAU, Hisar located at latitude 29.10° N, longitudes 75.46° E and at an altitude of 215.2 m above sea level. The climate of the experimental site is characterized as semi-arid subtropical with hot and dry summer and cool winters. The mean annual rainfall is 400 mm, out of which around 90% is received during monsoon (July-September). The physico-chemical properties of experimental soil are given in Table 1. The texture of the soil was sandy loam with $CaCO_3$ < 1.0%. The soil had almost neutral pH with mild EC ranged from 0.25-0.45 dS $m^{-1}$ and deficient in organic carbon (0.15-0.37%). The available P and K concentrations in soil ranges from 15-25 kg/ha and 240-333 kg/ ha, respectively. Canal water was used as flood irrigation and water table level was about 4 m deep.

The citrus plant used in the study was Kinnow mandarin budded on rough lemon (*Citrus jambhiri* Lush) rootstock at a spacing of 6 m × 6 m. The experiment was conducted for three consecutive years (2011-12, 2012-13 and 2013-14). Twenty trees subjected to the same cultural practices were chosen nearly similar in growth, vigour and health. Four nutritional treatments were designed to apply the same amount 800 g N, 320 g $P_2O_5$, and 105 g $K_2O$/ tree/year, which is the recommended dose of fertilizer of the region. Total dose of fertilizer was splited into four different percent ratio of N: P: K and applied at four phenological stages, *i.e.* spring flush stage (February), cell division stage (April), rainy season/ pre-autumn flush stage (July) and autumn flush stage (September) in all the treatments (Table 2). In control, RDF (N @ 800g; $P_2O_5$ @ 320 g and $K_2O$ @ 105 g/ plant/year) was applied in two splits, *i.e.* half dose of nitrogen and full doses of phosphorus and potassium in February and remaining half nitrogen in April. A complete randomized block design was followed with five replications taking one plant per replication. The data (pooled mean data of three years) pertaining to various parameters was analyzed statistically as per the procedure of (Panse and Sukhatme, 12). FYM was applied in December at the rate of 20 kg/ plant. N, P & K were applied in form of urea, diammonium phosphate and muriate of potash, respectively.

Pre-harvest fruit drop was calculated by counting the number of dropped fruits daily from September onward till harvesting from each plant under experiment and computed as pre harvest fruit drop (%). Harvesting of fruit was achieved in the last week of December and yield (kg/plant) was recorded by weighing on electronic balance. Numbers of fruits/ plant were counted at harvesting and average fruit weight was calculated by dividing the yield to the number of fruits. At harvest, sample of five representative fruits of each tree were devoted to determine the fruit physico chemical characteristics. Peel thickness was recorded by measuring the peel of the fruit at the equator of fruit to right angle with the help of Vernier calipers and averaged. Peel of the fruit

**Table 1.** Physico-chemical properties of soil profile of the experimental site.

| Soil depth (cm) | Texture | pH (1:2) | EC (dS $m^{-1}$) | Organic carbon (%) | Available phosphorus (kg/ha) | Available potash (kg/ha) |
|---|---|---|---|---|---|---|
| 0-15 | Sandy loam | 7.53 | 0.27 | 0.37 | 23.0 | 333 |
| 15-30 | Sandy loam | 7.60 | 0.25 | 0.30 | 25.0 | 264 |
| 30-60 | Sandy loam | 7.67 | 0.26 | 0.27 | 22.0 | 278 |
| 60-90 | Sandy loam | 7.73 | 0.29 | 0.15 | 20.0 | 240 |
| 90-120 | Sandy loam | 7.60 | 0.34 | 0.15 | 17.0 | 278 |
| 120-150 | Sandy loam | 7.60 | 0.40 | 0.15 | 20.0 | 252 |
| 150-200 | Size | 7.57 | 0.45 | 0.15 | 15.0 | 240 |

**Table 2.** Doses of applied nitrogen, phosphorus and potash at various phenological stages of Kinnow mandarin.

| Treatment | Per cent RDF application at different phenological stage | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feb (spring flush) | | | April (cell division stage) | | | July (rainy season flush) | | | September (autumn flush) | | |
| | N (%) | P (%) | K (%) | N (%) | P (%) | K (%) | N (%) | P (%) | K (%) | N (%) | P (%) | K (%) |
| $T_1$ | 40 | 100 | 40 | 20 | 0 | 40 | 20 | 0 | 20 | 20 | 0 | 0 |
| $T_2$ | 40 | 50 | 40 | 30 | 50 | 40 | 30 | 0 | 0 | 0 | 0 | 20 |
| $T_3$ | 40 | 40 | 40 | 30 | 30 | 20 | 0 | 30 | 20 | 30 | 0 | 20 |
| $T_4$:contol | 50 | 100 | 100 | 50 | 0 | 0 | - | - | - | - | - | - |

was separated and weighed and expressed as peel content (%). After peeling the same fruits were used for extracting the juice and expressed as juice content (%) on weight basis. The percent acidity and ascorbic acid were determined as suggested by (AOAC, 2). Total soluble solids (TSS) was determined with the help of Abbe's hand refractometer (0-32°Brix). Leaf samples were collected from each plant in the month of October by selecting 3rd and 4th leaf terminally from spring flush non-fruiting shoots at chest height. The known amount of powdered material was digested in diacid mixture of $H_2SO_4$: $HClO_4$ in the ratio of 4:1 for estimation of N,P,K, whereas, for Zn content diacid mixture of $HNO_3$:$HClO_4$ in the ratio of 4:1 was used. Nitrogen was estimated with Nessler's reagent method and phosphorus with Vandomolybdate yellow colour method as per standard procedure (Jackson, 7). Potash was determined with flame photometer (Piper, 13) and zinc was estimated with atomic absorption spectrophotometer.

## RESULTS AND DISCUSSION

Fruit yield is a good index of orchard productivity and fruit retention, fruit weight and number of fruits harvested is the good indicator of yield. Among various treatments, $T_2$ was found significantly most superior in increasing the fruit yield (119.57 kg/plant) over the remaining treatments, which were otherwise on par with each other (Table 3). The minimum yield (107.44 kg/plant) was found in treatments $T_4$ (control/ farmers' practice). Average fruit weight could not be affected significantly by any of the treatments; however, numerically maximum fruit weight (179.96 g) was achieved in $T_1$ and minimum (172.12 g) in $T_4$ (control/ farmers' practice). Treatment $T_2$ was found significantly most effective in reducing pre-harvest fruit drop (8.01%) closely followed by $T_3$ (8.16%) as compared to $T_1$ (9.34%) and $T_4$ (10.16%). Similarly, $T_2$ was also observed significantly superior in increasing number of fruits (681.07) harvested to remaining treatments, which were otherwise on par with each other. The minimum number of fruits (607.93) was harvested in $T_1$.

The results clearly shows that application of N @ 800 g/tree/year three times, 40% in Feb, 30% in April and 30% in July; $P_2O_5$ @ 320 g/tree/year two times, 50% in Feb. and 50% in April and $K_2O$ @ 105 g/tree/ year three times, 40% in Feb., 40% in April and 20% in September reduced the pre-harvest fruit drop to the tune of about 21.16%, increased number of fruit harvested/ plant (10.19%) and yield (10.29%) over control $T_4$ (control/ farmers' practice). The maximum weight of the fruit in treatment $T_1$ may be due to less number of fruits harvested and secondly, due to more content of K in leaves as more potash content in citrus leaves is directly related to fruit size. Hifny *et al.* (6) reported the highest fruit weight of Valencia oranges when receiving 50% of total N/tree/ year during period of pre-spring flush or the period of fruit cell division stage and subsequently stimulation of photosynthesis intensity. The reduced fruit drop in $T_2$ was due to more uptake of N and thus reduced competition for vegetative and reproductive growth with July application of nitrogen. Similarly, Saleem *et al.* (14) observed minimum fruit drop with split application of 2 kg compound fertilizers (CF) per tree

**Table 3.** Effect of split application of nutrients doses on fruit drop and yield of Kinnow mandarin (pooled mean).

| Treatment | Pre-harvest fruit drop (%) | Fruit wt. (g) | No. of fruits harvested/ plant | Yield (kg/tree) |
|---|---|---|---|---|
| $T_1$ | 9.34 | 179.96 | 607.93 | 109.18 |
| $T_2$ | 8.01 | 174.83 | 681.07 | 119.57 |
| $T_3$ | 8.16 | 175.40 | 633.13 | 111.94 |
| $T_4$ | 10.16 | 172.12 | 618.07 | 107.44 |
| CD at 5% | 0.91 | NS | 33.45 | 7.89 |

and 1.0 kg urea in August in Kinnow, while fruit drop was negligible in later months. These results are in accordance with those of Yaseen and Ahmed (20) who also showed increase in fruit yield of Kinnow over control with the application of NPK fertilizer (200-150-250 g/ tree) at three times, *i.e.* before new flush at last week of Jan., mid of April and end of July with foliar spray of multi-nutrients.

It seems that application of N in three splits, *i.e*. in Feb, April and July resulted in better yield. This may be due to more uptake of N when applied in July as compared to September. In fact nitrogen is the key component in mineral fertilization applied to citrus groves, since it influences tree growth, fruit production as well as fruit quality than any other treatments (Zekri and Obreza, 22). About 40% of annual vegetative growth in Kinnow takes place during July-August, which requires nitrogen for growth. If there is less availability during rainy season growth (July-August) then poor uptake of N and there will be remobilization of metabolites from spring growth leaves, which hinders in fruit growth and ultimately more pre-harvest fruit drop and reduced yield, which further had adverse effect on flowering and fruiting in next season (Dalal *et al*., 4). September application of nitrogenous fertilizers was not effective as it did not show response on yield and uptake of nitrogen in $T_1$ & $T_3$. This shows lower uptake of N in September due to reduced root activity in the autumn and winter. Similarly, adding of 50% of total given N/tree/year at pre-autumn flush stage caused the most increase in fruit yield of Valencia budded on sour orange compared with other phenological stages and control (Hifny *et al*., 6). Despite other factors, improvement in yield can also be attributed to reduced fruit drop preventing the plant from nutritional deficiencies and disorders. Application of nitrogen and potash in July and September, respectively strengthen the plants by inducing an enhancement in physiological response of the plants.

Peel thickness, TSS, juice content, juice acidity and ascorbic acid are the most important parameters used to determine Kinnow quality. Just unlike the yield response, split application of N,P,K had significantly minor impact on these parameters as compared to control/ farmers' practice. Among fruit quality parameters, peel thickness, TSS, and ascorbic acid differed significantly, whereas, peel content, juice content and acidity differed non-significantly with various treatments (Table 4). Peel thickness increased significantly in $T_2$ (3.77 mm) over the remaining treatments, whereas, thinnest peel (3.40 mm) was observed in $T_3$. Similarly, numerically maximum peel content (22.16%) was found in $T_2$ and minimum (21.11%) in $T_3$. Maximum juice content (50.29%) was measured in $T_3$ and minimum (47.86%) in $T_4$ (control/ farmers' practice). Treatment $T_2$ was found significantly most effective in increasing the TSS (10.72°Brix) over the other treatments. Treatment $T_4$ and $T_1$ were found significantly on par with each other and significantly higher in increasing the TSS over $T_3$. Treatment $T_1$ showed numerically least acidity (0.96%) and $T_2$ the highest acidity (1.03%). The maximum ascorbic acid content (23.12 mg/ 100 ml of juice) was estimated in $T_4$, which was also significantly highest over other treatments, whereas, treatments $T_1$, $T_2$ and $T_3$ were at par with each other. The minimum ascorbic acid content (21.47 mg/100 ml of juice) was estimated in $T_3$.

The results related to biochemical attributes of Kinnow mandarin clearly indicates the importance of split application of N,P&K, especially the third application of N & K in July and September. Nutrients applied in split dose positively influence the fruit quality parameters. Fruit quality in Kinnow improved with the increase in TSS content, whereas, juice and acidity content were not affected significantly. This may be due to higher N: K ratio in leaves in $T_2$ as compared to other treatments. The improvement in fruit physico-chemical parameters may be due to the fact that split nutrient application improves the nutrient uptake by the plant and this imparted the beneficial influence on plant growth and ultimately fruit quality parameters. Application of N and K in late stages of fruit growth (July and September) increased nitrogen and potash in leaves, which have the positive

**Table 4.** Effect of split application of nutrient doses on fruit quality of Kinnow mandarin (pooled mean).

| Treatment | Peel thickness (mm) | Peel content (%) | Juice (%) | TSS (°Brix) | Acidity (%) | Ascorbic acid (mg/ 100 ml juice) |
|---|---|---|---|---|---|---|
| $T_1$ | 3.45 | 21.96 | 48.75 | 10.31 | 0.96 | 21.58 |
| $T_2$ | 3.77 | 22.16 | 49.68 | 10.72 | 1.03 | 22.22 |
| $T_3$ | 3.40 | 21.11 | 50.29 | 9.91 | 1.00 | 21.47 |
| $T_4$ | 3.44 | 21.56 | 47.86 | 10.35 | 0.98 | 23.12 |
| CD at 5% | 0.14 | NS | NS | 0.29 | NS | 0.87 |

effect on many important plant structure, genetic and metabolic compounds in plant cell (Don, 5) and enhanced photosynthesis, consequently there will be more production of assimilates. These assimilates are depicted in terms of increases TSS, juice content and acidity. Similar increase in peel thickness showed more response when 50% of total N/tree/year was applied in pre-autumn stage and increase in TSS content when applied any of the phenological stage except control (Hifny *et al.*, 6) in Valencia sweet orange .The decrease in ascorbic acid content with split application of nutrients as compared to control in present study may be due to increased juice content and fruit weight.

Leaf nitrogen and potash contents differed significantly, whereas, phosphorus and zinc differed non-significantly with various treatments (Table 5). Maximum nitrogen content (2.55%) was found in treatment $T_2$, which was significantly superior to $T_4$ (control/ farmers' practice) and on par with other treatments. Minimum nitrogen content (2.33%) was observed in $T_4$ (control/ farmers' practice). Numerically maximum phosphorus content (0.138%) was found in treatment $T_1$ and minimum (0.19%) in $T_3$. Potash content increased in all the treatments over $T_4$ (control/farmers' practice) but could not reach to the level of significance except $T_1$. Maximum potash content (0.705%) was observed in treatment $T_1$ which was found at par with $T_3$ (0.644%) and $T_2$ (0.59%). Similarly, zinc content increased in all the treatments over $T_4$ (control/ farmers' practice) but could not attain the level of significance. However, the numerically maximum zinc content (31.18 ppm) was observed in treatment $T_1$ and minimum (28.64 ppm) in $T_4$ (control/ farmers' practice).

Split application of N & K increased the uptake in Kinnow orchard, whereas, reverse was observed in phosphorus. However, the leaf N content was in optimum range in all the treatments but it was on higher side when 30% of total nitrogen/tree/year was applied in July (pre-autumn flush stage) in treatment$T_2$, whereas, N uptake was lower when 20 and 30% of total N/tree/year was applied in September (autumn flush stage) in $T_1$ & $T_3$. This shows that nitrogen required by citrus plants is more during rainy season growth flush in July as compared to autumn season growth as nitrogen is essential for vegetative growth of plant. Martinez-Alcantara *et al.* (8) reported that fertilizer N uptake clearly differed among periods, being the lowest values recorded at flowering, while increased significantly later on at fruit set and growth periods of citrus. About 40% of the annual vegetative growth in Kinnow took place in rainy season and even <10% during autumn season flush (Dalal *et al.*, 4). As the split application of nutrients enhanced the nutrient availability to the plant especially N and K, besides it helps in efficacious regulation of stomatal conductance (Traiz and Zeiger, 18), which thus caused an increment in nutrients absorption efficiency of the plant. In the present study if P is applied in more than one split its uptake in leaves decreased in treatments $T_2$ & $T_3$. Phosphorus helps in root activity which is most in summer season in citrus. This shows that P should be applied in the early stages of the growth of the citrus plant. In contrary, Salik *et al.* (15) observed that when fertilizers were applied in April or July, not much improvement was noted and results were similar to control in Kinnow. Potash content in all the treatments were found in deficient range, whereas, it was in excess in soil. This shows that potash present in soil is not available to the Kinnow plant and also K applied by the farmers is not sufficient to supplement the requirement of K by the Kinnow orchards. However, the split application of K increased uptake and uptake was most when 20% of total K/tree/year was applied in July ($T_1$). However, when 20% of K was applied in September there was non-significant increase in K content ($T_2$). Saleem *et al.* (14) also reported that split application of compound fertilizers was more effective than single application of the same or simple fertilizers. Hence, it is clear that N & K application should be avoided in September due to poor uptake, whereas, P application should be restricted to February as single dose for better nutrients uptake. The findings of this experiment indicate the potential of same dose of N, P, and K to increase the productivity by reducing pre-harvest fruit drop, increasing number of harvested fruits and yield if N & K are splited in three applications, *i.e.* February, April and July in Kinnow orchards. It is also suggested that N, P, K application frequency should be adjusted based on growth/ phenological stages.

**Table 5.** Effect of split application of nutrient doses on leaf nutrients composition in Kinnow (pooled mean).

| Treatment | N (%) | P (%) | K (%) | Zn (ppm) |
|---|---|---|---|---|
| $T_1$ | 2.43 | 0.138 | 0.705 | 31.18 |
| $T_2$ | 2.55 | 0.128 | 0.576 | 29.87 |
| $T_3$ | 2.43 | 0.119 | 0.644 | 30.56 |
| $T_4$ | 2.33 | 0.126 | 0.522 | 28.64 |
| CD at 5% | 0.20 | NS | 0.119 | NS |

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# Plant growth, nutrient uptake, water use efficiency and yield of pomegranate as affected by irrigation scheduling in loamy soils of semi-arid regions

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## ABSTRACT

**A field experiment was conducted on loamy soil of National Research Centre on Pomegranate, Solapur, Maharashtra for three consecutive years (2010 to 2013) to assess the water requirement of pre-bearing and bearing plants of pomegranate cv. Bhagwa. The treatments consisted of replenishment of irrigation water equivalent to 30, 40, 50, 60, 70, 80 and 90% of cumulative pan evaporation (CPE) on alternate day laid out in randomized block design with four replications. The results of the study showed that during pre-bearing year, maximum plant growth can be achieved with the application of irrigation equivalent to 60% of CPE, while during bearing period it should be 70% of CPE. In three-year-old plants, maximum fruit yield (6.79 kg/plant) and irrigation efficiency (0.473 t/ha-cm) was observed in 70% CPE treatment. Leaf content of N (2.25%), Cu (124.9 ppm) and Mn (80.7 ppm) was also highest in this treatment. Fruit cracking to the extent of 58.8, 45.3 and 37.7% was recorded from the plants supplied with 30, 40 and 50% of CPE irrigation, respectively which reduced fruit yield drastically.**

**Key words:** Pomegranate, irrigation scheduling, plant growth, fruit yield, nutrient uptake.

## INTRODUCTION

For marginal and degraded lands of semi-arid to arid regions, fruit crops like pomegranate (*Punica granatum* L.) assumes greater significance. In India, during last two decades, pomegranate cultivation has registered a high growth due to its hardy nature, export potential, low maintenance cost and good keeping quality and reached to 1.31 lakh ha with an annual production of 13.45 lakh tonnes (Pal *et al*., 12). Majority of the pomegranate cultivation is on undulating, shallow and light textured soils where water scarcity is a major constraint (Marathe *et al*., 11). Hence, it is imperative to adopt holistic strategies to harvest more crop per drop of water. It is reported that pomegranate can tolerate extreme dry conditions but for optimum growth and quality fruit production, irrigation is most essential. Earlier, Lawande and Patil (6) suggested surface irrigation equivalent to 0.8 and 1.0 IW /CPE ratio for fruit yield and vegetative growth, respectively for 'Muskat' pomegranate grown in black soils of Parbhani areas. But nowadays, in almost all the pomegranate orchards, irrigation is being provided through drip-irrigation system and in absence of scientific knowledge about irrigation schedules, farmers have tendency to provide excess irrigation. Application of inappropriate amount of irrigation water especially in light textured soils results into wastage of water through deep percolation or otherwise creating waterlogging, poor aeration and weed infestation (Marathe *et al*., 9). Recently, irrigation equivalent to 100% pan evaporation was suggested for pomegranate, grown under high density planting system (Haneef *et al*., 5). There were few recommendations, on the basis of climatic approach but recommendations on basis of field experimentation is lacking. In this perspective, the present investigation was undertaken to suggest irrigation schedules for pre-bearing and bearing pomegranate cv. Bhagwa orchards grown under semi-arid region of India.

## MATERIALS AND METHODS

A field experiment was conducted during 2010 to 2013 at experimental farm of ICAR-NRCP, Solapur, Maharashtra, India. The site lies at 17°65" N latitude and 75°90" E longitude and 457 m above mean sea level receiving average annual rainfall of 472.8 mm. The soil was having loamy texture, 15.8% coarse fragments, montmorillonitic mineralogy, 60 cm deep with pH 7.66, electrical conductivity 0.18 dS/m, organic carbon 0.38% and calcium carbonate 6.24%. The available N, P and $K_2O$ content of surface soil was 190.0, 11.5 and 238.4 kg/ha, respectively. The field capacity (33 kPa) and permanent wilting point (1.5 M Pa) of soil was 24.2 and 13.1%, respectively. Average monthly maximum and minimum temperature during

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the experimental period (January to July) varied from 29.9 to 40.2°C and 15.2 to 25.1°C, respectively. The daily pan evaporation ranged between 3.7 to 19.8 mm.

The experiment was arranged in randomized block design with 4 replications having 2 plants per unit. There were 7 treatments consisting of application of irrigation water equivalent to 30 ($T_1$), 40 ($T_2$), 50 ($T_3$), 60 ($T_4$), 70 ($T_5$), 80 ($T_6$) and 90 ($T_7$) % of cumulative pan evaporation (CPE). Cumulative irrigation was provided on every alternate day through drip system of irrigation, having four (4 lph) drippers placed on four sides of each plant at a distance of 30 cm during first year and 50 cm afterwards. The crop water requirement of pomegranate crop was computed on daily basis using the following equation.

$V = E_p \times K_p \times K_c \times S_c \times W_p$

Where, V = volume of water (litres/ day/ plant), $E_p$ = open pan evaporation (mm/day), $K_p$ = pan coefficient, $K_c$ = crop coefficient, $S_c$ = crop spacing (plant to plant × row to row in metre) and $W_p$ = wetting factor. Irrigation efficiency of drip was considered as 90%. The effective rainfall was calculated by balance sheet method from the actual rainfall received and was used for daily water requirement of crop. Measured quantity of irrigation water was provided to the plants using water meters and separate pipeline for every treatment.

In all the treatments, 150-day-old air-layered saplings of pomegranate cv. Ganesh were planted during January 2009 and maintained by adopting similar cultivation practices. During 2010, various treatments were imposed on one-year-old plant from 10$^{th}$ February to 11$^{th}$ June 2010. Due to severe infestation of bacterial blight disease, as a management practice, plants were cut to ground level during October 2010. All plant debris were literally swept from soil surface of entire farm and disinfected by spraying bleaching powder on the surface. Again plants were allowed to grow and treatments were imposed from December 2011 to June 2012 and again during December 2012 to June 2013.

A representative leaf samples were collected (Marathe and Babu, 7) from individual plants and processed for nutrient analysis. The samples were digested (Chapman and Pratt, 1) in di-acid mixture ($H_2SO_4$:$HClO_4$ in 1:2.5). Nitrogen was determined by using micro-Kjeldhal steam distillation method, phosphorus by Vanadomolybdo phosphoric acid method, potassium by flame photometer and $Ca^{2+}$ + $Mg^{2+}$ by versenate titration method. All micronutrients (Fe, Zn, Mn and Cu) were determined using atomic absorption spectrophotometer (Perkin Elmer, USA make Analyst 400).

Vegetative growth in terms of plant height and plant spread was recorded in each year. Data on male and hermaphrodite flowers were taken by counting the flowers dropped on the ground and set on plants. The fruit yield data was recorded both in terms of number count and fruit weight basis during the year 2013. Cracked fruits were harvested separately and counted in terms of numbers. Chlorophyll content in the leaves as indicated by SPAD values was measured during 2012 using chlorophyll meter (Konica Minolta SPAD-502). The data obtained were subjected to statistical analysis such as analysis of variance (ANOVA) using online software (WASP 2.0) developed by ICAR Research Complex, Goa.

## RESULTS AND DISCUSSION

It was observed that for pomegranate, supplemental irritation is required only during summer season of the year. Accordingly, treatments were imposed during December to June months of the years as per the crop requirements. Quantity of irrigation water applied during the period largely varied from 187.8-563.5, 527.6-1582.7 and 614.7-1844.1 litres / plant, during the year 2010, 2011-12 and 2012-13, respectively (Table 1). Quantity of irrigation water was highest during the month of May followed by April and was lowest in the month of June. It was low during the year 2010 due to low vegetative growth of the plant and increased afterwards with the increase in plant canopy.

Soil moisture content during fruiting period varied from 16.9 to 21.0, 16.0 to 22.1 and 14.9 to 23.0 at 0-15, 15-30 and 30-45 cm vertical depth, respectively amongst different treatments (Table 2). Soil moisture content in 0-15 cm depth showed non-significant variation during all the months, mainly due to evaporation and percolation losses in surface layer. Moisture content was found to increase with the increasing quantity of irrigation water. During most of the period higher soil moisture was recorded in 90% CPE treatment. In 30 and 40% CPE treatments, it was very low in 30-45 cm depth, indicating that the quantity of irrigation water was not sufficient to percolate below 30 cm depth, inducing water stress to the plants.

Per cent increase in plant height and plant spread showed significant variation during all the years (Table 3). During first year (2010) maximum increase in plant height and plant spread was under 60 and 70 CPE treatments, respectively but no fixed trend was observed. During the year 2011-12, highest increase in plant height and plant spread was in 90 and 80% CPE treatments, respectively. The increase might be due to constant supply of ample water to the plant. This maintains the soil moisture at optimum level eliminating water stress to the plants resulted in greater vigor. For optimum plant growth, Lawande

**Table 1.** Quantity of irrigation water applied to the experimental plants during different years.

| Period | Water applied (litres / plant / day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30% CPE | 40% CPE | 50% CPE | 60% CPE | 70% CPE | 80% CPE | 90% CPE |
| February 2010 | 0.67 | 0.90 | 1.12 | 1.35 | 1.57 | 1.80 | 2.02 |
| March 2010 | 1.41 | 1.88 | 2.35 | 2.82 | 3.29 | 3.76 | 4.23 |
| April 2010 | 1.72 | 2.29 | 2.86 | 3.43 | 4.01 | 4.58 | 5.15 |
| May 2010 | 1.86 | 2.48 | 3.09 | 3.71 | 4.33 | 4.95 | 5.57 |
| June 2010 | 0.54 | 0.72 | 0.90 | 1.08 | 1.26 | 1.44 | 1.62 |
| Total during 2010 | 187.8 | 250.4 | 313.1 | 375.7 | 438.3 | 500.9 | 563.5 |
| December 2011 | 1.41 | 1.88 | 2.35 | 2.82 | 3.29 | 3.76 | 4.23 |
| January 2012 | 1.75 | 2.33 | 2.91 | 3.49 | 4.08 | 4.66 | 5.24 |
| February 2012 | 2.29 | 3.05 | 3.82 | 4.58 | 5.34 | 6.10 | 6.87 |
| March 2012 | 2.90 | 3.87 | 4.84 | 5.81 | 6.78 | 7.75 | 8.71 |
| April 2012 | 3.01 | 4.01 | 5.01 | 6.01 | 7.02 | 8.02 | 9.02 |
| May 2012 | 3.36 | 4.48 | 5.60 | 6.72 | 7.84 | 8.96 | 10.08 |
| June 2012 | 2.63 | 3.51 | 4.39 | 5.26 | 6.14 | 7.02 | 7.89 |
| Total during 2011-12 | 527.6 | 703.4 | 879.3 | 1055.2 | 1231.0 | 1406.9 | 1582.7 |
| December 2012 | 1.93 | 2.58 | 3.22 | 3.86 | 4.51 | 5.15 | 5.80 |
| January 2013 | 2.19 | 2.92 | 3.65 | 4.38 | 5.11 | 5.84 | 6.57 |
| February 2013 | 2.60 | 3.46 | 4.33 | 5.19 | 6.06 | 6.92 | 7.79 |
| March 2013 | 3.22 | 4.29 | 5.36 | 6.43 | 7.51 | 8.58 | 9.65 |
| April 2013 | 4.50 | 6.00 | 7.51 | 9.01 | 10.51 | 12.01 | 13.51 |
| May 203 | 4.98 | 6.64 | 8.30 | 9.96 | 11.62 | 13.28 | 14.94 |
| June 2013 | 2.27 | 3.03 | 3.79 | 4.55 | 5.30 | 6.06 | 6.82 |
| Total during 12-13 | 614.7 | 819.6 | 1024.5 | 1229.4 | 1434.3 | 1639.2 | 1844.1 |

**Table 2.** Moisture content in the root zone of pomegranate as affected by irrigation scheduling treatment.

| Treatment | March | | | April | | | May | | |
|---|---|---|---|---|---|---|---|---|---|
| Soil depth (cm )→ | 0-15 | 15-30 | 30-45 | 0-15 | 15-30 | 30-45 | 0-15 | 15-30 | 30-45 |
| 30% CPE | 16.9 | 17.5 | 15.6 | 17.0 | 17.5 | 14.9 | 16.5 | 16.5 | 14.3 |
| 40% CPE | 17.0 | 18.0 | 16.2 | 16.8 | 16.5 | 15.0 | 17.0 | 16.0 | 14.6 |
| 50% CPE | 17.5 | 18.8 | 18.0 | 17.3 | 17.0 | 17.2 | 16.5 | 17.2 | 15.8 |
| 60% CPE | 18.2 | 18.4 | 18.8 | 18.6 | 18.9 | 19.0 | 17.6 | 18.4 | 19.5 |
| 70% CPE | 18.8 | 19.5 | 20.3 | 19.0 | 19.5 | 20.3 | 18.2 | 19.2 | 19.8 |
| 80% CPE | 19.2 | 19.8 | 21.4 | 18.9 | 19.8 | 20.9 | 18.5 | 19.0 | 21.0 |
| 90% CPE | 20.4 | 21.4 | 22.7 | 21.0 | 22.0 | 23.0 | 20.4 | 22.1 | 22.5 |
| CD (p = 0.05) | NS | NS | 2.85* | NS | 2.82* | 3.25* | NS | 2.62* | 2.01* |

NS = Non-significant, *significant at 1% level

and Patil (6) suggested irrigation water equivalent 1.0 IW/CPE ratio for Muskat pomegranate using surface system of irrigation on black soils. During bearing period (2012-13), highest increase in plant height and plant spread was in 80 and 70% CPE treatments, respectively. The statistical analysis showed that significantly at par growth can also be obtained with the application of 60 and 70% of CPE water in pre-bearing and bearing plants, respectively. Plant growth was drastically reduced in 40 and 30% CPE treatments, receiving very less quantity of irrigation.

**Table 3.** Vegetative growth of pomegranate as affected by irrigation scheduling.

| Irrigation level | % increase during 2010 | | % increase during 2011-12 | | % increase during 2012-13 | |
|---|---|---|---|---|---|---|
| | Plant height | Plant spread | Plant height | Plant spread | Plant height | Plant spread |
| 30% CPE | 19.4 | 27.2 | 14.2 | 14.7 | 13.5 | 17.7 |
| 40% CPE | 20.0 | 25.3 | 13.9 | 14.3 | 14.9 | 18.5 |
| 50% CPE | 20.9 | 31.2 | 17.1 | 16.7 | 18.0 | 20.1 |
| 60% CPE | 30.2 | 33.7 | 17.8 | 18.0 | 20.9 | 25.0 |
| 70% CPE | 25.5 | 34.4 | 17.8 | 18.4 | 23.0 | 31.0 |
| 80% CPE | 27.0 | 31.2 | 17.2 | 19.4 | 23.4 | 27.9 |
| 90% CPE | 24.2 | 28.6 | 18.0 | 19.0 | 21.9 | 27.5 |
| CD (p = 0.05) | 4.69* | 4.05* | 1.86* | 1.66 | 3.05* | 2.35* |

NS = Non-significant, *significant at 1% level

The scheduling of irrigation had marked effect on major (N and K) and micro (Cu and Mn) nutrient contents in the leaves (Table 4). The leaf N, Cu and Mn contents was significantly higher with the application of 70% CPE irrigation water. Moderate level of irrigation water might have maintained good aeration and sufficient moisture content in soil, which resulted in higher uptake of these nutrients by the plants. Leaf K content was highest in 90% CPE treatment. The increased nutrient content due to higher moisture content in mulching treatment was reported by Chattopadhyaya and Patra (3) in pomegranate. Significantly lowest contents of N and K were recorded in 30% CPE treatment due to lack of sufficient moisture required for nutrient absorption. This finding is in close conformity with the findings of Marathe *et al.* (8) who reported decreased uptake of N, P, K and Fe in pomegranate with low moisture contend in higher irrigation interval treatments.

The leaf chlorophyll content was highest in 80% CPE followed by 90% CPE treatment (Fig. 1), indicating better photosynthetic capacity of the plants. This might be due to better nutrient uptake and ample water availability to the plants. Lowest chlorophyll content was recorded in 30% CPE treatment due stress conditions of the plant. Leaf temperature recorded during different period of fruit development increased with the increase in ambient temperature (Fig. 2). It was highest in the May followed by April and March. Minimum leaf temperature was in 70 to 90% CPE treatments receiving higher quantity of irrigation water. Cool canopy was found to be an important physiological principle for tolerance to high temperature stress. During all the months, higher leaf temperature was recorded in 30% CPE followed by 40% CPE treatments, indicating maximum stress conditions. As soil water becomes limited, transpiration got reduced and leaf temperature increased.

Number of hermaphrodite flowers were significantly highest in the plants supplied with 30% CPE followed by 50% CPE irrigation (Table 5). In general, flowering intensity increased with decreasing quantity of irrigation water. This indicated that moisture

**Table 4.** Leaf nutrient content of pomegranate as affected by irrigation scheduling.

| Irrigation level | Macronutrient (%) | | | | | Micronutrient (ppm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | Cu | Zn | Fe | Mn |
| 30% CPE | 1.86 | 0.146 | 0.49 | 2.06 | 0.57 | 106.6 | 28.3 | 115.6 | 78.2 |
| 40% CPE | 2.09 | 0.147 | 0.55 | 2.21 | 0.50 | 109.1 | 29.7 | 119.9 | 70.5 |
| 50% CPE | 2.15 | 0.158 | 0.61 | 2.19 | 0.50 | 105.8 | 27.6 | 123.0 | 66.7 |
| 60% CPE | 2.19 | 0.159 | 0.65 | 2.38 | 0.59 | 105.2 | 28.1 | 129.3 | 60.3 |
| 70% CPE | 2.25 | 0.148 | 0.63 | 2.00 | 0.49 | 124.9 | 27.9 | 118.1 | 80.7 |
| 80% CPE | 2.11 | 0.155 | 0.64 | 2.19 | 0.53 | 119.7 | 28.7 | 112.3 | 70.5 |
| 90% CPE | 2.06 | 0.144 | 0.67 | 1.81 | 0.61 | 109.3 | 28.8 | 114.3 | 72.5 |
| CD (p = 0.05) | 0.15* | NS | 0.07* | NS | NS | 13.1 | NS | NS | 8.7* |

NS = Non-significant, *significant at 1% level

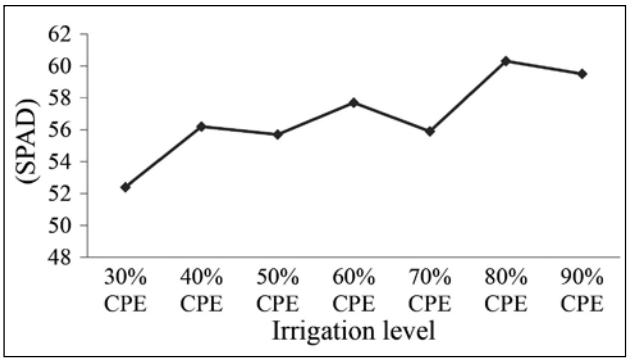


**Fig. 1.** Leaf chlorophyll content (SPAD) as affected by irrigation scheduling.

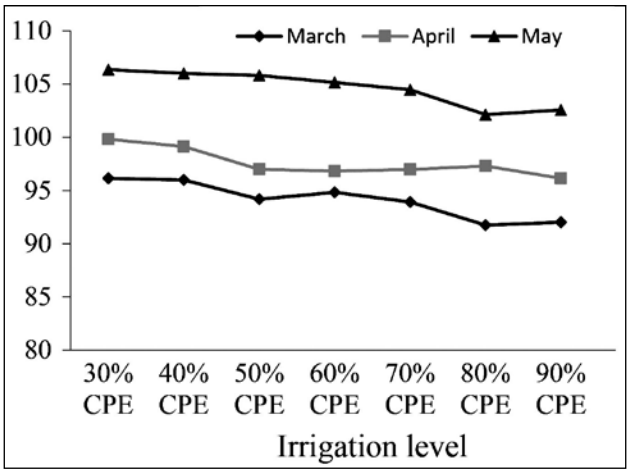


**Fig. 2.** Leaf temperature (°F) during different months as affected by irrigation scheduling.

stress encouraged reproductive phase, *i.e.* flowering intensity, might be due to the assimilation of more carbohydrates during moisture stress. The present findings are in conformity with the findings of Sharma *et al*. (13) and Marathe *et al*. (10) who reported that soil moisture deficit promotes early and more intense flowering in mango and Nagpur mandarin, respectively.

Fruit yield in terms of number and weight of the fruits was significantly higher in the plants supplied with irrigation water equivalent to 0.70 CPE followed by 0.80 CPE (Table 5). The increase in yield could be attributed to better plant growth, balanced nutrient uptake, bigger fruit size and least fruit cracking under these treatments. The results are in accordance with the findings of Lawande and Patil (6) who suggested IW/CPE ratio of 0.8 for higher fruit yield of Muskat pomegranate. Drastic reduction in fruit yield was recorded in the plants supplied with 30, 40 and 50% CPE irrigation. In these treatments, fruit cracking was as high as 58.8, 45.3 and 37.7%, respectively. The cracking was mainly due to water stress at the time of fruit maturity. Fruit cracking to the extent of 72% was reported under extreme arid climate of western Rajasthan (Charan, 2). The experimental results revealed that the cracking problem could be overcome by supplying optimum irrigation water equivalent to 70% CPE during fruiting period. Plants supplied with irrigation equivalent to 60 to 90% CPE produced good quality fruits but no fixed trend was observed with regard to different quality parameters (Data not shown). Fruit quality was drastically reduced in the treatments receiving less quantity of irrigation water (30 and 40% CPE) mainly due to shrinkage and cracking of the fruits.

It can be concluded that in light textured soils of semi-arid regions, 5.15, 5.84, 6.92, 8.58, 12.01, 13.28 and 6.06 l of water / day / plant should be provided through drip system of irrigation during the months of December, January, February, March, April, May and June, respectively to the bearing plants (height and canopy spread of 1.95 m). In water scarcity areas, pomegranate can be grown with supplemental irrigation only during summer season. In pre-bearing

**Table 5.** Flowering, fruit yield and water use efficiency as affected by irrigation scheduling.

| Irrigation level | Male flowers/ plant | Hermaphrodite flowers/ plant | No. of fruits (per plant) | Yield (kg/ plant) | Fruit cracking (%) | WUE (t/ha-cm) |
|---|---|---|---|---|---|---|
| 30% CPE | 235.5 | 140.3 | 9.0 | 1.64 | 58.8 | 0.266 |
| 40% CPE | 224.5 | 128.0 | 12.0 | 2.29 | 45.3 | 0.280 |
| 50% CPE | 210.0 | 135.1 | 15.0 | 2.91 | 37.7 | 0.284 |
| 60% CPE | 215.1 | 117.8 | 28.0 | 5.27 | 9.7 | 0.429 |
| 70% CPE | 225.0 | 125.2 | 35.1 | 6.79 | 0.8 | 0.473 |
| 80% CPE | 217.9 | 110.0 | 33.0 | 6.53 | 0.0 | 0.399 |
| 90% CPE | 200.0 | 112.3 | 31.0 | 6.48 | 0.0 | 0.351 |
| CD (p = 0.05) | NS | 10.28* | 3.47* | 0.44* | 2.78* | 0.033* |

NS = Non-significant, *significant at 1% level

periods, variation in quantity of irrigation water do not have much adverse effects on plant growth. It is advisable to make irrigation recommendations on the basis of plant canopy instead of age of the plants.

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# Effect of polyembryonic rootstocks on leaf mineral composition of five cultivars under Inceptisol

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## ABSTRACT

**The leaf macro- and micro-nutrient concentrations of five mango scion cultivars grown on three polyembryonic rootstock genotypes were studied during 2012-13 and 2013-14. Among the cultivars, Pusa Arunima appeared be the good accumulator for most of the nutrient elements except leaf N. Though, Amrapali seems to be the good accumulator of N and Mg but poorest accumulator of P, K, and Zn. Whereas, Mallika was good accumulator of N and Mg but poorest accumulator of Mn, Zn and Cu. Among rootstocks, Kurakkan was found potential for higher accumulation of leaf N, Ca, Mg and Zn concentrations in scion cultivars. Among different rootstock-scion combinations, leaf nitrogen (1.25%) and K (0.54%) in Pusa Arunima was observed higher on K-5 rootstock, while higher leaf N in Amrapali (1.36%) and Mallika (1.37%) were estimated on Kurakkan rootstock but Pusa Surya tree on Olour had higher leaf N (1.26%). In most of the cultivars, leaf K was higher on K-5 rootstock. Besides, higher leaf Ca in Pusa Arunima, Mallika and Dushehari were observed on K-5 rootstock, while in Amrapali and Pusa Surya, trees on Kurakkan had higher leaf Ca concentration. Similarly, leaf Mg concentration was also found higher on K-5 rootstock in Pusa Arunima, Amrapali, Mallika and Dushehari. The Pusa Arunima and Pusa Surya on Kurakkan proved its ability for leaf Fe and Mn concentrations, whereas, K-5 rootstock found superior over other rootstocks for the highest accumulations of Fe and Mn in Dushehari and Mallika. Both Kurakkan and Olour were equally effective for higher accumulation of Zn in Amrapali. Based on present study, it can also be inferred that metabolism of Kurakkan and/ or K-5 is better adapted for N, K, Ca, Mn, Fe and Zn accumulation for most of the mango cultivars tested.**

**Key words:** Cultivar, leaf mineral composition, mango, polyembryonic rootstock.

## INTRODUCTION

Mango (*Mangifera indica* L.) is one the most delicious and ancient fruit of India, which is widely grown mainly in tropical and subtropical regions of the world. Mangoes are also good source of vitamins A and C, carotenoids, phenolic compounds, and other dietary bioactive compounds. The mango fruit is rich in antioxidants and can reduced risk of cardiac disease, anti-cancer, and anti-viral activities. It is majorly grown in more than 90 countries, whereas in India, it is grown in about 2.51 m ha area with a productivity of 7.3 MT/ ha along with production of 18.43 MT (Anon, 2). In the present times, the productivity of mango in India and several other mango growing countries is very low. There are many factors responsible for low yield of which the use of seedling type non-descript rootstock might be considered as most important one. Hence, it is necessary to find out the different rootstock characteristics for enhancing mango fruit production. Selection of rootstocks with high nutrient uptake efficiency, well adapted to the soil and climatic conditions, will not only reduce production costs but also contribute to the sustainability and competitiveness of the Indian mango production.

Very few rootstocks studies have been reported and information is scanty on the long-range effect of rootstocks on mango yield, though some researchers studied the effect of rootstock in mango (Duran-Zauzo *et al*., 4). The studies on effect of the rootstock on nutrient composition of scion cultivar are meagre in mango adapted to tropical and subtropical conditions, though; studies in many tropical parts of the world have demonstrated the strong influence of mango polyembryonic rootstocks on the fruit yield, growth and mineral nutrition of the cultivar. This study, therefore aims to compare the influence of three polyembryonic rootstocks on accumulation of macro and micro nutrients that are important for growth, yield and quality of mango.

## MATERIALS AND METHODS

The experimental materials utilized for the present investigation consists of five mango cultivars (Pusa Arunima, Pusa Surya, Amrapali, Mallika and Dushehari) and three polyembryonic rootstocks (K-5, Kurakkan and Olour). Experiments were conducted on seven-year-old grafted plant spaced at 4 m x 4 m

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apart during 2011-15. Before starts of experiment, the available N in the soil was determined by using alkaline $KMnO_4$ method (Subbiah and Asija, 12), P by Olsen's method (Olsen *et al*., 10), K by ammonium acetate method (Hanway and Heidal, 6). However, soil available Ca, Mg, Fe, Cu, Zn and Mn were determined according to Hanway and Heidal (6) using atomic absorption spectrophotometery. For leaf tissue nutrient analysis, 10 four-month-old mature leaves were collected from the each replication and from all directions on the tree for determination of leaf nutrient contents during October in each year. These leaves were washed in series of tap water, 0.2% Teepol™ solution, 0.1N HCl and double-distilled water. The cleaned and decontaminated leaf samples were dried in a hot air oven at temperature of 70°C. Then, the dried sample was grinded with the help of a Willey mill and the ground material was passed through 1 mm mesh sieve. Leaf samples were digested in wet diacid by using nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) in the ratio of 9:4 for the estimation of mineral nutrients such as potassium, calcium, magnesium, iron, copper, zinc and manganese (Jackson, 8). Nitrogen in plant leaves was determined using Digestion Block method (Bremner *et al*., 3) and concentration on N in leaf samples was determined by using formula N (%) = [(T-B) × N × 1.4] /S, while P in plant leaves was determined by vandomolybdo phosphoric yellow colour method (Jackson, 8). The experiment was conducted in a factorial randomized block design (FRBD) with five replications. Data were analysed using the SAS package to calculate F values followed by Tukey's honest significance test. P values ≤ 0.05 were considered as significant.

## RESULTS AND DISCUSSION

On the bases of two years mean data leaf N concentration was higher in Dushehari without differing significantly with rest of the cultivars except Pusa Arunima (Table 1). Higher, leaf P and K concentrations were observed in Pusa Arunima, while they were lower in Amrapali than other cultivars (Table 1). Pusa Arunima had the highest leaf Ca concentration, while almost similar concentration of leaf Mg was observed in all cultivars except Dushehari (Table 2). Among the rootstocks, trees on Kurakkan had the highest leaf N, Ca and Mg concentrations, while trees on K-5 proved superior for leaf P and K concentrations (Table 1). Interaction between rootstock and cultivar also revealed significant differences for leaf N, P and K concentrations in Pusa Arunima, Mallika and Dushehari (Table 2). Among the rootstocks, K-5 and Kurakkan had significantly higher leaf N concentration in Pusa Arunima. However, in Mallika and Dushehari trees on Kurakkan accumulated higher N in leaf

**Table 1.** Mean effect of cultivar and rootstock on leaf N, P and K concentrations in mango grafted on different rootstocks.

| Rootstock/ cultivar | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|
| Cultivar | | | | | |
| Pusa Arunima | $1.19^{b}$ | $0.17^{a}$ | $0.48^{a}$ | $1.41^{a}$ | $0.19^{a}$ |
| Pusa Surya | $1.21^{ba}$ | $0.15^{bac}$ | $0.47^{bc}$ | $1.21^{d}$ | $0.19^{ba}$ |
| Amrapali | $1.23^{ba}$ | $0.13^{c}$ | $0.43^{c}$ | $1.36^{b}$ | $0.19^{ba}$ |
| Mallika | $1.23^{ba}$ | $0.15^{bc}$ | $0.46^{b}$ | $1.33^{c}$ | $0.19^{ba}$ |
| Dushehari | $1.25^{a}$ | $0.16^{ba}$ | $0.44^{c}$ | $1.32^{c}$ | $0.18^{b}$ |
| Rootstock | | | | | |
| K-5 | $1.20^{b}$ | $0.17^{a}$ | $0.49^{a}$ | $1.37^{b}$ | $0.19^{b}$ |
| Kurakkan | $1.30^{a}$ | $0.15^{b}$ | $0.42^{c}$ | $1.39^{a}$ | $0.20^{a}$ |
| Olour | $1.18^{b}$ | $0.13^{c}$ | $0.45^{b}$ | $1.22^{c}$ | $0.18^{c}$ |
| LSD (p ≤ 0.05) | | | | | |
| Cultivar | 0.05 | 0.02 | 0.01 | 0.02 | 0.01 |
| Rootstock | 0.04 | 0.01 | 0.02 | 0.02 | 0.01 |

**Table 2.** Leaf N, P and K concentrations of mango cultivars grafted on different rootstocks.

| Rootstock/ cultivar | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|
| Pusa Arunima | | | | | |
| K-5 | $1.25^{dc}$ | $0.22^{a}$ | $0.54^{a}$ | $1.51^{ba}$ | $0.18^{bdc}$ |
| Kurakkan | $1.23^{edc}$ | $0.15^{b}$ | $0.48^{bcd}$ | $1.56^{a}$ | $0.21^{ba}$ |
| Olour | $1.10^{e}$ | $0.13^{b}$ | $0.41^{e}$ | $1.15^{ed}$ | $0.19^{bdc}$ |
| Pusa Surya | | | | | |
| K-5 | $1.19^{edc}$ | $0.16^{b}$ | $0.52^{ba}$ | $1.05^{f}$ | $0.19^{bdc}$ |
| Kurakkan | $1.18^{edc}$ | $0.15^{b}$ | $0.43^{ed}$ | $1.45^{b}$ | $0.19^{bdc}$ |
| Olour | $1.26^{bc}$ | $0.13^{b}$ | $0.46^{ecd}$ | $1.11^{ef}$ | $0.18^{dc}$ |
| Amrapali | | | | | |
| K-5 | $1.15^{edc}$ | $0.15^{b}$ | $0.49^{bc}$ | $1.29^{c}$ | $0.17^{d}$ |
| Kurakkan | $1.27^{bac}$ | $0.13^{b}$ | $0.45^{ecd}$ | $1.47^{b}$ | $0.22^{a}$ |
| Olour | $1.27^{bac}$ | $0.12^{b}$ | $0.35^{f}$ | $1.32^{c}$ | $0.17^{dc}$ |
| Mallika | | | | | |
| K-5 | $1.16^{edc}$ | $0.17^{b}$ | $0.49^{bc}$ | $1.48^{b}$ | $0.20^{bac}$ |
| Kurakkan | $1.40^{ba}$ | $0.14^{b}$ | $0.45^{ecd}$ | $1.31^{c}$ | $0.21^{ba}$ |
| Olour | $1.11^{ed}$ | $0.12^{b}$ | $0.43^{ed}$ | $1.21^{d}$ | $0.16^{d}$ |
| Dushehari | | | | | |
| K-5 | $1.22^{edc}$ | $0.16^{b}$ | $0.41^{e}$ | $1.50^{ba}$ | $0.18^{bdc}$ |
| Kurakkan | $1.41^{a}$ | $0.16^{b}$ | $0.45^{ecd}$ | $1.18^{ed}$ | $0.19^{bdc}$ |
| Olour | $1.14^{edc}$ | $0.14^{b}$ | $0.45^{ecd}$ | $1.29^{c}$ | $0.19^{bdc}$ |
| LSD (p ≤ 0.05) | 0.15 | 0.06 | 0.05 | 0.07 | 0.03 |

tissues. Moreover, except Pusa Arunima, leaf P concentration was not affected by rootstock in rest of the cultivars. K-5 rootstock proved superior in terms of leaf P concentration for Pusa Arunima, However, rootstock influenced leaf K concentration in all cultivars except Dushehari. Among the rootstocks it is evident that K-5 had significantly higher leaf K content as compared to rest of the rootstocks irrespective of the cultivars. However, for Amrapali and Mallika, Kurakkan was also as good as K-5. Differences in K accumulation in leaf tissues of scion cultivars may be due to variation in absorption capacity of rootstock or differences in the uptake of K ion into xylem and its translocation from root to shoot. Moreover, for leaf Ca, both Kurakkan and K-5 rootstocks proved efficient with higher accumulation of Ca in Pusa Arunima and Pusa Surya, however Amrapali was on either Kurakkan or Olour. These results are in agreement to earlier findings in mango (Duran Zuazo *et al*., 5); Mexican lime (Khankahdani *et al*., 9). In present investigation, rootstock had significant effect on leaf nutrient concentrations in some cultivars, while it did not affect significantly in others. Differences in nutrient accumulation in scion cultivars as result of rootstock-scion combination may be due to various reasons such as structure of root system, variations in root CEC, and characteristics of root exudates and phenotype of the scion cultivars. It is clear from the present results that except Pusa Arunima, higher leaf N was observed in vigorous or medium vigorous rootstocks, while reverse trend was noticed for leaf K concentration and higher K concentration was noticed in rootstock imparting dwarfing in scion cultivars. As a result of low yield in these cultivars on K-5 rootstock had thus lower demand for K, higher leaf K concentration might be the possible reason in such rootstock-scion combinations. In contrast, Abdalla *et al.* (1) reported low leaf K concentration in dwarfing rootstock of apple. Rootstock and variety could influence leaf nutrient composition of mango even when grown under similar agro-climatic and soil conditions.

The differences in leaf micronutrients concentrations were observed due to cultivars and rootstocks tested in the present study (Fig. 1). From micronutrients analysis, it is clear that Pusa Arunima and Pusa Surya had higher leaf Fe, concentrations than rest of the cultivars. Pusa Arunima seems to be good Cu and Zn accumulators, however, highest leaf Cu concentration was observed in Pusa Surya. Among rootstock genotypes, K-5 proved to be potential for enhancing Fe and Mn concentrations in leaf tissues of scion cultivars, while Kurakkan was promising for higher accumulation of Zn. Kurakkan as well as Olour proved better rootstocks for leaf Cu concentration. Rootstock influenced leaf micronutrient concentrations in all mango cultivars (Fig. 1). Pusa Arunima and Pusa Surya on Kurakkan proved superior for leaf Fe and Mn levels, however, trees on K-5 also accumulated higher Fe than trees on Olour. For Dushehari, K-5 rootstock was found superior over others for highest accumulations of Fe and Mn. This rootstock promoted leaf Mn concentrations in Mallika also. Rootstocks K-5 and Kurakkan were found equally good for Amrapali with regard to leaf Mn concentration. Leaf Zn concentrations were not much affected significantly in Pusa Surya and Mallika but significantly highest level was observed in Pusa Arunima and Dushehari on Kurakkan rootstock. Both Kurakkan and Olour were equally effective for higher accumulation of Zn in Amrapali. Furthermore, leaf Cu concentration in Pusa Arunima was noticed highest on Kurakkan, while for Pusa Surya both Olour and K-5 rootstock proved to be a good Cu accumulator. Amrapali leaf had the highest Cu on Olour rootstock as compared to rest of the rootstocks taken into study. Our findings are in agreement to those of Khankahdani *et al*. (9) in citrus for all micronutrients and Reddy and Kurian (11) in mango for Fe. Based on the results, it can be argued that metabolism of Kurakkan was better adapted for Fe and Zn for most of the mango cultivars studied. However, K-5 rootstock genotype performed better to support Mn uptake for most of the cultivars except Pusa Surya, where Kurakkan seemed to be better. In Mallika, the levels of Fe, Zn and Cu were higher on rootstock that promotes vigorous growth, however, a reverse relation was observed in case of Pusa Arunima, where all micronutrients were higher either on dwarf or semi-dwarf rootstocks. Our findings conformed to the findings of Ikinci *et al.* (7) for most of the cultivars except Pusa Arunima.

Based on present study, it could be inferred that metabolism of Kurakkan and/ or K-5 were better for N, K, Ca, Mn, Fe and Zn accumulation for most of the mango cultivars studied. Furthermore, vigorous rootstock was more effective for Fe and Mn absorption in Pusa Surya.

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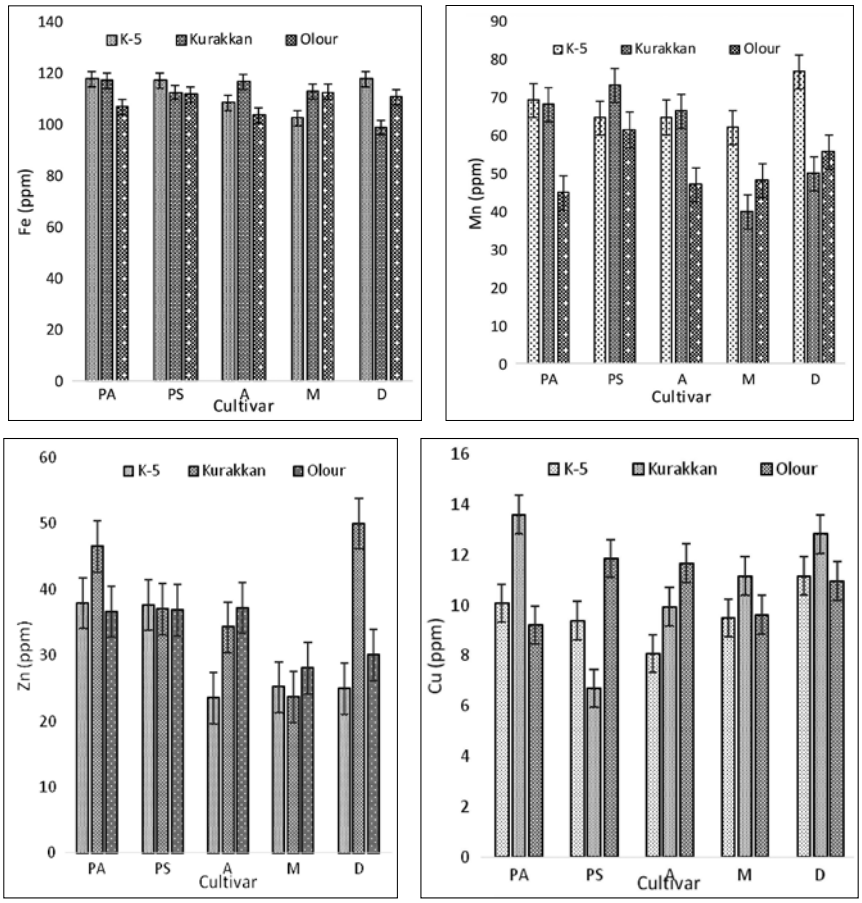


**Fig. 1**. Effect of rootstocks on leaf Fe (A), Mn (B), Zn (C) and Cu (D) of different mango cultivars. Vertical bar represent mean of five replicates ± SE m. The LSD (P ≤ 0.05) for Fe; 5.18, Mn; 6.43, Zn; 3.88, Cu; 2.64. PA, Pusa Arunima; PS, Pusa Surya; A, Amrapali; M, Mallika, and D; Dushehari


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# Genetic analysis of root yield and its contributing traits in tropical carrot (*Daucus carota* L.)

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## ABSTRACT

**Information on the genetic basis of root yield and quality of different coloured carrot genotypes is essential for planning the breeding strategies for genetic improvement. The objective of this study was carried out during winter and spring-summer season of 2011-15 to determine the gene action involved in the inheritance of economic traits of three carrot hybrids by using six generation mean analysis ($P_1$, $P_2$, $F_1$, $F_2$, $B_1$ and $B_2$). Three crosses of carrot were analyzed to study the gene actions involved in the inheritance of economic traits, *viz*., root length, root weight, shoulder diameter, root diameter, flesh thickness, core diameter and root to top ratio. The genetics of root weight, root to top ratio and root diameter in the all White Pale crosses were complementary type of gene interaction, which shows the genetic improvement of carrot tropical with respect to these traits can improved through biparental matting followed by mass and cyclic recurrent selection in advanced generation.**

**Key words:** Carrot, complimentary epistasis, duplicate epistasis, gene action, recurrent selection, generation mean analysis.

## INTRODUCTION

Carrot (*Daucus carota* L.; 2n = 2X = 18) is a cool weather crop grown in temperate and subtropical regions for its edible storage tap roots both for fresh as well as processed vegetable throughout the world and is most important of all the root crops. The objective of carrot breeding programmes is to evolve high yielding and well adapted cultivar with desirable economic traits. Breeding for such cultivars requires through understanding of genetic components of carrot. Many breeding procedures have been brought up for increasing yield of carrot but in order to bring up best hybrid combinations, a large population of carrot inbred lines are crossed to each other. Before the improvement of high yielding carrot cultivars and/or hybrids, it is important to study the economic components of gene interaction and effects. The genetic component of complex traits plays important role launching a sound breeding strategy. Therefore, the present experiment was undertaken to determine the inheritance pattern of economic traits in carrot involving four phenotypically contrasting tropical carrots. These inheritance studies will help in the understanding of gene interaction and breeding selection of potential parental lines or crosses. Furthermore, these breeding strategies will help to accelerate the tropical carrot breeding with generation of new carrot cultivars and hybrids.

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## MATERIALS AND METHODS

The experiment was carried out at Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi in the winter season for four years. The experiment was laid out in a randomized block design with three replications. Four different coloured carrot (*D. carota* L.) inbred lines, *viz.*, White Pale (yellow), IPC-126 (purple), IPC-122 (red) and PM (orange), which were used for development of six generations. Six basic generations ($P_1$, $P_2$, $F_1$, $F_2$, $B_1$ and $B_2$) of three crosses (White Pale × IPC-126, White Pale × IPC-122 and White Pale × PM) were raised and planted in a randomized block design in three replications at vegetable research farm during the November 2014 as four ridges of for each parent and $F_1$s, ten ridges for $B_1$ and $B_2$ each, 15 ridges for $F_2$ plants. The four parental and 3 $F_1$ generations were represented by 20 plants within each replication, while each segregating generation, $F_2$'s, $B_1$ and $B_2$s were represented by 100 and 50 plants. Data were recorded on an individual plant of six populations for each cross where 20, 20, 25, 300, 50 and 50 plants, which were chosen from $P_1$, $P_2$, $F_1$, $F_2$, $B_1$ and $B_2$ of each crosses respectively, to record the following traits: root length, top height, number of leaves, plant weight, root weight, leaf weight, shoulder diameter, core diameter and root girth-top, bottom and middle. To determine the presence or absence of non-allelic interactions, scaling test as A, B, C and D have been calculated to test adequacy of additive-dominance model in each case (Mather, 10). The best model was selected based on additive and dominance

model using non-significant Chi-square test and lower the standard error. The observed means of the six generations were used to estimate '*m*', 'd' and '*h*' as per the joint scaling test of Cavalli (1). The joint scaling Mather (10) test was employed to estimate the mean [*m*], additive effect [*d*], dominance effect [*h*], additive × additive [*i*], additive × dominance [*j*] and dominance × dominance [*l*] values. Significance of the scales and gene effects were tested by using the *t*-test of Singh and Singh (15). The type of epistasis was determined as complementary when dominance [*h*] and dominance × dominance [*l*] gene effects have same sign and duplicate epistasis when the sign was different (Kearsey and Pooni, 8). Statistical analyses were carried out separately for each cross using the PBT (12) software developed by IRRI.

## RESULTS AND DISCUSSION

The scale estimates of root length (Table 1) showed that the adequacy of six parameter model in explaining the inheritance of root length. Preponderance of additive × additive gene [*i*] action as well as dominance × dominance [*l*] gene interaction was found to play an important role in governing the inheritance of this trait in White Pale × IPC-126 and White Pale × IPC-122 cross combinations. Significant values of dominance [*h*] and dominance × dominance [*l*] with same signs indicated that epistatic interaction of complimentary type explained the inheritance of root length in White Pale × PM cross. This interaction inflates the variation in the segregating population. Similar results have been reported earlier by Dixit *et al.* (3), Holland (5), Iqbal *et al.* (6), Novoselovic *et al.* (11), Srivastava *et al.* (17) and Wu *et al.* (19). The positive direction of [*d*], [*l*] and complimentary type epistatic gene interactions in White Pale × PM cross showed the intermating of these parents and selection in filial generation for improvement for this trait. The results are in agreement with those of Checa *et al.* (2), Iqbal *et al.* (6), Singh *et al.* (14), Srikanth *et al.* (16), Srivastava *et al.* (17) and Stuber *et al.* (18).

As given in the Table 2 for root weight gene interaction, the significant [*h*] and [*l*] effects showed the epistatic interaction in the inheritance of root weight in a cross of White Pale × IPC-126, whereas the sign of [*h*] and [*l*] were of same sign due to the interaction of complimentary epistasis type. The digenic non-allelic epistasis of [*i*], [*j*] and [*l*] were considered the major contributors in the inheritance of these traits in crosses of White Pale × IPC-122 and White Pale × PM. Significance of [*j*] for root weight traits in the both White Pale × IPC-126 and White Pale × PM crosses revealed that selection through selfing is not effective for improvement of these traits because among the digenic interactions, additive × dominance type is more fixable and more useful for carrot breeders. These results are comparable with Jenson (7), Novoselovic *et al.* (11), Rodriguez *et al.* (13), Singh *et al.* (14), Srikanth *et al.* (16), Stuber *et al.* (18) and Wu *et al.* (19).

Joint scaling and simple scaling test were significant for all crosses showing that adequacy of six parameter model to explain shoulder and root diameter (Table 1). It indicated that presence of non-allelic interaction (Table 2), the effect of dominance [*h*] and additive × dominance [*j*] were significant and positive in the cross White Pale × IPC-126, whereas in White Pale × PM there were significant effect of additive [*d*], dominance × dominance [*l*] and additive × dominance [*j*] gene interactions. The negative additive [*d*] effects lead to non-dispersal of gene(s) between parents. The additive [*d*] and dominance [*h*] gene interactions played an important role in the inheritance of these traits in a cross of White Pale × IPC-122. Similarly, results on these traits were confirmed with Checa *et al.* (2), Dixit *et al.* (3), Gamble (4), Holland (5) and Srikanth *et al.* (16). Complimentary type of gene interaction were expressed in all three crosses, which implied that heterosis breeding can be exploited for improvement of this trait.

Three crosses with respect to core diameter exhibited significance for either A, B, C, or D scales indicating the presence of inter-allelic interaction (Table 2). The magnitude of [*h*] effects was comparatively higher than that of [*d*] effects in non-interacting cross. The additive gene [*d*] and dominance × dominance [*l*] gene effect were significant in White Pale × IPC-126 cross and White Pale × IPC-122 with negative and positive values, respectively. Duplicate type of epistasis were governing core diameter in White Pale × IPC-126 and White Pale × IPC-122, which showed that early selection may not be useful for this trait and advanced generation selection will be useful for uniform core diameter. The results are in agreement with those of Jenson (7), Novoselovic *et al.* (11), Srikanth *et al.* (16), Srivastava *et al.* (17), Stuber *et al.* (18) and Wu *et al.* (19). Negative additive [*d*], additive × dominance [*j*] and positive dominance × dominance [*l*] gene interactions expressed significantly in White Pale × PM cross for core diameter in heterosis breeding will be more useful due to complimentary type of epistatic interaction.

Flesh thickness revealed that the estimates for either of simple scales, A, B, C, or D were significant for all the crosses. The additive gene [*d*] effect was significant in cross White Pale × IPC-122 with positive values and White Pale × IPC-126, White Pale × PM with negative values. The highest magnitude of additive gene [*d*] effects was found in cross White

**Table 1.** Estimates of scaling test and Joint scaling test of three white pale crosses for economic traits.

| Trait | Cross | Scaling test | | | | Joint Scaling Test | | |
|---|---|---|---|---|---|---|---|---|
| | | A ± SE | B ± SE | C ± SE | D ± SE | m ± SE | d ± SE | h ± SE |
| Root length (cm) | I | 7.55** ± 1.34 | 13.76** ± 1.60 | 13.85** ± 2.53 | 3.72** ± 1.08 | 26.13** ± 0.40 | 1.82** ± 0.37 | -1.04** ± 0.81 |
| | II | 9.93** ± 1.61 | 8.18** ± 2.23 | 10.25** ± 1.85 | 3.93** ± 1.38 | 23.44** ± 0.37 | -0.10** ± 0.39 | 5.62** ± 0.68 |
| | III | 10.84** ± 2.20 | 7.14** ± 1.50 | 15.93** ± 1.63 | 1.03 ± 1.40 | 22.02** ± 0.33 | -0.95** ± 0.36 | 8.75** ± 0.52 |
| Root weight (g) | I | 434.70** ± 25.43 | 279.00** ± 30.53 | 772.14** ± 23.52 | -29.22 ± 22.00 | 211.47** ± 3.69 | -63.78** ± 3.79 | 193.12** ± 5.16 |
| | II | 295.23** ± 33.28 | 378.28** ± 28.97 | 526.90** ± 31.20 | 73.30** ± 26.28 | 281.94** ± 2.18 | -3.94 ± 2.20 | 130.15** ± 4.83 |
| | III | 503.49** ± 24.38 | 307.87** ± 34.73 | 689.66** ± 28.56 | 60.85* ± 25.15 | 241.58** ± 1.88 | -36.56** ± 1.90 | 189.35** ± 3.60 |
| Shoulder dia. (mm) | I | 51.25** ± 2.76 | 7.75* ± 3.36 | 63.19** ± 3.39 | -2.09 ± 2.32 | 41.77** ± 0.68 | -4.71** ± 0.71 | 7.94** ± 1.08 |
| | II | 6.08* ± 3.02 | 5.91 ± 5.91 | 12.92** ± 3.33 | -0.45 ± 2.51 | 43.40** ± 0.54 | -5.00** ± 0.57 | 13.64** ± 0.89 |
| | III | 25.61** ± 3.07 | 0.65 ± 3.74 | 17.48** ± 3.00 | 4.38 ± 2.68 | 42.37** ± 0.41 | -4.93** ± 0.43 | 12.31** ± 0.72 |
| Root dia. (mm) | I | 51.32** ± 2.55 | 7.66* ± 3.35 | 63.22** ± 3.28 | -2.11 ± 2.25 | 36.02** ± 0.69 | -4.55** ± 0.73 | 12.46** ± 0.98 |
| | II | 6.08* ± 3.02 | 5.91 ± 3.25 | 12.92** ± 3.33 | -0.45 ± 2.51 | 40.19** ± 0.54 | -5.00** ± 0.57 | 13.64** ± 0.89 |
| | III | 25.61** ± 3.07 | 0.65 ± 3.74 | 17.48** ± 3.00 | 4.38 ± 2.68 | 39.16** ± 0.41 | -4.93** ± 0.43 | 12.31** ± 0.72 |
| Core dia. (mm) | I | 2.22** ± 0.41 | 1.17* ± 0.57 | 3.10** ± 0.78 | 0.14 ± 0.21 | 4.40** ± 0.16 | -0.00** ± 0.13 | -0.77* ± 0.32 |
| | II | 6.37** ± 1.28 | 6.29** ± 1.10 | 0.53 ± 2.04 | 6.07** ± 0.75 | 8.54** ± 0.28 | -1.49** ± 0.25 | 0.56** ± 0.61 |
| | III | 8.66** ± 0.80 | 4.74** ± 0.96 | 11.79** ± 1.36 | 0.81 ± 0.53 | 6.30** ± 0.24 | -0.74** ± 0.22 | -1.03* ± 0.49 |
| Flesh thickness (mm) | I | 53.38** ± 2.76 | 10.03** ± 3.40 | 67.67** ± 3.41 | -2.12 ± 2.33 | 36.35** ± 0.70 | -4.23** ± 0.75 | 9.95** ± 1.09 |
| | II | 5.83* ± 3.052 | 4.82 ± 3.29 | 31.59** ± 2.96 | -10.471** ± 2.4 | 30.97** ± 0.58 | -5.74** ± 0.64 | 15.35** ± 0.82 |
| | III | 27.67** ± 3.21 | 4.39 ± 3.68 | 21.96** ± 2.98 | 5.05* ± 2.70 | 38.10** ± 0.45 | -4.60** ± 0.47 | 13.06** ± 0.71 |
| Root to top ratio | I | 14.12** ± 1.37 | 10.38** ± 2.10 | 23.38** ± 2.52 | 0.56 ± 0.84 | 6.93** ± 0.56 | -1.23* ± 0.52 | -1.53* ± 1.06 |
| | II | 14.12** ± 1.37 | 10.38** ± 2.10 | 23.38** ± 2.52 | 0.56* ± 0.84 | 8.61** ± 0.40 | -2.31** ± 0.37 | -1.13* ± 0.87 |
| | III | 18.60** ± 1.60 | 10.11** ± 1.92 | 25.65** ± 2.72 | 1.53 ± 1.03 | 7.32** ± 0.51 | -1.19** ± 0.47 | -1.84* ± 1.03 |

Cross: I = White Pale × IPC-126, II = White Pale × IPC-122, III = White Pale × PM

Significant at A & B = involves three type of non-allelic-gene interactions; Significant at C = involves D × D; Significant at D = involves A × A; Significant at C and D = involves A × A and D × D

m = mean, [d] = additive, [h] = Dominance

*Significant at 5 and 1% levels; D = Duplicate epistasis; C = Complementary epistasis

**Table 2.** Estimation of gene effects based on six generation mean analysis in three White Pale crosses of carrot.

| Trait | Cross | Gene interaction | | | | | | Type of epistasis |
|---|---|---|---|---|---|---|---|---|
| | | m ± SE | [*d*] ± SE | [*h*] ± SE | [*i*] ± SE | [*j*] ± SE | [*l*] ± SE | |
| Root length (cm) | I | 25.52** ± 0.40 | 0.48 ± 0.72 | -4.62 ± 2.38 | -7.45** ± 2.17 | 6.21* ± 1.69 | 28.76** ± 3.85 | D |
| | II | 25.60** ± 0.29 | -0.69 ± 1.25 | -1.09* ± 2.85 | -7.87** ± 2.77 | -1.74 ± 2.63 | 25.99** ± 5.33 | D |
| | III | 24.31** ± 0.31 | -1.00 ± 1.25 | 6.34* ± 2.85 | -2.05 ± 2.80 | -3.70 ± 2.61 | 20.05** ± 5.26 | C |
| Root weight (g) | I | 159.59** ± 5.27 | -47.60* ± 19.31 | 239.19** ± 44.31 | 58.44 ± 44.01 | -155.70** ± 39.45 | 655.26** ± 80.76 | C |
| | II | 235.90** ± 7.39 | 43.77* ± 21.72 | 8.13* ± 52.80 | -146.61** ± 52.57 | 83.04 ± 43.68 | 820.13** ± 92.34 | D |
| | III | 181.71** ± 6.90 | -57.05** ± 21.01 | 83.04* ± 50.43 | -121.70* ± 50.30 | -195.61** ± 42.21 | 933.07** ± 88.79 | D |
| Shoulder dia. (mm) | I | 37.30** ± 0.64 | -20.08** ± 1.94 | 13.60** ± 4.78 | 4.18 ± 4.64 | -43.49** ± 4.22 | 54.82** ± 8.46 | C |
| | II | 48.17** ± 0.70 | 4.52* ± 2.08 | 14.82** ± 5.10 | 0.91 ± 5.02 | -0.17 ± 4.34 | 11.08** ± 8.98 | C |
| | III | 45.72** ± 0.65 | -6.94** ± 2.34 | 4.35* ± 5.42 | -8.77 ± 5.37 | -24.95** ± 4.77 | 35.03** ± 9.86 | C |
| Root dia. (mm) | I | 33.46** ± 0.63 | -20.43** ± 1.87 | 13.87** ± 4.63 | 4.23 ± 4.51 | -43.66** ± 4.13 | 54.74** ± 8.17 | C |
| | II | 44.96** ± 0.70 | 4.52* ± 2.08 | 14.82** ± 5.10 | 0.91 ± 5.02 | -0.17 ± 4.34 | 11.08** ± 8.98 | C |
| | III | 42.51** ± 0.65 | -6.94** ± 2.34 | 4.35* ± 5.42 | -8.77 ± 5.37 | -24.95** ± 4.77 | 35.03** ± 9.86 | C |
| Core dia. (mm) | I | 3.97** ± 0.06 | -0.35* ± 0.17 | -0.13* ± 0.571 | -0.29 ± 0.43 | -1.05 ± 0.57 | 3.68** ± 1.059 | D |
| | II | 10.14** ± 0.26 | 1.18* ± 0.53 | -9.29** ± 1.74 | -12.14** ± 1.51 | -0.07 ± 1.22 | 24.82** ± 2.96 | D |
| | III | 5.51** ± 0.16 | -0.82* ± 0.42 | 0.99* ± 1.22 | -1.62 ± 1.06 | -3.91** ± 1.00 | 15.03** ± 2.17 | C |
| Flesh thickness (mm) | I | 32.54** ± 0.64 | -20.12** ± 1.94 | 13.79** ± 4.79 | 4.24 ± 4.66 | -43.34** ± 4.26 | 59.18** ± 8.50 | C |
| | II | 33.32** ± 0.60 | 3.97* ± 2.10 | 34.72** ± 4.93 | 20.94 ± 4.86 | -1.01** ± 4.43 | -10.28** ± 8.93 | D |
| | III | 40.96** ± 0.65 | -6.21** ± 2.36 | 3.13* ± 5.45 | -10.11 ± 5.40 | -23.27** ± 4.83 | 42.18** ± 9.92 | C |
| Root to top ratio | I | 5.58** ± 0.23 | -1.41* ± 0.71 | -0.96* ± 2.05 | -1.12 ± 1.69 | -3.73 ± 2.25 | 25.62** ± 3.79 | D |
| | II | 7.90** ± 0.29 | 1.97* ± 0.88 | 0.96* ± 2.52 | -5.17 ± 2.12 | -0.79 ± 1.95 | 28.73** ± 4.61 | C |
| | III | 5.80** ± 0.31 | -1.97* ± 0.82 | 2.30* ± 2.40 | -3.06 ± 2.07 | -8.48** ± 2.02 | 31.77** ± 4.27 | C |

Cross: I = White Pale × IPC-126, II = White Pale × IPC-122, III = White Pale × PM
m = mean, [*d*] = additive, [*h*] = Dominance, [*i*] = additive × additive, [*j*] = additive × dominance, [*l*] = dominance × dominance
*,**Significant at 5 and 1% levels; D = Duplicate epistasis; C = Complementary epistasis

Pale × IPC-126. Dominant [*h*] gene effects were highly significant with positive values for flesh thickness was found in the White Pale × IPC-126 and White Pale × IPC-122 crosses. Among the interaction of gene effects, additive × dominance [*j*] gene effects were highly significant in White Pale × IPC-126, White Pale × IPC-122 and White Pale × PM cross with negative estimates. Dominant × dominant interaction [*l*] was highly significant in White Pale × IPC-126 and White Pale × PM crosses with positive direction. White Pale × IPC-126 and White Pale × PM crosses showed complimentary type of epistasis as indicate by positive signs of [*h*] and [*l*] genetic parameters, whereas, White Pale × IPC-122 cross expressed duplicate type of epistasis as indicated by negative signs of [*h*] and [*l*] genetic parameters. Similar findings have been observed by Holland (5), Srivastava *et al.* (17), Stuber *et al*. (18) and Wu *et al.* (19).Therefore, heterosis breeding will be utilized for improving flesh thickness due to negative [*d*], [*j*] which leads to non-dispersal of alleles in White Pale × IPC-126 and White Pale × PM crosses, whereas recurrent and mass selection through intermating of parents could be exploited for improvement of this trait in White Pale × IPC-122 cross.

The root to top ratio estimates for scaling tests and gene effects (Table 2) revealed that the estimates for either of simple scales, A, B, C, and D were significant for all White Pale crosses. Additive gene effects [*d*] were observed to be significant in a cross White Pale × IPC-122 with positive estimates, which were in desirable direction, whereas in White Pale × IPC-126 and White Pale × PM crosses were additive gene effects with negative values. The positive dominance × dominance [*l*] and negative additive gene [*d*] effect was controlling root to top ratio trait in White Pale × IPC-126 and White Pale × PM and *vice-versa* in White Pale × IPC-122 cross for this trait. The dissimilar signs of [*h*] and [*l*] gene action and duplicate type of epistatic effects was observed in White Pale × IPC-126 in which selection breeding methodology can be advanced generation through intermating of parents followed by mass selection and recurrent selection. Heterosis would be exploited for applicable for root diameter, length, flesh thickness and core diameter traits in White Pale × IPC-122 and White Pale × PM crosses indicated predominance of complementary type of epistasis due to non-fixable gene effects. The negative additive [*d*] gene effects leads to non-dispersal of gene between parents, which confirm well with the findings of Ma *et al.* (9), Checa *et al.* (2), Gamble (4), Holland (5), Iqbal *et al.* (6) and Srikanth *et al.* (16). The generation mean analysis revealed that complimentary and duplicate type of epistatic gene interaction was inherited for economic traits.

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# Genotype × environmental interactions in ridge gourd genotypes for fruit yield and its contributing traits

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## ABSTRACT

**Phenotypic stability of 51 ridge gourd (*Luffa acutangula* Roxb.) genotypes was studied during *Rabi*-summer seasons of 2011-14. Pooled analysis of variance revealed highly significant differences among the genotypes for all the traits except for yield/ vine suggesting enough genetic variability. Mean squares for environments and genotype × environment (G × E) interactions were significant suggesting that the environments under study are diverse enough and the traits responded to the environments differently. Among the genotypes, RGGP-12 recorded the highest mean yield (37.93 t/ha) followed by RGGP-41 (37.05 t/ha). Genotypes RGG-12, RGGP-41, RGGP-3 and RGGP-7 proved to be most stable genotypes for mean fruit yield/ ha, RGGP-48 for fruit number/ vine and fruit weight, RGGP-21 for node number for first female flower appearance and fruit number, which can be exploited for these yield contributing traits in the ridge gourd improvement programmes.**

**Key words**: G × E interaction, ridge gourd, stability analysis, regression coefficient.

## INTRODUCTION

Ridge gourd is the important cucurbitaceous vegetable grown throughout the country. Immature fruits of ridge gourd are very nutritious and good source of vitamin A, calcium, phosphorus, ascorbic acid and iron. In any breeding programme it is necessary to screen and identify phenotypically stable genotype for yield, which could perform more or less uniformly under different environmental conditions. It is an established fact that yield is a complex character and largely depends upon its components characters, with an interaction with the environments resulting in to the ultimate product, *i.e*. yield. Thereafter, breeding a stable variety, it is necessary to get the information on the extent of genotype × environment (GE) interaction for yield and its component characters. In ridge gourd, yield/ plant depends on number of fruits/ plant, fruit length and fruit weight (Varalakshmi *et al.*, 8; Hanumegowda *et al.*, 3).

To meet the objective of developing varieties with high yield potential a wide collection of germplasm must be available so that the evaluation for desirable traits for yield can be exercised and a breeding programme for an ideal plant type concept can be made accordingly. A phenotype is the product of interplay of genotype and its environment.

A specific genotype does not exhibit the same phenotype under the changing environments and different genotypes respond differently to a specific environment. This variation arising from the lack of correspondence between the genetic and non-genetic effects is known as genotype × environment interaction. G × E interactions are generally considered impediment in plant breeding as it baffles the breeder in judging the real potential of a genotype when grown in different environments. Several workers considered G × E interactions as linear functions of environment and proposed regression of yield of a genotype on the mean yield of all genotypes in each environment to evaluate stability of performance of genotype (Shaikh *et al.*, 6). The main objective of a breeding programme is to develop varieties that perform well over a broad spectrum of environments. The information about phenotypic stability is useful for the selection of crop varieties as well as for breeding programmes. In ridge gourd, only one study is available so far on these aspects by Varalakshmi and Subba Reddy (9). Hence, the objective of the present study was to explore the effect of genotype (G) and genotype × environment (GE) on fruit yield and its attributing traits in ridge gourd.

## MATERIAL AND METHODS

The experimental material for the present study comprised of 51 ridge gourd genotypes maintained in the Division of Vegetable Crops, ICAR-IIHR, Bengaluru. These genotypes were grown in simple randomized block design (RBD) in two replications at the Vegetable Farm during *Rabi*-summer seasons of 2011-12, 2012-13 and 2013-14. All the recommended cultural and agronomic practices including pest control measures were adopted to raise good crop.

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To ascertain the comparative behaviour of different genotypes under different environments, observations were recorded on five randomly selected plants from each replication for seven parameters such as node number for first female flower appearance, fruit length (cm), fruit girth (cm), fruit weight (g), fruit No./vine, fruit yield/ vine (kg) and fruit yield/ ha (t). In all the experiments, plot means (mean of five plants) were used for environment-wise analysis of variance and pooled analysis of variance for the estimation of G × E interaction effects and stability analysis as suggested by Eberhart and Russell (2).

## RESULTS AND DISCUSSION

In the pooled analysis of variance for different traits revealed highly significant differences among the genotypes for all the traits except for yield/ vine suggesting enough genetic variability among the genotypes for all these traits (Table 1). Similar findings were reported by Shaikh *et al.* (6) and Thakur *et al.* (7) for all the yield and yield contributing traits in bottle gourd and bitter gourd respectively. The variances associated with genetic effects were smaller than the variances associated with environmental effects for most of the characters studied, *i.e.* the fruit girth, fruit weight, fruit yield/ vine and fruit yield/ ha. This showed that under the present environmental conditions for determination of such traits, the genotypes need to be evaluated in multi environmental trials. Furthermore, the larger variances associated with genetic effects indicate a greater influence of genetic factors than the interaction of genotype × environment for the expression of these traits in ridge gourd. Similarly, the mean square for environment was also significant for all the traits except fruit length indicting that the environments under study are diverse enough. Further, G×E interactions were also significant for all the traits suggesting that the traits responded to the environments differently. The environments (linear) also differed significantly for all the traits except fruit length, which indicates that the environments selected for testing of genotypes were quite varied in their effect on the performance of genotypes; this helps in identifying a stable genotype over different environments. Similar results were earlier reported for yield and yield contributing traits in ridge gourd (Varalakshmi and Subba Reddy, 9).

The G × E linear component was significant for fruit girth, fruit number/vine, fruit weight and fruit yield/ha suggesting that the variation in performance of different genotypes is due to the regression of genotypes on environments and hence the performance is predictable in nature (Krishna Prasad and Singh, 4; Varalakshmi and Subba Reddy, 9; Agasimani *et al.*, 1; Shaikh *et al*., 6; Vasanthkumar *et al.*, 10). The mean square due for pooled deviation is significant for all the traits except for fruit number/ vine and fruit yield/ ha suggesting that variation in performance of genotypes is entirely unpredictable.

When stability parameters were studied in 51 genotypes, none of the genotypes were stable for all the seven yield related traits in ridge gourd (Tables 2 & 3). Similar observations were made in bottle gourd by Sheikh *et al*. (6). However, considering the stability parameters of individual traits, many ridge gourd genotypes were stable across the environments tested for that specific trait. Node number for first female flower appearance indicate the earliness in cucurbits and for this character, as many as 14 genotypes recorded lower mean values (advantageous) than the population mean (9.8) (Table 2). Out of which nine genotypes, *viz*., RGGP-15, RGGP-20, RGGP-22, RGGP-32, RGGP-35, RGGP-25, RGGP-36, RGGP-21 and RGGP-19

**Table 1.** Variance (mean square) for stability of yield and yield attributing traits in ridge gourd (pooled).

| Source of variation | df | Node No. for first female flower appearance | Fruit length (cm) | Fruit girth (cm) | Fruit No./ vine | Fruit weight (g) | Fruit yield/ vine (kg) | Fruit yield (t/ ha) |
|---|---|---|---|---|---|---|---|---|
| Genotype (G) | 50 | 27.18** | 145.94** | 11.30** | 95.04** | 9139.17** | 0.50 | 125.71** |
| Environment (E) | 2 | 27.18** | 19.39 | 611.92** | 14.95 | 27861.51** | 2.61** | 448.35** |
| G × E | 50 | 3.18** | 37.83** | 7.18** | 17.12** | 1358.36** | 0.37** | 33.81** |
| E + (G × E) | 102 | 3.45 | 37.50 | 19.03** | 17.08** | 1878.03** | 0.41 | 41.94** |
| E (Linear) | 1 | 34.08** | 38.83 | 1223.86** | 29.91** | 55722.85** | 5.23** | 896.72** |
| G × E (Linear) | 50 | 3.45 | 36.15 | 13.28** | 33.36** | 1932.26** | 0.39 | 66.12** |
| Pooled deviation | 51 | 2.85** | 38.79** | 1.05** | 0.87 | 769.07** | 0.34** | 1.48 |
| Pooled error | 150 | 0.35 | 5.71 | 0.22 | 0.69 | 52.11 | 0.008 | 1.33 |

**Significant at P = 0.01

**Table 2.** Mean performance and stability parameters for yield and yield contributing traits in ridge gourd.

| Genotype | Node No. for first female flower appearance | | | Fruit length (cm) | | | Fruit girth (cm) | | | Fruit No./ vine | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ |
| RGGP 1 | 9.92 | 0.88* | -0.35 | 19 | 10.24 | 166.84** | 16.52 | 3.83 | -0.51 | 16.52 | 3.83 | -0.51 |
| RGGP 2 | 9.97 | -1.00 | 5.66** | 22.7 | 15.41 | 266.29** | 14.3 | 1.95 | -0.57 | 14.3 | 1.95 | -0.57 |
| RGGP 3 | 11.53 | 1.71 | 5.36** | 26.9 | 3.13 | 93.44** | 11.73 | 3.28* | -0.69 | 11.73 | 3.28* | -0.69 |
| RGGP 4 | 17.82 | 6.40* | -0.25 | 29.7** | -1.47 | -4.63 | 5.13 | 1.23 | -0.66 | 5.13 | 1.23 | -0.66 |
| RGGP 5 | 10.08 | -2.30* | -0.33 | 26.9 | -1.01 | 93.81** | 11.25 | 2.07 | -0.37 | 11.25 | 2.07 | -0.37 |
| RGGP 6 | 10.13 | 1.19 | 4.71** | 25.3 | 1.57 | 13.43 | 9.3 | -2.87 | -0.34 | 9.3 | -2.87 | -0.34 |
| RGGP 7 | 10.72 | 1.62 | -0.35 | 24.9 | -2.3 | -2.91 | 12.58 | 2.72 | -0.53 | 12.58 | 2.72 | -0.53 |
| RGGP 8 | 15.27 | 5.3 | 3.39** | 24 | -2.61 | -2.46 | 7.28 | 1.49 | -0.68 | 7.28 | 1.49 | -0.68 |
| RGGP 9 | 11.4 | -0.77 | 2.19** | 25.6 | 4.02 | 19.99* | 9.28 | -4.06 | -0.45 | 9.28 | -4.06 | -0.45 |
| RGGP 10 | 9.57 | -1.6 | 0.92 | 24.4 | -2.9 | -1.08 | 13.25 | 0.36 | -0.37 | 13.25 | 0.36 | -0.37 |
| RGGP 11 | 19.97 | 2.82 | 46.94** | 26.1 | -6.41* | -5.7 | 5.27 | 1.17 | -0.69 | 5.27 | 1.17 | -0.69 |
| RGGP 12 | 8.62 | 0.89 | -0.14 | 27.1** | -0.93 | -3.52 | 14.12 | -0.28* | -0.69 | 14.12 | -0.28* | -0.69 |
| RGGP 13 | 8.65 | 2.99 | 3.13** | 26.5 | -3.16 | -3.81 | 10.77 | 0.39 | -0.66 | 10.77 | 0.39 | -0.66 |
| RGGP 14 | 8.2 | 2.11 | 0.2 | 33.5** | 7.94* | -5.7 | 22.9 | 8.59** | -0.69 | 22.9 | 8.59** | -0.69 |
| RGGP 15 | 5.3 | 0.94 | -0.3 | 16.8 | -6.09 | 55.88** | 17.98 | 2.9 | -0.57 | 17.98 | 2.9 | -0.57 |
| RGGP 16 | 7.87 | -0.10** | -0.35 | 17 | 1.35 | 12.04 | 18.5 | 2.15 | -0.29 | 18.5 | 2.15 | -0.29 |
| RGGP 17 | 8.77 | 0 | 0.58 | 15.5 | -1.43 | 32.25** | 19.03 | 7.09** | -0.69 | 19.03 | 7.09** | -0.69 |
| RGGP 18 | 8.37 | -0.57 | 1.03* | 18.6 | 1.28 | 14.9 | 8.9 | -1.41 | -0.63 | 8.9 | -1.41 | -0.63 |
| RGGP 19 | 7.72 | -1.12 | -0.32 | 17.7 | -1.12 | 3.44 | 12.92 | 2.81 | -0.62 | 12.92 | 2.81 | -0.62 |
| RGGP 20 | 6.03 | -0.5 | 0.37 | 19.1 | -1.67 | 5.06 | 15.55 | 3.45 | -0.64 | 15.55 | 3.45 | -0.64 |
| RGGP 21 | 7.67 | 2.63 | 0.32 | 17.4 | -0.6 | 1.31 | 21.63 | 10.47 | 0.2 | 21.63 | 10.47 | 0.2 |
| RGGP 22 | 6.17 | -0.44 | 0.69 | 19.7 | 1.8 | -2.84 | 7.82 | -4.13 | -0.28 | 7.82 | -4.13 | -0.28 |
| RGGP 23 | 7.12 | 1.56 | 1.10* | 16 | -3.65* | -5.67 | 16.65 | 8.53 | -0.29 | 16.65 | 8.53 | -0.29 |
| RGGP 24 | 13.92 | 1.84 | 0.85 | 18.4 | 1.77 | -2.73 | 9.85 | -1.48 | -0.61 | 9.85 | -1.48 | -0.61 |
| RGGP 25 | 7.43 | -3.86 | 0.57 | 18 | -3.65 | -1.81 | 16.7 | 5.11 | -0.58 | 16.7 | 5.11 | -0.58 |
| RGGP 26 | 6.9 | 4.2 | 1.83** | 18.8 | -2.97 | 45.20** | 24.08 | 17.79 | 1.68 | 24.08 | 17.79 | 1.68 |
| RGGP 27 | 9.17 | 0.78 | 0.12 | 18.7 | 11.03 | 0.75 | 15.38 | 5.55 | -0.52 | 15.38 | 5.55 | -0.52 |
| RGGP 28 | 9.07 | 1.52 | -0.33 | 15.8 | -6.3 | 73.10** | 18.75 | 13.33 | 2.26* | 18.75 | 13.33 | 2.26* |
| RGGP 29 | 6.95 | 0.66 | 1.52* | 17 | -1.22 | 44.41** | 12.4 | -9.16 | 0.53 | 12.4 | -9.16 | 0.53 |
| RGGP 30 | 8.3 | 2.16 | 0.68 | 14 | -0.17 | 5.36 | 9.97 | 5.1 | 0.99 | 9.97 | 5.1 | 0.99 |
| RGGP 31 | 7.28 | 3.13 | 1.27* | 16.2 | 3.53 | -5.56 | 18.17 | -17.5 | 1.16 | 18.17 | -17.5 | 1.16 |
| RGGP 32 | 6.35 | 0.57 | 0.01 | 15.2 | -3.38 | -4.94 | 15.33 | -7.67 | 5.93** | 15.33 | -7.67 | 5.93** |
| RGGP 33 | 10.75 | 2.86 | 8.82** | 12.7 | 0.9 | -5.54 | 33.83 | -31.39 | 13.65** | 33.83 | -31.39 | 13.65** |
| RGGP 34 | 8.72 | -0.58 | 0.87 | 16.2 | 6.08 | 171.34** | 14.55 | 8.86 | 1.25 | 14.55 | 8.86 | 1.25 |
| RGGP 35 | 6.83 | 3.59 | -0.19 | 16.7 | 6.85 | -1.92 | 14.35 | 5.96 | 0.18 | 14.35 | 5.96 | 0.18 |
| RGGP 36 | 7.52 | -0.24 | -0.11 | 13.6 | -2.1 | -2.87 | 18.12 | -8.58 | 0.38 | 18.12 | -8.58 | 0.38 |
| RGGP 37 | 8.13 | -1.31 | 1.96** | 15.3 | 6.09 | 10.79 | 9.77 | 0.42 | -0.58 | 9.77 | 0.42 | -0.58 |
| RGGP 38 | 8.28 | 2.18 | 4.95** | 14.9 | 3.45 | 1.01 | 17.52 | 7.85 | 1.11 | 17.52 | 7.85 | 1.11 |
| RGGP 39 | 8.73 | -0.42 | 0.41 | 20.3 | -9.87 | 123.35** | 21.27 | -7.95 | 0.23 | 21.27 | -7.95 | 0.23 |

*Contd...*

*Table 2 Contd...*

| Genotype | Node No. for first female flower appearance | | | Fruit length (cm) | | | Fruit girth (cm) | | | Fruit No./ vine | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ |
| RGGP 40 | 9.83 | -3.1 | 2.77** | 12.7 | -5.64 | -5.13 | 12.3 | 3.32** | -0.69 | 12.3 | 3.32** | -0.69 |
| RGGP 41 | 10.3 | 0.6 | 1.22* | 19.2 | 22.13* | -5.32 | 11.72 | -0.57 | -0.67 | 11.72 | -0.57 | -0.67 |
| RGGP 42 | 12.22 | 2.11 | 4.37** | 28.7** | -4.2 | 172.15** | 8.7 | 0.49* | -0.69 | 8.7 | 0.49* | -0.69 |
| RGGP 43 | 9.5 | 0.65 | 3.34** | 32.4** | 3.98 | 18.26 | 8.43 | -0.39 | -0.66 | 8.43 | -0.39 | -0.66 |
| RGGP 44 | 12.58 | 3.86 | 9.13** | 30.5** | 10.26 | 113.07** | 6.25 | 0.23 | -0.69 | 6.25 | 0.23 | -0.69 |
| RGGP 45 | 15.62 | 6.74 | -0.03 | 33.6** | 14.42 | 10.92 | 5.65 | -0.40** | -0.69 | 5.65 | -0.40** | -0.69 |
| RGGP 46 | 14.93 | 4.93 | 2.03** | 29.8** | -6.01 | 75.48** | 7.67 | 3.84 | -0.57 | 7.67 | 3.84 | -0.57 |
| RGGP 47 | 12.55 | -1.95 | 0.55 | 39.9** | 6.22 | 22.32* | 9.68 | -5.64 | -0.23 | 9.68 | -5.64 | -0.23 |
| RGGP 48 | 8.5 | 0.08* | -0.35 | 32.9** | 1.23 | 88.61** | 12.42 | 2.62 | -0.54 | 12.42 | 2.62 | -0.54 |
| RGGP 49 | 9.5 | -0.14 | -0.35 | 36.5** | -2.33 | 11.3 | 13.43 | 2.52 | -0.34 | 13.43 | 2.52 | -0.34 |
| RGGP 50 | 12.37 | -1.35 | 1.75** | 33.6** | 8.88** | -5.7 | 4.73 | -1.14* | -0.68 | 4.73 | -1.14* | -0.68 |
| RGGP 51 | 9.52 | -1.17 | 5.56** | 26.4 | -19.4 | 0.87 | 18.07 | 8.16 | 0.28 | 18.07 | 8.16 | 0.28 |
| Population mean | 9.8 | | | 22.5 | | | 13.6 | | | 13.6 | | |
| $CD_{0.01}$ | 2.0 | | | 4.6 | | | 4.3 | | | 4.3 | | |

*$P$ = 0.05, **$P$ = 0.01, bi = regression coefficient, $S^2di$ = deviation from regression coefficient

were found stable due to lower mean values and regression coefficient approaching unit with non-significant deviation from regression (Vasanthkumar *et al*., 10). One genotype, RGGP-16 had significant regression coefficient differing from unity with least deviation, hence suitable for favourable environments only. However four other genotypes, though had lower means values (desirable) with unit regression, their performance was not predictable owing to its significant deviation from regression.

Twelve genotypes showed the highest mean fruit length than the population mean (22.5 cm) (Table 2). Considering high mean performance and stability parameters together, out of the 51 genotypes, two genotypes, *viz*., RGGP-45 and RGGP-43 were found to possess desirable and stable performance across the environments with highest mean, unit regression and least deviation. Genotypes with high mean yield, a regression coefficient equal to the unity (bi = 1) and small deviations from regression ($s^2di$ = 0) are considered stable (Eberhart and Russell, 2). Two other genotypes, RGGP-50 and RGGP-14 had high mean, regression coefficient more than unity and least deviation indicating that these genotypes are suitable for good environments only. Though RGGP-49, RGGP-4 and RGGP-12 had higher mean fruit length and least deviation, they are suitable for poor environments, because of the negative regression coefficient. Five genotypes, namely, RGGP-47, RGGP-48, RGGP-44, RGGP-46 and RGGP-42 in spite of having high mean values than population mean and regression coefficient either unity or negative, their performance is not predictable in view of the significant deviation.

With respect to fruit girth, seven genotypes, namely, RGGP-29, RGGP-30, RGGP-35, RGGP-36, RGGP-37, RGGP-43 and RGGP-50 recorded the highest mean than the population mean (11.8 cm), but none of them were stable across the environments (Table 2). Contrary to this, Sheikh *et al.* (6) reported a stable genotype for fruit girth in bottle gourd. Out of the seven genotypes, which recorded higher mean, two genotypes, RGGP-29 and RGGP-36 were suitable for better environments only with regression coefficient differing least significantly from unity. RGGP-30 and RGGP-50 were suitable for poor environments with less than unity of regression and least deviation. Though the other three genotypes had higher mean and less than unit regression, their performance is not predictable, because of the significant deviation.

Fruit number/ vine is a very important parameter and is directly correlated with fruit yield in ridge gourd (Varalakshmi *et al*., 8). In the present investigation 11 genotypes have recorded higher mean values for this trait compared to the population mean (13.6) (Table 2). Out of these, four genotypes namely, RGGP-16, RGGP-21, RGGP-26 and RGGP-51 were stable across the environments with unit regression coefficient and least deviation. Similar results have been reported by Varalakshmi and Subba

**Table 3.** Mean performance and stability parameters for yield and yield contributing traits in ridge gourd.

| Genotype | Fruit weight (g) | | | Fruit yield/vine (kg) | | | Fruit yield (t/ha) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ |
| RGGP 1 | 185.72 | 2.98* | -40.24 | 2.1 | 1.57 | 0.06** | 27.03 | 2.86 | -0.94 |
| RGGP 2 | 235.63 | 1.37 | 1769.93** | 2.3 | 1.62 | 0.48** | 25.6 | 1.25 | -1.13 |
| RGGP 3 | 228.65 | 1.96 | 849.84** | 1.53 | -1.27 | 1.58** | 34.78 | 0.48 | -0.81 |
| RGGP 4 | 214.25 | 1.37 | 1384.45** | 1.42 | 2.16 | 0.24** | 12.05 | 0.5 | -1.29 |
| RGGP 5 | 181.87 | 3.04* | -44.22 | 1.82 | 3.01 | -0.01 | 21.7 | 0.92 | -1.3 |
| RGGP 6 | 188.92 | 1.57 | 12.73 | 1.8 | 1.46 | 0.19** | 23.05 | 1.85 | -1.14 |
| RGGP 7 | 189.32 | 2.12 | 2278.35** | 1.42 | -0.56 | 0.25** | 29.03 | 0.05 | -1.09 |
| RGGP 8 | 214.77 | 2.99 | 671.73** | 1.68 | 4.06 | 0.01 | 15.72 | 0.35* | -1.31 |
| RGGP 9 | 174.08 | 3.21* | -48.94 | 1.65 | 1.47 | 0.13** | 23.25 | 2.54* | -1.12 |
| RGGP 10 | 172.05 | 2.44 | -20.56 | 1.37 | -0.59 | 0.08** | 25.15 | 1.8 | -1.01 |
| RGGP 11 | 237.47 | 0.86 | 39.52 | 2.35 | 3.11 | 0.87** | 14.37 | -0.16* | -1.2 |
| RGGP 12 | 219.35 | 1.92 | 350.26** | 2.4 | 2.21 | 0.40** | 37.93 | 1.06 | -1.1 |
| RGGP 13 | 210.33 | 3.75 | 802.98** | 1.63 | -0.34 | 0 | 28.2 | 4.29** | -1.29 |
| RGGP 14 | 142.70 | -1.05 | 4907.75** | 1.25 | -0.88 | 0.22** | 23.22 | -0.69 | 0.53 |
| RGGP 15 | 101.12 | 0.96 | 6.36 | 1.27 | -0.14 | 0.20** | 14.1 | -0.11 | -1.13 |
| RGGP 16 | 120.35 | 1.21 | 1156.62** | 1.82 | -0.51 | 0.14** | 18.62 | -0.32 | -1.06 |
| RGGP 17 | 120.48 | 0.34 | 359.39** | 1.53 | -1.19 | 0.34** | 28.38 | -1.43* | -1.16 |
| RGGP 18 | 141.73 | 0.16 | 1145.30** | 1.55 | 0.72 | 0.08** | 16.97 | 0.39 | -0.27 |
| RGGP 19 | 151.83 | 0.07 | 27.23 | 1.62 | 0.15 | 0.01 | 21.98 | -0.06* | -1.23 |
| RGGP 20 | 123.72 | -0.37 | 211.06* | 1.53 | -0.94 | 0.06** | 21.78 | 0.20* | -1.32 |
| RGGP 21 | 130.07 | 0.13 | 12.32 | 1.02 | -1.07 | 0.81** | 26.88 | -1.76* | -1.18 |
| RGGP 22 | 153.12 | -1.78 | 81.20 | 1.4 | 0.6 | 1.14** | 8.55 | 0.23** | -1.33 |
| RGGP 23 | 112.18 | 1.06 | 644.90** | 1.82 | 0.37 | 0.09** | 23.72 | -0.71 | 0.84 |
| RGGP 24 | 163.18 | 0.91 | -40.58 | 1.67 | -1.06 | 0.06** | 20.93 | 0.39 | -0.78 |
| RGGP 25 | 143.88 | 1.04 | -47.76 | 1.83 | -0.11 | 0.01 | 25.07 | -1.23* | -1.11 |
| RGGP 26 | 100.12 | 0.13 | 24.77 | 2.1 | 0.7 | 0.18** | 26.22 | -0.21** | -1.32 |
| RGGP 27 | 175.32 | 0.60 | 93.57 | 1.95 | -0.91 | 0.10** | 31.45 | -0.14 | -0.98 |
| RGGP 28 | 118.82 | 1.27 | -39.75 | 2.2 | 0.11 | 0.28** | 23.35 | 0.05 | -1.12 |
| RGGP 29 | 117.90 | 1.02 | -51.79 | 1.83 | -1.15 | 0.63** | 19.62 | 3.04 | 0.24 |
| RGGP 30 | 154.77 | 0.92 | 31.61 | 1.65 | 3.88 | 0.08** | 17.88 | -0.53* | -1.18 |
| RGGP 31 | 110.10 | -0.80 | 4020.02** | 1.72 | 1.09 | -0.01 | 22 | 1.94 | 33.54** |
| RGGP 32 | 121.45 | -0.45 | 5604.20** | 1.63 | 3.64 | 0.16** | 20.97 | 1.74 | 19.25** |
| RGGP 33 | 80.13 | -1.19** | -51.70 | 1.3 | -0.96* | -0.01 | 23.88 | 3.05* | -1.03 |
| RGGP 34 | 116.35 | 0.83 | -35.25 | 1.58 | 0.42 | 0.03* | 19.92 | -1.31** | -1.32 |
| RGGP 35 | 149.45 | -0.24 | 168.80* | 1.55 | 3.34 | 0.29** | 25.38 | -1.41* | -1.17 |
| RGGP 36 | 100.63 | 0.05** | -52.11 | 1.3 | 1.24 | 0.38** | 16.4 | 3.29 | -0.43 |
| RGGP 37 | 168.67 | -0.24 | 260.14** | 1.73 | 0.8 | 0.03* | 20.33 | 0.05 | -1.09 |
| RGGP 38 | 114.22 | 0.38 | -35.01 | 1.88 | 3.97 | 0.21** | 23.7 | 0.19 | 0.04 |
| RGGP 39 | 100.17 | 0.38** | -52.06 | 1.3 | -0.46 | 0.23** | 21.73 | 4.95* | -0.54 |

*Contd...*

*Table 3 Contd...*

| Genotype | Fruit weight (g) | | | Fruit yield/vine (kg) | | | Fruit yield (t/ha) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ | Mean | bi | $S^2di$ |
| RGGP 40 | 131.13 | -0.62 | 239.02* | 2.45 | 2.18 | 0.54** | 19.98 | -1.28* | -1.16 |
| RGGP 41 | 234.12 | 0.59* | -51.97 | 1.87 | 0.3 | 1.26** | 37.05 | 0.93 | -1.3 |
| RGGP 42 | 174.47 | 1.87 | 171.50* | 1.73 | 2.09 | 0.27** | 17.57 | 2.15 | -0.61 |
| RGGP 43 | 244.40 | 1.49 | 768.31** | 1.55 | 0.63 | 0.14** | 26.4 | 1.92 | -0.36 |
| RGGP 44 | 224.37 | 2.04 | 1032.93** | 1.57 | 2.62** | -0.01 | 19.83 | 0.86 | -1.14 |
| RGGP 45 | 300.78 | 2.78 | 1480.30** | 1.43 | 1.38 | 0.02 | 20.57 | 2.58* | -1.27 |
| RGGP 46 | 234.63 | 4.03 | 208.42* | 2.38 | 6.31 | 0.20** | 19.55 | 0.76 | -1.25 |
| RGGP 47 | 241.52 | 2.17* | -51.35 | 2.65 | 4.15 | 0.17** | 31.68 | 5.53* | -1.23 |
| RGGP 48 | 267.17 | 1.50 | -33.80 | 2.9 | 5.76* | 0.01 | 36.55 | 5.12* | 0.39 |
| RGGP 49 | 258.88 | 1.77 | -21.85 | 1.5 | -0.67 | 0.41** | 36.95 | 6.45* | -0.68 |
| RGGP 50 | 260.80 | -0.92 | 6520.54** | 1.67 | -0.58 | 0.76** | 15.28 | 0.7 | -1.27 |
| RGGP 51 | 102.83 | -0.60** | -52.02 | 0.78 | -2.73 | 2.91** | 27.13 | -2.14* | -1.18 |
| Population Mean | 169.8 | | | 1.8 | | | 23.3 | | |
| $CD_{0.01}$ | 37.5 | | | 0.5 | | | 6.1 | | |

*$P$ = 0.05, **$P$ = 0.01, bi = regression coefficient, $S^2di$ = deviation from regression coefficient

Reddy (9) in ridge gourd, by Narayan *et al*. (5) and Agasimani *et al.* (1) in bitter gourd and Vasanthkumar *et al.* (10) in watermelon. Though RGGP-33 and RGGP-28 recorded highest mean fruit number/ vine with unit or negative regression, they are not useful as their performance is not predictable as the deviation from regression is significant. Two other genotypes, RGGP-14 and RGGP-17 were suitable for good environments only with significant regression coefficient (8.59** and 7.09**, respectively) and least deviation (0.69). RGGP-29, RGGP-31 and RGGP-36 are suitable for poor environments with negative regression and least deviation.

Individual fruit weight is another important yield contributing character in ridge gourd (Varalakshmi *et al*., 8) and 16 genotypes have recorded higher mean than the population mean (169.8 g) over the different environments (Table 3). Out of these three genotypes, namely, RGGP-48, RGGP-49 and RGGP-11 were found to be stable across environments for fruit weight (Varalakshmi and Subba Reddy, 9; Vasanthkumar *et al*., 10). Genotypes, RGGP-47 and RGGP-41 with the regression coefficients more than unity and least deviation from regression showed below average stability indicating that these genotypes can do better under favourable environments only. However, the stability of the remaining 11 genotypes, it was not predictable though they had higher mean fruit weight with unit regression, as the deviation from regression is significant.

Fruit yield/ vine is the main trait responsible for overall performance of a variety across environments and in the present study; six genotypes have recorded significantly higher mean values over population mean (1.8 kg). Out of these only one genotype's performance is predictable, *i.e*., G-48, which is suitable for favourable environments only (Table 3). The other five genotypes, *viz*., RGGP-47, RGGP-40, RGGP-12, RGGP-46 and RGGP-11, though had significantly higher mean with unit regression, their stability cannot be predicted because of the significant deviation from regression. However, two other genotypes, RGGP-25 and RGGP-5 were stable with mean performance equal to population mean with regression around unity and least deviation. Such varied response of genotypes for stability parameters have been also reported by Varalakshmi and Subba Reddy (9) in ridge gourd; by Shaikh *et al.* (6) in bottle gourd, Agasimani *et al.* (1) and Krishna Prasad and Singh (4) in bitter gourd and Vasanthkumar *et al.* (10) in watermelon.

For stability analysis of fruit yield per hectare, eight genotypes showed significantly superior performance compared to population mean (23.3 t/ ha) (Table 3). Out of which, the genotypes RGGP-12, RGGP-41, RGGP-3 and RGGP-7 proved to be most stable genotypes exhibiting significantly higher mean fruit yield/ ha compared to population mean and their regression coefficients were near to unity with non-significant deviation from regression (Vasanthkumar *et al.*, 10). Three other genotypes, *viz*.,

RGGP-49, RGGP-48 and RGGP-47 with significant regression and least deviation are suitable for good environments. Only one genotype with superior performance, *i.e*., RGGP-27 is suitable for poor environments *i.e*., low input supply or unfavourable environmental conditions. Considering the higher mean yield performance than population mean, though not significant, six more genotypes, *viz*., RGGP-1, RGGP-43, RGGP-2, RGGP-10, RGGP-38 and RGGP-28 possess stability over different environments.

Overall, nine genotypes for node number for first female flower appearance, two each for fruit length and fruit girth, four for fruit number/ vine, three for fruit weight, one for fruit yield/ vine and four for fruit yield/ ha showed higher mean values than population mean in desirable direction, regression coefficient less than unity and non-significant deviation from regression. These genotypes can be cultivated for specific trait(s) of choice under a wide range of agro-climatic conditions. RGGP-48 appeared stable for fruit number/ vine and fruit weight, RGGP-21 was stable for node number for first female flower appearance and fruit number, while RGGP-43 was stable for fruit length and yield/ha. These genotypes can be used for exploitation of these traits or can be used as parents in the hybridization programmes in ridge gourd.

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# Estimation of genetic components of variation and heterosis studies in bitter gourd for horticultural traits

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## ABSTRACT

**Gene action and magnitude of heterosis were studied by using diallel analysis (without reciprocal) of the 21 crosses derived by crossing seven diverse bitter gourd inbreds for earliness and yield components. Data was recorded for eleven quantitative traits. Proportion of genes with positive and negative effects at all loci ($H_2/4H_1$) in the parents were found to be less than 0.25 for most of the traits revealed the asymmetrical distribution of the positive and negative alleles. Days to 50% flowering, days to first harvesting, fruit length (cm), fruit diameter (cm), number of fruits per plant and yield per plant (g) exhibited below 50% narrow sense heritability, which indicated the predominance of non-additive gene action. The average degree of dominance $(H_1/D)^{1/2}$ revealed that over dominance gene action for most of the yield related traits. Heterosis over standard check (Pusa Do Mousami) was also observed for all the traits under study. The best performing $F_1$ hybrids with high standard heterosis and mean performance for yield were found in crosses DBGS-54 × DBGS-2 (43.00%), DBGS-54 × Pusa Vishesh (37.89%) and Pusa Aushadhi × DBGS-54 (34.57%). Pusa Aushadhi × DBGS-54, Pusa Aushadhi × DBGS-57 and Pusa Aushadhi × DBGS-37 were best early yielding hybrids.**

**Key words**: Bitter gourd, diallel analysis, gene action, heterosis.

## INTRODUCTION

Bitter gourd (syn. balsam pear, bitter cucumber, African cucumber and bitter melon; *Momordica charantia* L.) is an economically important member of the family Cucurbitaceae that is widely cultivated in India, China, Malaysia, Africa, and South America. Although the general chemical composition of immature fruit is similar to other cucurbits, bitter gourd possesses comparatively high concentrations of ascorbic acid and iron (Behera, 3). Bitter gourd has been used as a traditional medicine for diabetes and other health-related ailments and contains health promoting substances such as charantin and vicine. Indian bitter gourd provides immense phenotypic variation based on various characters such as growth habit, maturity, fruit shape, size, colour, and surface texture (Robinson *et al*., 10) and sex expression (Behera *et al*., 2). Inspite of the potential economic and medicinal importance of this crop, due attention was not given towards a need based crop improvement programme.

The exploitation of heterosis is much easier in cross-pollinated crops like bitter gourd and being monoecious, it provides ample scope for the utilization of hybrid vigour on commercial scale. Further, the diversified parents from different regions with high yield and quality would also pave way for the development and release of hybrids through heterosis breeding. The hybrid vigour is tremendously increased on crossing genetically diverse inbreds (heterotic groups) and thus heterosis is mostly realised from parents with sufficient genetic diversity. Therefore, the objective of present investigation is to determine genetic component of variance and heterosis by using both indigenous and exotic lines of bitter gourd.

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## MATERIALS AND METHODS

The experiment was conducted at Vegetable Research Farm, Division of Vegetable Science, IARI, New Delhi during spring-summer season (February to May) 2015 in a randomized complete block design with three replications with 20 plants per treatment. Seven genetically diverse inbreds of bitter gourd maintained by selfing at IARI, namely, Pusa Aushadhi (PA), DBGS-54 (S-54), Pusa Vishesh (PV), Pusa Do Mousami (PDM), DBGS-2 (S-2), DBGS-57 (S-57) and DBGS-37 (S-37) along with their 21 hybrids developed by using diallel mating system (without reciprocal) were utilised for the present study (Griffing, 7). Seed of 21 $F_1$ hybrids and the 7 parental lines (total of 28 treatments) were sown in 50-cell plug tray under the polyhouse and the seedlings were transplanted on both sides of the channel with a spacing of 2 m between channel and 60 cm between plants with 90 cm irrigation channels. The recommended NPK fertilizer doses and cultural practices along with plant protection measures were followed to raise an ideal crop. The fruits were harvested at horticultural maturity stage. Ten plants were selected after discarding the

border plants at both ends and were examined for 11 quantitative traits, namely, node at first pistillate flower appearance, days to first pistillate flower appearance, days to 50% flowering, days to first harvesting, fruit length (cm), fruit diameter (cm), number of fruits per plant, average fruit weight (g), yield per plant (g), yield per plot (kg) and yield per ha (q). The expected values of the components of genetic variation were estimated as suggested by Hayman (8). Standard heterosis was expressed as percentage increase or decrease of $F_1$ over standard parent (Pusa Do Mousami) and was calculated by using the formula (Fonseca and Patterson, 6).

## RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed that mean sum of squares due to genotype, parent, hybrid and parent *vs* hybrid were highly significant for all traits studied. The magnitude and nature of genetic components of variation in a specific population is of prime importance for the effective prediction of most desirable breeding programme (Debnath, 4). The suitability of breeding methods for improvement of particular trait depends on nature of gene action. The results of diallel analysis revealed over-dominance for all the traits except node at first pistillate flower appearance and days to first pistillate flower appearance (Table 1).

The dominance component of genetic variation ($H_1$) was higher than additive component (D) for all the characters except node at first pistillate flower appearance and days to first pistillate flower appearance. Heritability in narrow sense (h) was found to be the highest (74.95%) for average fruit weight (g), while lowest value was recorded for fruit length (4.81%). Other traits like days to 50% flowering, days to first harvesting, fruit length (cm), fruit diameter (cm), number of fruits per plant, yield/ plant (g), yield per plot (kg) and yield per ha (q) had the narrow sense heritability less than 50%, which is indicative of predominance of non-additive gene action for these traits under study.

The F value was negative for three traits like, node at first pistillate flower appearance, days to first harvesting and fruit length (cm). This result indicated predominance of recessive gene in parental lines because negative F value is indicative of recessive genetic control and positive F value indicates dominant

**Table 1.** Estimation of the genetic components of variation for yield and yield related traits in bitter gourd.

| Parent | Node at first pistillate flower appearance | Days to first pistillate flower appearance | Days to 50 % flowering | Days to first harvesting | Fruit length (cm) | Fruit dia. (cm) | No. of fruits/ plant | Av. fruit weight (g) | Yield/ plant (kg) | Yield/ plot (kg) | Yield/ ha (q) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D | | 22.37** ± 3.20 | 12.13** ± 1.91 | 14.70** ± 4.22 | 1.88 ± 3.65 | 3.97** ± 1.20 | 46.72** ± 5.50 | 119.05** ± 23.18 | 0.03 ± 0.02 | 10.39 ± 11.32 | 170.35 ± 339.19 |
| F | | 11.16 ± 7.49 | 5.70 ± 4.59 | -1.02 ± 10.12 | -4.33 ± 8.76 | 8.07** ± 2.88 | 51.79** ± 13.20 | 178.42** ± 55.62 | 0.04 ± 0.06 | 14.77 ± 27.18 | 427.20 ± 813.70 |
| $H_1$ | 2.22* ± 1.17 | 17.79* ± 7.51 | 18.32** ± 4.60 | 24.29* ± 10.15 | 24.78** ± 8.79 | 11.64** ± 2.89 | 75.24** ± 13.25 | 207.18** ± 55.81 | 0.23** ± 0.06 | 98.15** ± 27.24 | 2700.76** ± 816.58 |
| $H_2$ | 2.07* ± 1.03 | 12.57* ± 6.61 | 15.22** ± 4.06 | 24.46** ± 8.94 | 16.98* ± 7.74 | 6.22** ± 2.54 | 58.71** ± 11.68 | 118.05** ± 49.48 | 0.19** ± 0.05 | 79.32** ± 24.01 | 2197.02** ± 719.52 |
| $h^2$ | 0.19 ± 0.69 | -1.48 ± 4.45 | 9.34** ± 2.72 | 16.43** ± 6.01 | 28.35** ± 5.20 | 0.08 ± 1.71 | 153.50** ± 7.84 | -0.09 ± 33.03 | 0.35** ± 0.03 | 141.08** ± 16.12 | 3574.30** ± 483.27 |
| E | 0.98** ± 0.17 | 3.04** ± 1.10 | 2.35** ± 0.68 | 7.62** ± 1.49 | 2.04 ± 1.29 | 0.87* ± 0.42 | 7.34** ± 1.95 | 2.76 ± 8.20 | 0.02* ± 0.01 | 7.20 ± 4.00 | 127.78 ± 119.92 |
| $(H_1/D)^{1/2}$ | 0.43 | 0.89 | 1.23 | 1.29 | 3.63 | 1.71 | 1.27 | 1.32 | 2.96 | 3.07 | 3.98 |
| $(H_2/4H_1)$ | 0.23 | 0.18 | 0.21 | 0.25 | 0.17 | 0.13 | 0.20 | 0.14 | 0.20 | 0.20 | 0.20 |
| $(4DH_1)^{1/2}+F/(4DH_1)^{1/2}-F$ | 0.39 | 1.78 | 1.47 | 0.95 | 0.52 | 3.92 | 2.55 | 3.63 | 1.63 | 1.60 | 1.92 |
| $h^2/H_2$ | 0.09 | -0.12 | 0.61 | 0.67 | 1.67 | 0.01 | 2.61 | 0.00 | 1.84 | 1.78 | 1.63 |
| Heritability (narrow sense) | 53.17 | 54.37 | 35.53 | 20.85 | 4.81 | 36.00 | 46.95 | 74.95 | 9.04 | 8.47 | 5.76 |
| r | 0.857** | 0.242 | 0.548** | 0.932** | -0.382* | -0.090 | -0.955** | 0.360 | -0.592** | -0.609** | -0.739** |

*, ** Significant at 5 and 1% levels

gene control (Hayman, 8). The proportion of genes with positive and negative effect in the parents was found to be less than 0.25 for all the traits under study except days to first harvesting where it was perfectly 0.25. This was indicative of asymmetry at loci showing dominance, which was similar to earlier report of Dey *et al*. (5).

The mean degree of dominance $(H_1/D)^{1/2}$ was found to be more than one for all the characters under study except node at first pistillate flower appearance and days to first female flower appearance. This also confirmed over-dominance for most of the yield related traits under study. These results were in consonance with the report of Dey *et al*. (5). They also revealed the preponderance of non-additive gene action in controlling most of the yield attributes in bitter gourd. The predominance of non-additive gene action and low to moderate narrow sense heritability for the characters suggested that exploitation of heterosis breeding would be more advantageous to enhancing yield in bitter gourd.

Earliness is an important trait for realizing the potential economic yield in a less time. Earliness in bitter gourd is attributed to node at first pistillate flower appearance, days to first pistillate flower appearance and days to first harvesting. The standard heterosis of 21 hybrids for 11 quantitative traits were presented in Table 2 and range of standard heterosis in Fig. 1. The standard heterosis was observed in the range of -48.57% (Pusa Aushadhi × DBGS-54) to 40.00% (DBGS-57 × DBGS-37) for node at first pistillate flower appearance over commercial check Pusa Do Mousami (PDM). Out of 21 hybrids, 12 hybrids showed significant negative (desired) standard heterosis over PDM (Table 3). The hybrids showed highest desirable heterosis over PDM for node at first pistillate flower appearance in the order of their merit were Pusa Aushadhi × DBGS-54 (-48.57%), Pusa Aushadhi × DBGS-2 (-42.85%) and Pusa Aushadhi × Pusa Vishesh (-34.28%). For days to first pistillate flower appearance, eight hybrids showed significant

**Table 2.** Heterosis for yield and yield related traits in bitter gourd over standard check (Pusa Do Mousami).

| Cross | Node at first pistillate flower appearance | Days to first pistillate flower appearance | Days to 50% flowering | Days to first harvesting | Fruit length (cm) | Fruit dia. (cm) | No. of fruits/ plant | Av. fruit weight (g) | Yield/ plant (kg) | Yield/ plot (kg) | Yield/ ha (q) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PA × S-54 | -48.57** | -12.96** | -20.86** | -6.78* | -13.63** | 24.34** | 78.83** | -32.31** | 20.55** | 20.41** | 34.57** |
| PA × PV | -34.28** | -21.30** | -15.11** | -3.39 | -19.55** | 20.47** | 78.38** | -6.95* | 15.67** | 15.63** | 15.63* |
| PA × PDM | -34.28** | -11.11** | -9.35** | 1.13 | -19.77** | 16.62** | 94.37** | -39.96** | 16.84** | 16.74** | 16.74* |
| PA × S-2 | -42.85** | -13.89** | -17.99** | -3.39 | -2.50 | 4.75** | 97.52** | -32.25** | 33.57** | 33.41** | 33.41** |
| PA × S-57 | -20.00** | -14.81** | -15.11** | -6.78* | 0.68 | 48.07** | 79.05** | -25.45** | 33.37** | 33.21** | 33.21** |
| PA × S-37 | -22.86** | -11.11** | -13.67** | -6.78* | -4.32** | 14.54** | 67.79** | -40.02** | 0.02 | -0.03 | -0.03 |
| S-54 × PV | -17.14** | -2.78 | -4.32** | -3.39 | 11.59** | 26.12** | 58.56** | -35.08** | 19.05** | 17.15** | 37.89** |
| S-54 × PDM | -11.43** | -7.41** | -15.83** | 12.43** | 20.00** | 15.13** | 61.04** | -33.63** | 7.33** | 7.17* | 7.17 |
| S-54 × S-2 | -20.00** | 7.41** | -2.88 | 0.00 | 38.41** | -9.20** | 89.64** | -9.43** | 36.38** | 41.88** | 43.00** |
| S-54 × S-57 | -8.57** | -1.85 | -5.75** | -3.39 | 17.27** | 10.68** | 65.99** | -19.79** | 33.07** | 32.94** | 32.95** |
| S-54 × S-37 | -22.86** | -2.78 | -7.91** | 4.52 | 25.00** | 16.62** | 69.82** | -43.52** | -4.19** | -4.27 | -4.27 |
| PV × PDM | 8.57** | -3.70 | -11.51** | 4.52 | 8.41** | 3.86** | 79.96** | -35.77** | 15.54** | 15.37** | 15.37* |
| PV × S-2 | 8.57** | -3.70 | -15.11** | 12.43** | 18.41** | 3.86** | 66.89** | -35.10** | 7.68** | 7.74* | 7.74 |
| PV × S-57 | 14.28** | 3.70 | -7.91** | 0.00 | -8.64** | 8.01** | 77.70** | -33.82** | 17.85** | 17.74** | 17.74** |
| PV × S-37 | 11.43** | -3.70 | -0.72 | 0.00 | 13.64** | 17.21** | 79.28** | -34.95** | 16.19** | 16.04** | 16.04* |
| PDM × S-2 | 14.28** | 0.00 | -4.32** | 0.00 | 63.64** | -10.38** | 66.67** | -28.66** | 18.70** | 18.51** | 18.51** |
| PDM × S-57 | 14.28** | -1.85 | -9.35** | -3.39 | 4.78** | 19.02** | 67.12** | -24.26** | 27.36** | 27.27** | 27.28** |
| PDM × S-37 | -5.71** | -7.41** | -12.23** | 0.00 | 19.32** | 23.45** | 68.24** | -28.16** | 21.35** | 21.11** | 21.11** |
| S-2 × S-57 | 20.00** | -1.85 | -5.04** | 4.52 | 56.82** | 14.54** | 72.30** | -24.72** | 30.07** | 29.78** | 29.78** |
| S-2 × S-37 | 28.57** | 1.85 | -5.04** | 16.95** | 34.32** | 0.89 | 57.66** | -26.17** | 16.34** | 16.31** | 16.30* |
| S-57 × S-37 | 40.00** | 20.37** | 2.16 | 7.91** | 32.27** | 27.90** | 72.75** | -36.94** | 9.33** | 9.20** | 9.20 |
| CD at 5% | 1.96 | 4.05 | 3.44 | 6.46 | 3.18 | 1.79 | 5.02 | 3.59 | 0.23 | 5.45 | 14.69 |

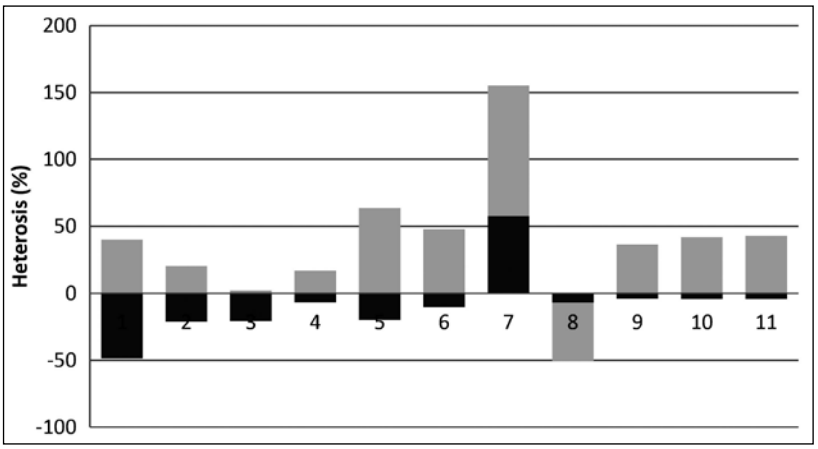
*, ** Significant at 5 and 1% levels

**Table 3.** Performance and heterosis for yield and yield related traits in bitter gourd over standard check (Pusa Do Mousami).

| Trait | Range | | | No. of hybrids having significant standard heterosis | | Better performing hybrids |
|---|---|---|---|---|---|---|
| | Parent | Hybrid | Standard heterosis | +ve | -ve | |
| Node at first pistillate flower appearance | 5.67 to 15.00 | 6.00 to 16.33 | -48.57 to 40.00 | 9 | 12 | PA × S-54, PA × S-2, PA × PV |
| Node at first pistillate flower appearance | 27.33 to 41.33 | 28.33 to 43.33 | -21.30 to 20.37 | 2 | 8 | PA × PV, PA × S-57, PA × S-2 |
| Days to 50% flowering | 37.33 to 48.67 | 36.67 to 47.33 | -20.86 to 2.16 | 0 | 18 | PA × S-54, PA × S-2, S-54 × PDM |
| Days to first harvesting | 55.00 to 68.67 | 55.00 to 69.00 | -6.78 to 16.95 | 4 | 3 | PA × S-54, PA × S-57, PA × S-37 |
| Fruit length (cm) | 10.37 to 15.83 | 11.77 to 24.00 | -19.77 to 63.64 | 14 | 5 | PDM × S-2, S-2 × S-57, S-54 × S-2 |
| Fruit dia. (cm) | 9.00 to 15.78 | 10.07 to 16.63 | -10.38 to 48.07 | 18 | 2 | PA × S-57, S-54 × PV, PA × S-54 |
| No. of fruits/ plant | 29.60 to 53.20 | 46.67 to 58.47 | 57.66 to 97.52 | 21 | 0 | PA × S-2, PA × PDM, S-54 × S-2 |
| Av. fruit weight (g) | 31.34 to 67.54 | 38.15 to 61.17 | -6.95 to -43.52 | 0 | 21 | PA × PV, S-54 × S-2, S-54 × S-57 |
| Yield/ plant (kg) | 1.67 to 2.29 | 1.91 to 2.72 | -4.19 to 36.38 | 19 | 1 | S-54 × S-2, PA × S-2, PA × S-57 |
| Yield/ plot (kg) | 33.40 to 45.83 | 38.28 to 56.73 | -4.27 to 41.88 | 19 | 0 | S-54 × S-2, PA × S-2, PA ×S-57 |
| Yield/ ha (q) | 148.45 to 197.01 | 170.14 to 254.14 | -4.27 to 43.00 | 16 | 0 | S-54 × S-2, S-54 × PV, PA × S-54 |

desirable standard heterosis which ranged from -21.30% (Pusa Aushadhi × Pusa Vishesh) to 20.37% (DBGS-57 × DBGS-37). The cross, Pusa Aushadhi × Pusa Vishesh (-21.30%) followed by Pusa Aushadhi × DBGS-57 (-14.81%), Pusa Aushadhi × DBGS-2 (-13.89%) were recorded desirable standard heterosis for days to first pistillate flower appearance. Eighteen hybrids for days to 50% flowering and three hybrids for days to first harvesting recorded significant desirable standard heterosis (%). The desirable standard heterosis for days to 50% flowering ranging from -20.86% (Pusa Aushadhi × DBGS-54) to 2.16% (DBGS-57 × DBGS-37) and for days to first harvesting ranged from -6.78% (Pusa Aushadhi × DBGS-54) to 16.95% (DBGS-2 × DBGS-37). The highest negative standard heterosis for days to first harvesting was observed in three crosses, *viz.*, Pusa Aushadhi × DBGS-54 (-6.78%), Pusa Aushadhi × DBGS-57 (-6.78%) and Pusa Aushadhi × DBGS-37 (-6.78%). Cross Pusa Aushadhi × DBGS-54 (-20.86%) followed by Pusa Aushadhi × DBGS-2 (-17.99%) and DBGS-54 × Pusa Do Mousami (-15.83%) showed the highest negative standard heterosis for days to 50% flowering. The earliness in these hybrids might be due to use of Pusa Aushadhi, a predominant gynoecious line as one of the parents in which flowering starts at early node ($5^{th}$-$6^{th}$ node). Similar results were reported earlier by Dey *et al*. (5) particularly in gynoecious based hybrids.

Number of fruits per plant, fruit length, fruit diameter and average fruit weight are main contributing traits for yield. A critical analysis of the data on these traits in the parents, hybrids and standard check indicated that the parents and hybrids have higher mean values for number of fruits as compared to standard check. Fourteen hybrids had shown significant standard heterosis in the positive direction for fruit length, 18 hybrids for fruit diameter and 21 hybrids for number of fruits per plant. The standard heterosis for fruit length ranged from -19.77% (Pusa Aushadhi × Pusa Do Mousami) to 63.64% (Pusa Do Mousami × DBGS-2), for fruit diameter -10.38% (Pusa Do Mousami × DBGS-2) to 48.07% (Pusa Aushadhi × DBGS-57), for number of fruits per plant 57.66% (DBGS-2 × DBGS-37) to 97.52% (Pusa Aushadhi × DBGS-2) and for average fruit weight ranged from -6.95 (Pusa Aushadhi × Pusa Vishesh) to -43.52% (DBGS-54 × DBGS-37). The highest standard heterosis for fruit length was observed in hybrid Pusa Do Mousami × DBGS-2 (63.64%) followed by DBGS-2 × DBGS-57 (56.82%) and DBGS-54 × DBGS-2 (38.41%). The highest standard heterosis was recorded in hybrid Pusa Aushadhi × DBGS-57 (48.07%) followed by DBGS-54 × Pusa Vishesh (26.12%) and Pusa Aushadhi × DBGS-54 (24.34%) for fruit diameter and for number of fruits per plant, the highest standard heterosis was observed




1. Node at first pistillate flower appearance
2. Days to first pistillate flower appearance
3. Days to 50% flowering
4. Days to first harvesting
5. Fruit length (cm)
6. Fruit diameter
7. No. of fruits/plant
8. Av. fruit weight (g)
9. Yield/ plant (kg)
10. Yield/ plot (kg)
11. Yield/ ha (q)

**Fig. 1.** The range of standard heterosis over check for 11 quantitative traits in bitter gourd.

in hybrid Pusa Aushadhi × DBGS-2 (97.52%) followed by Pusa Aushadhi × Pusa Do Mousami (94.37%) and DBGS-54 × DBGS-2 (89.64%). Similarly, higher standard heterosis for of fruit number per plant and fruit weight (g) was reported earlier by Al-Mamuna *et al*. (1) and Laxuman *et al*. (9). The higher standard heterosis for fruit diameter was observed when DBGS-54 and DBGS-57 were used as one of the parents and they had high fruit diameter of 16 and 12 cm, respectively.

The ultimate goal of a breeder and most important trait is yield, it may be yield per plant, yield per plot and estimated yield per ha. For yield per plant 19 hybrids, for yield per plot 19 hybrids and for yield per ha 16 hybrids showed significant positive standard heterosis out of 21 hybrids. The standard heterosis for yield per plant ranged from -4.19% (DBGS-54 × DBGS-37) to 36.38% (DBGS-54 × DBGS-2), for yield per plot ranged from -4.27% (DBGS-54 × DBGS-37) to 41.88% (DBGS-54 × DBGS-2) and for yield per ha ranged from -4.27% (DBGS-54 × DBGS-37) to 43.00% (DBGS-54 × DBGS-2). The highest standard heterosis for yield per plant was shown by hybrid DBGS-54 × DBGS-2 (36.38%) followed by Pusa Aushadhi × DBGS-2 (33.57%) and Pusa Aushadhi × DBGS-57 (33.37%). For yield per plot, highest standard heterosis was showed by DBGS-54 × DBGS-2 (41.88%) followed by Pusa Aushadhi × DBGS-2 (33.41%) and Pusa Aushadhi × DBGS-57 (33.21%). For yield per ha, highest standard heterosis was observed in hybrid DBGS-54 × DBGS-2 (43.00%) (Fig. 2). These results

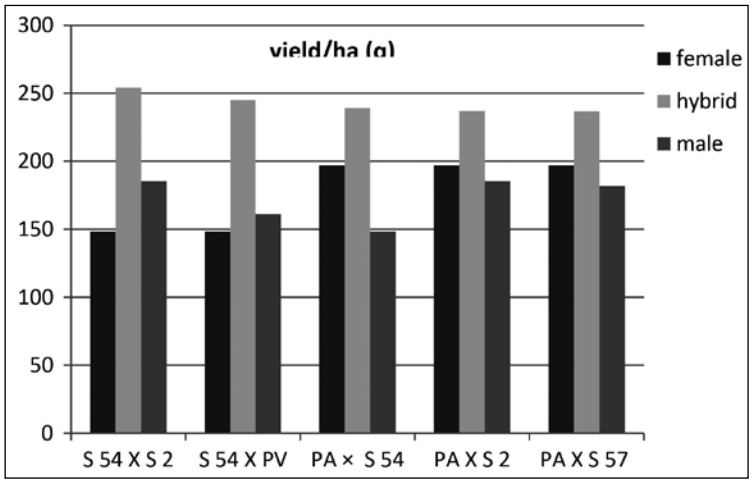



**Fig. 2.** Relative contributions of male and female parents in promising $F_1$ for higher standard heterosis in bitter gourd.

are in agreement with those of Singh *et al*. (11) and Thangamani *et al*. (12) for increase in yield per plant.

It is, therefore, suggested that these promising crosses DBGS-54 × DBGS-2, DBGS-54 × Pusa Vishesh and Pusa Aushadhi × DBGS-54 may be exploited for further amelioration of yield and yield components in bitter gourd and directly utilized as promising hybrids.

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# Induced systemic resistance (ISR) in hot pepper against *Phytophthora capsici* infection triggered by cell wall oligosaccharide elicitors from *Trichoderma* species

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## ABSTRACT

**Induced systemic resistance, one of the mechanisms of biological control is elicited either by oligosaccharides or glycoproteins released from cell wall of fungal bioagents like *Trichoderma* species. Oligosaccharide elicitors from 10 *Trichoderma* isolates having biocontrol potential were tested for their ability to elicit ISR in hot pepper against *Phytophthora capsici* infection. Treatment with elicitors from isolates Th10, Th9, Th33 and Th28 reduced *P. capsici* infection in hot pepper by 70-80% compared to 100% infection in pathogen inoculated control. Assays of peroxiase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and glucanase revealed that elicitor treatment significantly induced higher enzyme activity in elicitor treated plants compared to control. Induction of more number of isoforms of PO and PPO was also observed. Elicitors from Th10 and Th33 induced two fold increase in PPO and PAL and three-fold increase in PO, while Th28 and Th9 increased PPO and glucanase by two folds. Different enzymes (involved in phenyl propanoid metabolism or hydryolytic enzymes) contributed to ISR in different isolates.**

**Key words:** Elicitors, hot pepper, induced systemic resistance (ISR), *Phytophthora capsici*, *Trichoderma*.

## INTRODUCTION

*Trichoderma* species are the most common commercially available biological control agents used to manage soil borne pathogens. Earlier the mechanisms of action, *viz*. mycoparasitism, antibiotics production and competition for space and nutrition were explored for identifying the potential isolates of *Trichoderma*. In the recent years, another mechanism, *i.e.* induced systemic resistance is being explored. Treatment with bioagents to the seeds or seedlings induces an array of defense mechanism systemically in plants that includes hydrolytic enzymes like chitinase or glucanase or enzymes involved in phenyl propanoid metabolism like phenylalaline ammonia lyase, peroxidase or polyphenol oxidase. Fungal bioagents like *Trichoderma* releases elicitors that induce the defense mechanism in plants. Elicitors may be glucans, chitin oligomers, chitosan, glycoproteins, proteins or fatty acids (Brotman *et al*., 1). In the present study, hot pepper - *P. capsici* pathosystem was taken up to understand the induction of defense mechanism cell wall glucan elicitors from *Trichoderma* isolates that had been earlier identified as potential biocontrol agents. The main objectives were to determine whether treatment of roots with cell wall elicitors from *Trichoderma* isolates could induce systemically defense enzymes in leaves against foliar infection by *P. capsici*, quantify the defense responses and correlate them to ISR against the pathogen.

## MATERIALS AND METHODS

From the *Trichoderma* isolates maintained at ICAR-NBAII, Bengaluru ten isolates were selected based on their ability to induce systemic resistance in our earlier study (unpublished data). *Trichoderma* isolates used in the study and the disease incidence due to challenge inoculation with *P. capsici* in plants treated with their talc formulations were: *T. harzianum* isolates Th9 (45%), Th10 (35%), Ta101 (65%), Th19 (50%), Th28 (45%) and Th33 (50%); T. *asperellum* isolates Ta30 (48%) and Ta102 (52%) and *T. virens* isolates Tvs5 (55%) and Tvs8 (50%). In *P. capsici* alone inoculated plants there was 100% infection. *Trichoderma* cultures were maintained on potato dextrose agar (HiMedia) slants at 4ºC. A highly virulent isolate of *P. capsici* (PC 06-16) capable of causing serious foliar infection as blight was used. Zoospores at $2 \times 10^6$ per ml were used for inoculation.

Cell wall elicitors were extracted from *Trichoderma* isolates and carbohydrate content was measured as described by Sriram *et al*. (13). Hot pepper cv. Byadagi Kaddi (susceptible to *P. capsici*) was used. Before sowing, seeds were thoroughly surface sterilized with 1% sodium hypochlorite for 2 min. and washed in sterile water and air-dried. Biopeat SG compost was used as substrate for raising the

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seedlings, and 30-day-old seedlings were used in the experiment. Hot pepper seedlings were treated with elicitor preparation by dipping seedlings for 10-15 min., in elicitor preparation and transplanted. Untreated plants served as control. Two sets each of five replications were maintained and in each replication 10 plants were maintained. One day after elicitor treatment one set of plants were spray-inoculated with *P. capsici* (2 × $10^6$ zoospore/ ml) and plants treated with pathogen alone served as pathogen inoculated control. Disease incidence was recorded 30 days after pathogen inoculation.

Leaf samples were collected at weekly intervals (1, 7, 14, 21, and 28 days). Biochemical studies included colorimetric assays of peroxiase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), total proteins, total phenols and glucanase activity. Activity gel electrophoresis with native gels was carried out to study the isoforms of PO and PPO induced by elicitor treatment. One gram leaf sample was homogenized in two 5 ml portions of 80% ethanol and total phenols were estimated using Folin-Ciocalteau reagent (Sriram *et al*., 11). Leaf samples were homogenized with liquid nitrogen for enzyme assays. For total protein, PO and PPO assays, one gram powdered sample was extracted with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C and centrifuged for 20 min. at 10,000 rpm. Total protein content in the samples was estimated by Lowry's method. Activities of PAL, PO, PPO and glucanase were assayed as described earlier (Dickerson *et al*., 4; Sriram *et al*., 11; Karthikeyan *et al*., 6; Pan *et al.* 9, respectively). Activity gel electrophoresis with native gels was carried out to study the induction of PO and PPO isoforms due to elicitor treatment as described by Marri *et al.* (8). All gels were analyzed for presence or absence of isoforms after staining. The data related to different assays were analyzed by ANOVA using Statistical Product and Service Solution (SPSS) version16.0 software.

## RESULTS AND DISCUSSION

Carbohydrate estimation of cell wall elicitor confirmed that extracted cell wall elicitor contained carbohydrate moiety. No significant difference (P<0.280, F = 1.46) was found in the concentration of carbohydrate in terms of glucose units per g of fresh weight cell wall elicitor preparation from different *Trichoderma* isolates (20-60 µg/ml). Treatment with different *Trichoderma* cell wall elicitors significantly (P<0.01, F = 20.08) reduced infection by *P. capsici* in plants compared to pathogen alone inoculated plants where 100% disease incidence was observed. Disease incidence was reduced by 70-80% in plants treated with elicitors from Th10 (80%), Th9 (75%), Th33 (70%) and Th28 (70%). Plants treated with elicitors from Tvs5, Ta102, Tvs8 and Th19 did not show significant reduction in leaf blight incidence by *P. capsici* (Fig. 1).

In plants treated with elicitors from Th9, Th10, Th33 and Th28, there was enhanced high protein content (2.7, 2.8, 2.7 and 2.7 mg/g, respectively) till 28$^{th}$ day (Fig. 2A) compared to 1.5 mg/g in control. Challenge inoculation with *P. capsici* enhanced the protein content in plants treated with these elicitors ((2.8, 3.4, 3.0 and 2.9 mg/g respectively).

Peroxidase activity significantly increased in all plants treated with cell wall elicitor preparation from Th33 and Ta102 (11.17 and 11.23 change in abs/min./g of plant tissue). Challenge inoculation also enhanced PO activity (12.35 change in abs/min./g) (Fig. 3B). Native-PAGE analysis revealed the presence of

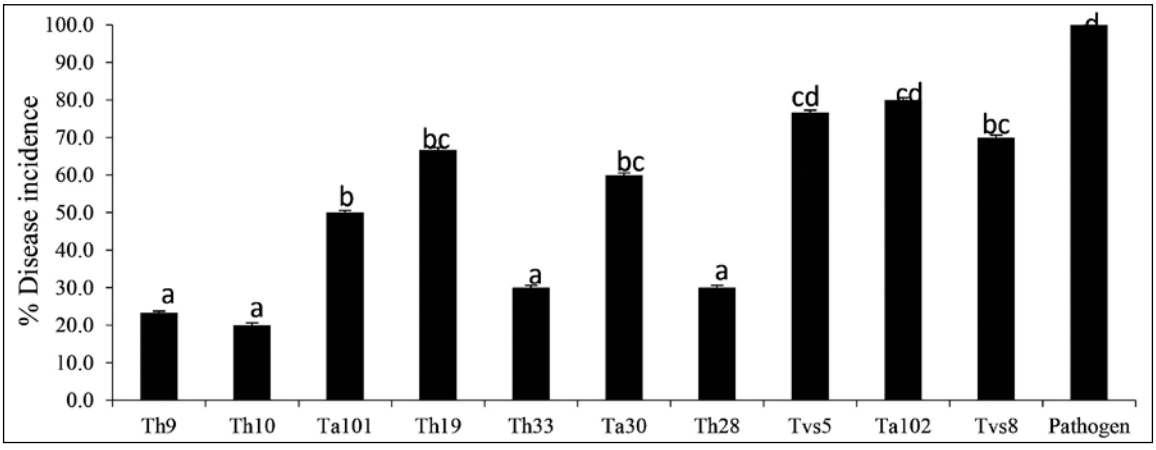


**Fig. 1.** Per cent disease incidence in hot pepper plants treated with different *Trichoderma* elicitor (glucan) and challenge inoculated with pathogen (*P. capsici*). Values represented are back transformed. Different letters indicate significant difference according to Duncan's test (P<0.05).

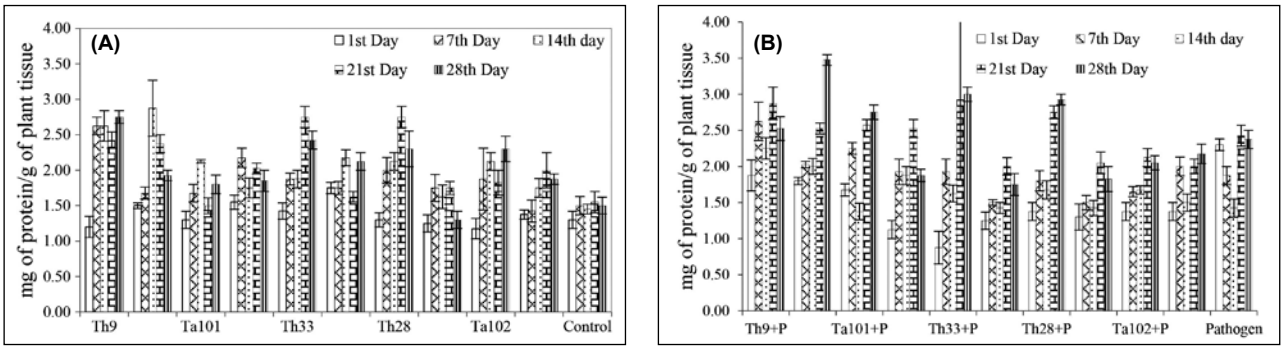


**Fig. 2.** Total proteins content in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

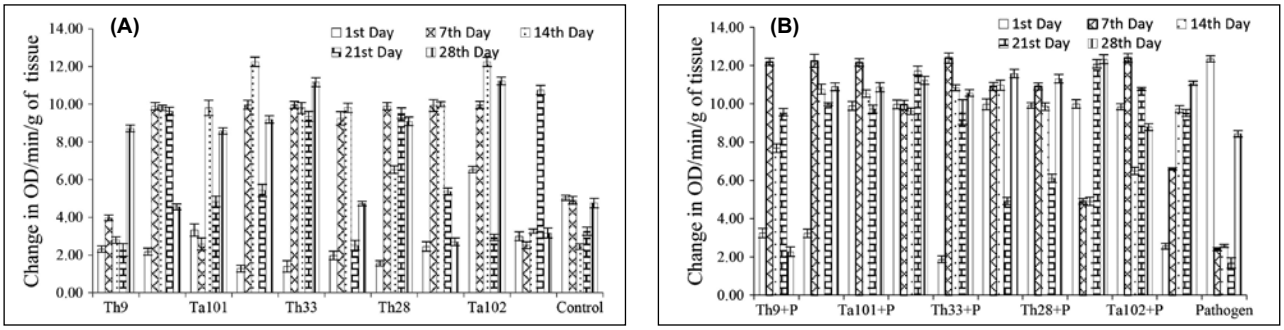


**Fig. 3.** Peroxidase activity in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE

maximum of six PO isoforms designated as PO1-PO6 with greater band intensity in plants treated with elicitors alone and challenged with *P. capsici*, whereas in control plants only three PO isoforms were seen that too were faint or less intense bands indicating the role of cell wall elicitor in inducing high PO activity in red pepper and in turn ISR (Table 1). Treatment with elicitors from isolates Th9 and Th28 enhanced PPO activity (0.18 and 0.17 change in abs/min./g of plant tissue, Fig. 4A). There was no significant difference in PPO activity in plants treated with elicitors of other isolates compared to control. Challenge inoculation with pathogen enhanced PPO activity in plants treated with elicitors from Th9, Th28 and TVS8 (Fig. 4B). Plants treated with elicitor alone and challenged with *P. capsici* expressed 4 or 5 PPO isoforms (PPO1, PPO2, PPO3, PPO4 and PPO5) compared to 3 isoforms in control and 4 isoforms in *P. capsici* alone inoculated plants (Table 2). PPO5 was present only in elicitor treated plants while PPO4 was induced either by pathogen or elicitor treatment. Significant increase in phenol content was observed in plants

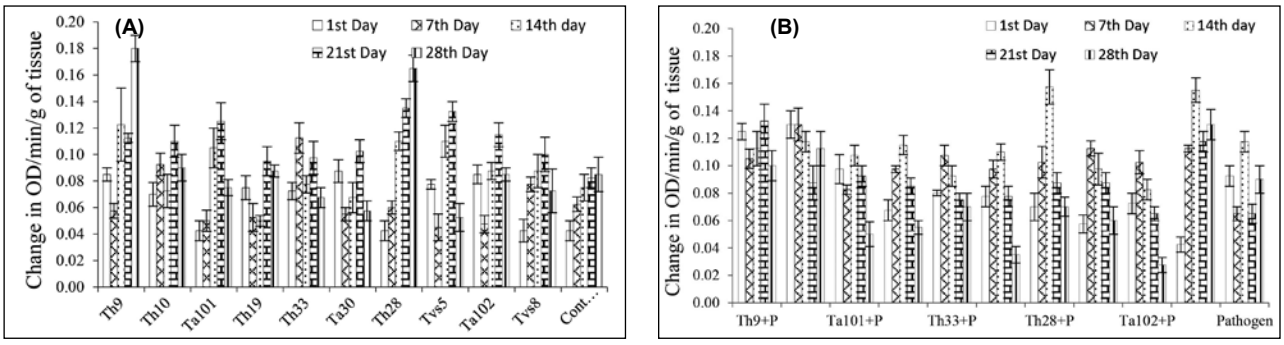


**Fig. 4.** Polyphenol oxidase activity in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

**Table 1.** Number of peroxidase isoforms observed under activity gel electrophoresis in hot pepper in response to treatment with elicitors from *Trichoderma* species.

| Treatment | 1 day | | 7 day | | 14 day | | 21 day | | 28 day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E-P | E+P | E-P | E+P | E-P | E+P | E-P | E+P | E-P | E+P |
| Th9 | 4 | 4 | 4 | 5 | 5 | 3 | 6 | 6 | 5 | 6 |
| Th10 | 5 | 5 | 6 | 6 | 5 | 5* | 6* | 3# | 5 | 3 |
| Ta101 | 5 | 5 | 5 | 5 | 5 | 5* | 6 | 5 | 5* | 5* |
| Th19 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5# | 5* | 6* |
| Th33 | 5* | 5 | 4 | 5 | 5 | 5 | 5 | 3# | 5* | 5* |
| Ta30 | 5* | 5* | 5 | 5 | 4 | 5 | 6 | 5 | 5 | 5 |
| Th28 | 5 | 5* | 5 | 5 | 4# | 6 | 5 | 5 | 3# | 4 |
| Tvs5 | 6 | 5 | 5 | 5 | 4 | 6 | 5 | 6 | 5 | 5* |
| Ta102 | 5 | 5 | 5 | 5 | 6 | 4 | 5 | 4 | 4 | 4 |
| Tvs8 | 6 | 5 | 5 | 5 | 4# | 6 | 4 | 6 | 4 | 4 |
| Control | 3 | - | 4 | - | 3 | - | 4# | - | 3# | - |
| Pathogen | - | 5 | - | 5 | - | 6* | - | 6* | - | 6 |

E-P = Elicitor without pathogen; E+P = Elicitor with pathogen, # light band (thin); * thick band

**Table 2.** Number of polyphenol oxidase isoforms observed under activity gel electrophoresis in hot pepper in response to treatment with elicitors from *Trichoderma* species.

| Treatment | 7 day | | 28 day | |
|---|---|---|---|---|
| | E-P | E+P | E-P | E+P |
| Th9 | 4 | 4 | 4 | 4 |
| Th10 | 5 | 5 | 4 | 4 |
| Ta101 | 5 | 5 | 5 | 5 |
| Th19 | 4 | 4 | 5 | 5 |
| Th33 | 5 | 5 | 5 | 5 |
| Ta30 | 5 | 5 | 4 | 4 |
| Th28 | 4 | 3 | 3 | 3 |
| Tvs5 | 4 | 4 | 4 | 4 |
| Ta102 | 5 | 5 | 4 | 4 |
| Tvs8 | 5 | 5 | 4 | 4 |
| Control | 3 | - | 3 | - |
| Pathogen | - | 4 | - | 4 |

E-P = Elicitor without pathogen; E+P = Elicitor with pathogen

treated with elicitors from Th9 (840 µg/g), Ta101 (720 µg/g), Ta102 (800 µg/g), Th10 (940 µg/g) and Th28 (780 µg/g) compared to 440 µg/g in control (Fig. 5A). Pathogen alone inoculated plants the phenol content was 460 µg/g and it did not significantly change the phenol content in elicitor treated plants. (Fig. 5B).

A two-fold significant increase in β-1,3-glucanase activity was observed in plants treated with elicitors of Th9 and Th28 (11.6 and 14.1 mg of glucose released/min/g of plant tissue) compared to control and reached maximum on 14$^{th}$ day (Fig. 6A). Challenge inoculation with pathogen followed by treatment with elicitors from Th10, Ta30, Th28 and Th33 enhanced glucanase activity (16.9, 12, 10.7 and 13.2 mg of glucose released/min./g of plant tissue, respectively) (Fig. 6B). Glucanase as well as PPO induction was observed as a result of both systemic acquired resistance and induced systemic resistance. Enhanced PAL activity was observed in plants treated with elicitor from Th10 and Ta101 (190 and 110 µM of trans-cinnamic acid/h/g of plant tissue) that declined after 7 days, while in control it was 30 µM of trans-cinnamic acid/h/g of plant tissue (Fig. 7A). Challenge inoculation enhanced PAL activity in plants treated with elicitors from Th9 and Th10 (180 and 130 µM, respectively) (Fig. 7B).

Differential expression of defense enzymes, *viz*., PR-proteins and enzymes of phenyl propanoid pathway was observed in response to elicitor treatment. Among the isolates studied, elicitors of Th9, Th10, Th28 and Th33 showed promising results in activating defense mechanism in plants. Treatment with elicitors of the isolates Th9, Th10, Th33 and Th28 resulted in enhanced activities of defense enzymes (PO, PPO, PAL and β-1,3-glucanase) (Fig. 8). Treatment with elicitors of Th9, Th28 and Th33 showed early increased PAL activity as PAL catalyses the first step in phenyl propanoid pathway causing increased production of phenolic phytoalexins, which resulted in reduced pathogen invasion. Isolates Th9, Th28 and Th33 showed more induction of β-1,

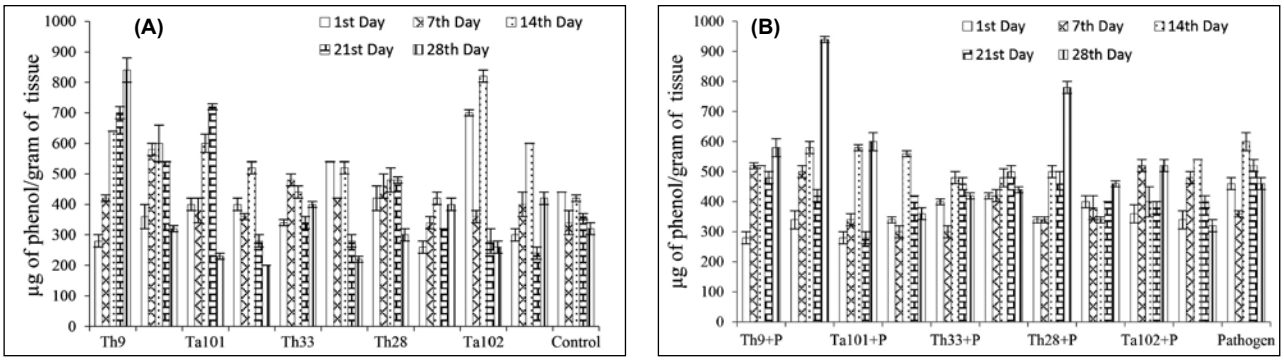


**Fig. 5.** Total phenols content in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

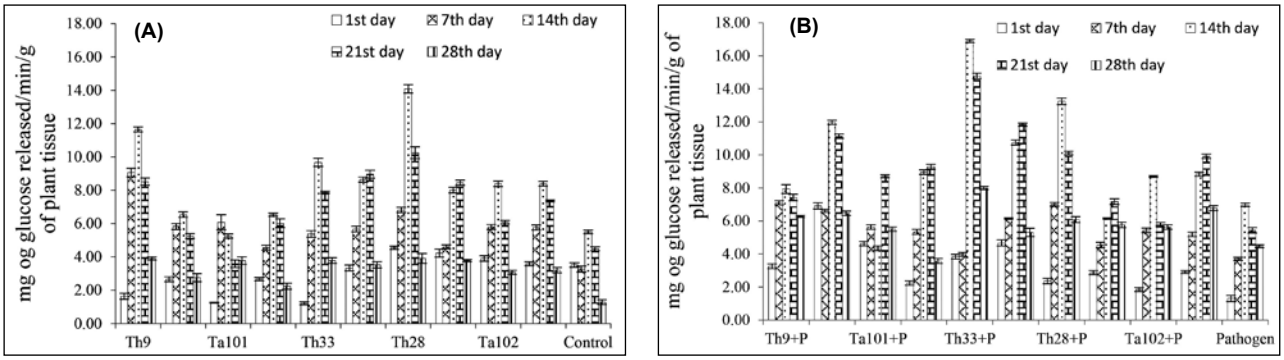


**Fig. 6.** β-1,3-glucanase activity in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

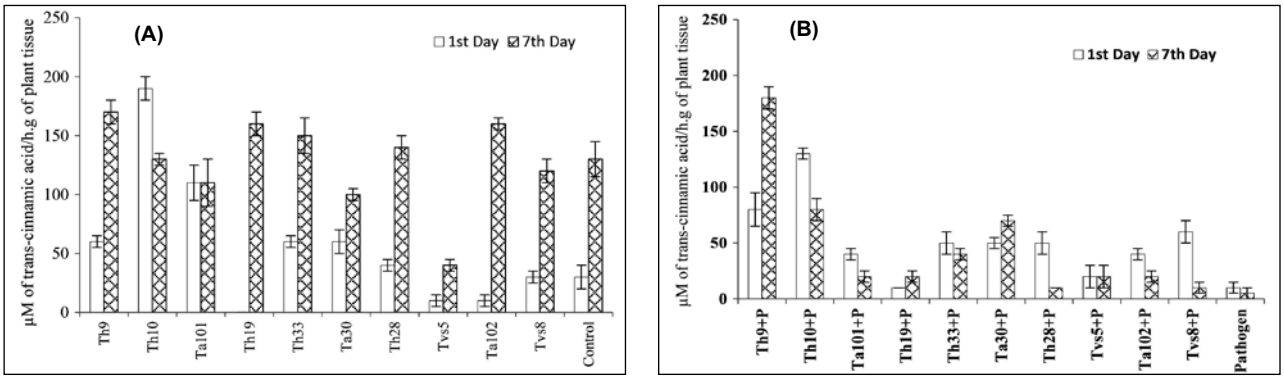


**Fig. 7.** Phenylalanine ammonia lyase activity in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

3-glucanase activity which is triggered by cell wall elicitor treatment which diffuse toward and affect the glucan support structure of *P. capsici*. Since, *P. capsici* cell wall contains glucan, the enhanced glucanase activity might be one of the reasons for increased disease reduction. Elicitor from Th10 enhanced PO activity that catalyses the last steps in the biosynthesis of lignin and hydrogen peroxide that are deposited in cell walls and papillae and interferes with further growth and development of pathogen.

Reports on the induction of defense enzymes due to treatment with bioagents or their elicitors have been reported earlier. Buensanteai *et al*. (2) observed up-regulation of PAL gene in maize plants treated with extracellular protein elicitor from *T. virens*. Karthikeyan *et al.* (6) reported the induction of

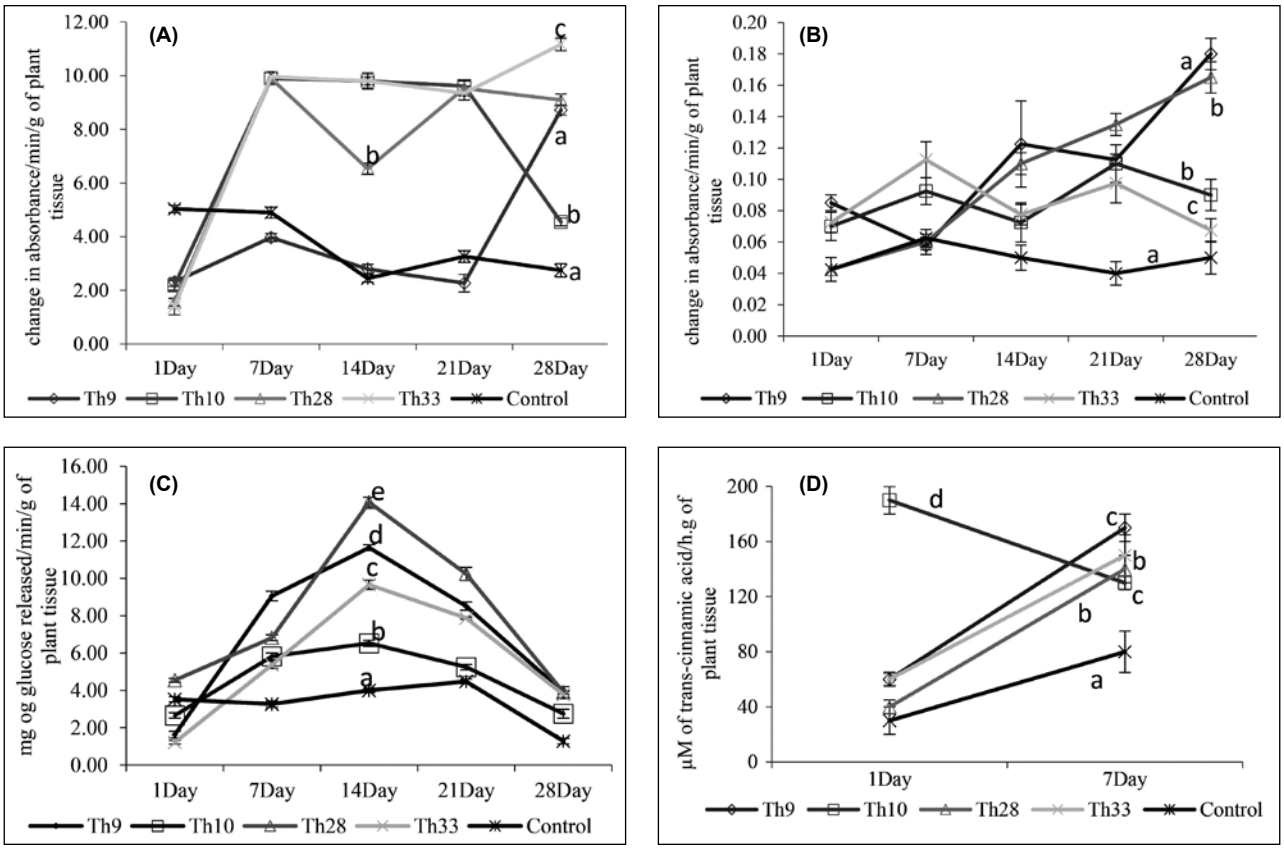


**Fig. 8.** Enzymatic activities of selected isolates. a. Peroxidase, b. Polyphenol oxidase, c. Glucanase, d. PAL. Different letters indicate significant difference according to Duncan's test (P = 0.05).

PPO in coconut plants in response to treatment with *Pseudomonas fluorescens* and *T. viride*. Increased hydrogen peroxide production in rice plants and cotton cotyledons treated with protein elicitor from *Trichoderma virens* was observed by Djonović *et al*. (5). Patil *et al.* (10) reported the induction of phenol content in tomato plants treated with elicitors from non-pathogenic *Fusarium* isolates that provided protection against *F. oxysporum* f. sp. *lycopersici*. Sriram *et al.* (12) observed higher induction of phenol content in hot pepper plants treated with cell wall elicitors from *T. harzianum* and protection against infection by *P. capsici*. The induction of defense mechanism using cell wall fragments of *T. viride* and *T. longibrachiatum* has been documented earlier (Koch *et al*., 7; Chang *et al*., 3). In our earlier study, cell wall glucan elicitor from *T. harzianum* induced systemic resistance by significant increase in glucanase and phenol content in hot pepper plants against *P. capsici* (Sriram *et al*., 12). The treatment with elicitors from isolates Th9, Th10, Th28 and Th33 triggered different defense mechanism (ISR) in hot pepper plants, which resulted in reduced disease incidence by ISR mediated protection either through induction of glucanase or enzymes involved in phenyl propanoid pathway or shikimic acid pathway.

## ACKNOWLEDGEMENTS

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# Effect of straw mulch and integrated nitrogen management on yield and quality of turmeric under North Indian plains

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## ABSTRACT

**The continuous application of nitrogen (N) through chemical source has shown deleterious effect on productivity of turmeric, whereas availability of organic source is very limited to meet the nitrogen requirement. Therefore it was considered worth to study the impact of integration of chemical and organic source of N on productivity of turmeric. A three-year field experiment was conducted at Punjab Agricultural University, Ludhiana, during 2011-12 to 2013-14 to study the response of turmeric to straw mulching and N applied through different combinations of organic manure (FYM) and N-fertilizer (urea). Application of paddy straw as mulch @ 6.25 t ha$^{-1}$ significantly increased the plant height, number of tillers, mother, primary and secondary rhizomes, rhizome weight and yield of turmeric. The fresh rhizome yield increased by 85.0 and 34.9% during 2011-12 and 2012-13, respectively, due to mulch application over no-mulch. A slight improvement was noticed in curcumin content, however, the difference between mulch and no-mulch treatments was non-significant. Application of 125 kg N/ ha through FYM alone, produced the highest rhizome yield during all study years (20.6, 22.36 and 23.17 t/ha during 2011-12, 2012-13 and 2013-14, respectively), which was, however, statistically at par with the application of two-third and half of organic manure along with one-third and half of N through urea, respectively. Nitrogen substitution through urea beyond 50% resulted into reduced growth and fresh rhizome yield. Hence, integration of 50% of N (62.5 kg/ha) through organic manure (FYM) and 50% of N through fertilizer urea enhanced the turmeric yield as compared to 100% N fertilizer alone. Quality parameter in terms of curcumin content did not show any significant differences with the different combinations of integrated nitrogen source.**

**Key words:** Curcumin, farm yard manure, nitrogen, rhizome yield, straw mulch, turmeric.

## INTRODUCTION

Turmeric (*Curcuma longa* L.) is an important spice crop, grown for its aromatic underground rhizome. India is the largest producer and the major part of its production is consumed within the country. The nutritional requirement of turmeric varies with varieties, locations in relation to quality and productivity of crop (Babu and Muthuswami, 1). The use of optimum dose of N is of vital importance, being an essential constituent of nucleic acid, it increases the number of leaves and tillers, which affect growth, yield and quality of turmeric. The response of turmeric to N varies widely according to soil and climatic conditions. It's nutrient requirement is high due to shallow rooting and capacity to produce large amount of dry matter per unit area, hence sufficient quantity of nutrient has to be applied to meet its nutritional requirement (Nagarajan and Pillai, 14). The application of farmyard manure (FYM) and mulch have favourable effect on turmeric rhizome yield. The application of mulch, conserves soil moisture by decreasing evaporation losses, reduces weed menace, regulates soil temperature (Dass *et al*., 3) and helps protect the germinating rhizomes from desiccation especially during early growth period of hot and dry months (May and June). Similarly, growth characters, yield attributes and yield of turmeric were improved with farmyard manure application (Gill *et al*., 6).

The availability of quality organic manure to sustain the productivity is a matter of concern, therefore the combined use of both organic manure and chemical fertilizer in required quantity assumes special significance as complementary and supplementary to each other especially for the high yielding crops with higher nutrient requirements (Kumar *et al*., 10; Kumar *et al*., 12; Dass *et al*., 4). The application of organic manure with nitrogen (N) fertilizer is known to stimulate mineralizable N fractions and increase the efficiency of inorganic N fertilizer in the soil. The response of turmeric to integrated N management in relation to straw mulching has not been adequately studied, hence the current field investigation was made to study the integrated effect of chemical fertilizers and organic manures along with straw mulching on growth, yield and quality of turmeric.

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## MATERIALS AND METHODS

The three-year field experiment was conducted at the Research Farm of Punjab Agricultural University, Ludhiana, during *kharif* season of 2011-12, 2012-13 and 2013-14. The experimental site is characterized by sub-tropical and semi-arid type of climate with hot and dry summer (April to June), hot and humid monsoon period (July to September), mild winter (October to November) and cold winter (December to January). The experimental site is situated at 30° 56' N latitude with 75° 52' E longitude with a mean height of 247 m above the mean sea level. The rainfall during the crop growing season was 1280.5, 409.7 and 807 mm in 2011-12, 2012-13 and 2013-14, respectively. The planting of turmeric (var. 'Punjab Haldi 1') was done in the last week of April every year with basal dose of 25 kg $ha^{-1}$ each of phosphorus ($P_2O_5$) in the form of single super phosphate and potash ($K_2O$) in the form of muriate of potash. The planting was done on flat bed at spacing of 30 cm × 20 cm, respectively. Pendimethalin 0.975 kg $ha^{-1}$ was applied within two days of planting and three manual hoeing (at 50, 75, 100 days after planting) were also undertaken to keep the weeds under check. Immediately after planting, irrigation was given and subsequently 9, 13 and 11 irrigations were given as per requirement of the crop during 2011-12, 2012-13 and 2013-14, respectively. Soil of experimental field was loamy-sand texture, low in organic carbon (0.20%) and available nitrogen (125.4 kg $ha^{-1}$) and medium in available phosphorus (19.5 kg $ha^{-1}$) and potassium (208.7 kg $ha^{-1}$). Soil pH (7.8) and electrical conductivity (0.21dS $m^{-1}$) were within the normal range. The content of available Zn, Mn, Fe, and Cu (1.76, 3.57, 4.58 and 0.24 mg $kg^{-1}$, respectively) in soil was sufficient for crop production. The NPK content of farmyard manure was 1.10, 1.16 and 0.98% N, 0.43, 0.48 and 0.41% P and 0.82, 0.77 and 0.91% K during 2011-12, 2012-13 and 2013-14, respectively.

The experiment was laid-out in a randomized complete block design (RCBD) with four replications. The treatments consisted of two levels of mulch (0 and 6.25 t $ha^{-1}$ through paddy straw) and five combinations of organic manure (FYM) and N fertilizer (urea) as sources of nitrogen ($N_1$: whole N through FYM at planting (equivalent to 125 kg N $ha^{-1}$), $N_2$: 2/3 N (83.5 kg N $ha^{-1}$) through FYM at planting + 1/3 N through urea (41.5 kg N $ha^{-1}$ in two equal splits at 75 and 100 days after planting), $N_3$: 1/2 N (62.5 kg N $ha^{-1}$) through FYM at planting + 1/2 N through urea (62.5 kg/ha in two equal splits at 75 and 100 DAP), $N_4$: 1/3 N (41.5 kg/ha) through FYM at planting + 2/3 N through urea (83.5 kg N $ha^{-1}$ in two equal splits at 75 and 100 days after planting), $N_5$: 1/4 N (31 kg/ha) through FYM at planting + 3/4 N through urea (94 kg N $ha^{-1}$ in three equal splits at 75, 100 and 125 DAP) during 2011-12 and N5: whole N through urea (125 kg $ha^{-1}$ in three equal splits at 75, 100 and 125 DAP) during 2012-13 and 2013-14. Paddy straw was used as mulch during 2011-12 and 2012-13, however during 2013-14, mulching treatments were not repeated due to significant and apparent response of mulching during the first two study years. The crop was harvested during second week of January every year. Observations on vegetative growth, *viz.*, plant height and number of tillers $plant^{-1}$ were recorded at the time of maximum growth, *i.e.* last week of October, whereas, the data on yield and rhizome characters were recorded at harvesting stage. The curcumin content of processed rhizomes from each plot was determined following the method as described by Thimmaiah (16). Analysis of variance was performed on the data sets using SPSS 17 software and significant effects ($p \leq 0.05$) were noted.

## RESULTS AND DISCUSSION

The fresh rhizome yield increased significantly with mulch application, which was 85 and 35% higher over no mulch application during 2011-12 and 2012-13, respectively (Table 3). Significantly higher number of mother, primary and secondary rhizomes per plant was recorded in mulch over no-mulch treatment during both the years (2011-12 and 2012-13), which might have enhanced the fresh rhizome yield. Similarly, rhizome weight per plant was 71 and 57% higher with mulch application as compared to no mulch during 2011-12 and 2012-13, respectively. Higher plant growth in terms of plant height and tillers with mulch application (Table 1) might have contributed to enhance the production of yield attributes and rhizome yield significantly. The positive effects of mulch application on growth and yield may possibly be due to modification in the soil environment, *viz.* moderating soil temperature during early growth of the crop which coincides with hot-dry months of May and June that conserves the soil moisture, increases microbial activities and nutrient availability. The beneficial effects of mulch application on rhizome yield, weight and number of rhizomes per plant, plant height and tillering have also been reported by various studies (Junior *et al*., 8; Gill *et al*., 6; Manhas, 13; Verma and Sarnaik, 17). Curcumin content was improved with mulch application, however, the differences were non-significant during both the years.

Applying recommended dose of N (125 kg /ha) through organic manure (FYM @ 25 t $ha^{-1}$) resulted in the highest fresh rhizome yield of 22.36, 23.17 t/ha that was 33.9 and 47.3% higher than application of 100% recommended dose of N (RDN) through urea, during 2012-13 and 2013-14, respectively (Table 3).

**Table 1.** Effect of different nitrogen sources on growth of turmeric as affected by INM treatments.

| Treatment | Plant height (cm) | | | Tillers/ plant | | |
|---|---|---|---|---|---|---|
| | 2011-12 | 2012-13 | 2013-14 | 2011-12 | 2012-13 | 2013-14 |
| Straw mulch (t/ha) | | | | | | |
| 0.0 | 94.9 | 99.8 | - | 1.6 | 1.4 | - |
| 6.25 | 119.3 | 114.4 | - | 2.0 | 1.6 | - |
| CD (P = 0.05) | 9.5 | 5.5 | - | 0.3 | NS | - |
| Nitrogen combination | | | | | | |
| N1: Whole N organic (eqv. to 125 kg/ha) | 109.8 | 110.6 | 98.6 | 2.0 | 1.3 | 2.5 |
| N2: 1/3 N organic (41.5 kg/ha) + 2/3 N inorganic (83.5 kg/ha) | 108.0 | 103.0 | 78.0 | 1.8 | 1.2 | 2.0 |
| N3: 1/2 N organic (62.5 kg/ha) + 1/2 N inorganic (62.5 kg/ha) | 108.3 | 105.6 | 90.2 | 1.8 | 1.2 | 2.2 |
| N4: 2/3 N organic (83.5 kg/ha) + 1/3 N inorganic (41.5 kg/ha) | 109.5 | 115.6 | 91.8 | 1.8 | 1.3 | 2.4 |
| N5*: 1/4 N organic (31 kg/ha) + 3/4 N inorganic (94 kg/ha)<br>N5**: Whole N chemical fertilizer | 99.8 | 100.7 | 71.3 | 1.7 | 1.2 | 2.1 |
| CD (P = 0.05) | NS | 8.6 | 12.9 | NS | NS | 0.35 |

NS = Non significant, Interaction = NS, N*: 2011-12, N**: 2012-13 and 2013-14

Increase in fresh rhizome yield with farmyard manure might be due its beneficial effects in terms of additional supply of plant nutrients besides nitrogen as well as improvement in physical and biological properties of soil (Chaudhary *et al*., 2). Further, integration of fertilizer nitrogen with FYM to substitute 67, 50, 33 and 25% of recommended N was statistically at par with the application of FYM alone during 2011-12. However, fresh rhizome yield decreased significantly when FYM was substituted with fertilizer N beyond 50% of RDN and the fresh rhizome yield decreased by 8.7 with 75% substitution of FYM during 2011-12 and drastically decreased by 33.9 and 47.3% during 2012-13 and 2013-14, respectively with complete omission of FYM and N-supply through urea only (Table 3). Substitution of 50% (60 kg/ha) of RDN through FYM has also been reported in nutrient exhaustive crop like rice to achieve the grain yield comparable to that of 100% of RDN, *i.e.* 120 kg/ha under similar soil fertility conditions (Kumar *et al*., 13). Application of FYM possibly reduced the nitrogen losses and enhanced the nutrient availability especially in long-duration crops. Nambiar and Abrol (15) also observed a declining trend in productivity of turmeric crop as a consequence of continuous application of chemical fertilizers, which could be checked by the use of organic manure. This imbalance of nutrients has adverse effect on the absorption of other nutrients, which ultimately affects growth and yield of the crop. The higher doses of fertilizer nitrogen (urea) have also shown many adverse impacts on the beneficial soil micro-flora and fauna, particularly the soil diazotrophic count which is drastically altered in terms of number as well as diversity (Gosal *et al*., 7). Turmeric growth in terms of plant height, number of tillers and number of mother, primary and secondary rhizomes also showed similar trends as in case of fresh rhizome yield. Different combination of integrated nitrogen sources had non-significant effect on growth and yield attributes of turmeric during 2011-12 (Tables 1 & 2). However, during 2012-13 plant height and rhizome weight per plant (Tables 1 & 2) and during 2013-14 plant height, number of tillers and number of secondary rhizomes (Table 1) declined significantly when N was applied through fertilizer alone as compared to whole organic manure. Quality parameter, *i.e.* curcumin content, which is a genetically governed trait, did not show significant difference with the combined use of farmyard manure and N fertilizer when compared with organic manure alone (Table 2). Earlier, Kandiannan and Chandaragir (11) and Gill *et al.* (6) observed that different nitrogen levels had non-significant effect on curcumin content of turmeric. The interaction effects between mulch and integrated nitrogen sources were found to be non-significant as all the treatments of N combinations had recorded better growth and yield under straw mulching against the respective treatment under no straw mulching. Therefore, it can be concluded that straw mulching has beneficial effects on growth and

**Table 2.** Effect of different nitrogen sources on yield attributes of turmeric as affected by INM treatments.

| Treatment | Mother rhizome/ plant | | | Primary rhizomes/ plant | | | Secondary rhizomes/ plant | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011-12 | 2012-13 | 2013-14 | 2011-12 | 2012-13 | 2013-14 | 2011-12 | 2012-13 | 2013-14 |
| Straw mulch (t/ ha) | | | | | | | | | |
| 0.0 | 1.7 | 2.21 | - | 6.8 | 8.4 | - | 6.5 | 8.0 | - |
| 6.25 | 2.1 | 2.74 | - | 8.8 | 10.5 | - | 11.2 | 11.1 | - |
| CD (p = 0.05) | 0.30 | 0.34 | | 1.2 | 1.2 | | 1.4 | 1.0 | |
| Nitrogen combination | | | | | | | | | |
| N1: Whole N organic (eqv. to 125 kg/ha) | 2.0 | 2.4 | 2.1 | 8.4 | 9.4 | 8.3 | 9.4 | 9.8 | 10.1 |
| N2:1/3 N organic (41.5 kg/ha) + 2/3 N inorganic (83.5 kg /ha) | 1.8 | 2.6 | 2.1 | 7.5 | 9.1 | 7.4 | 8.9 | 8.8 | 8.3 |
| N3:1/2 N organic (62.5 kg/ha) + 1/2 N inorganic (62.5 kg/ha) | 1.9 | 2.3 | 2.0 | 7.7 | 9.4 | 7.0 | 8.9 | 10.0 | 9.2 |
| N4:2/3 N organic (83.5 kg/ha) + 1/3 N inorganic (41.5 kg/ha) | 1.9 | 2.4 | 2.3 | 8.3 | 10.0 | 8.9 | 9.3 | 10.5 | 10.7 |
| N5*: 1/4 N organic (31 kg/ha) + 3/4 N inorganic (94 kg/ha) N5**: Whole N chemical fertilizer | 1.7 | 2.7 | 1.9 | 7.2 | 9.3 | 6.6 | 7.7 | 8.6 | 7.5 |
| CD (p = 0.05) | NS | NS | NS | NS | NS | NS | NS | NS | 1.4 |

NS = Non significant, Interaction = NS, N*: 2011-12, N**: 2012-13 and 2013-14

**Table 3.** Effect of nitrogen sources on yield and quality of turmeric as affected by INM treatments.

| Treatment | Rhizome weight/ plant (g) | | | Fresh rhizome yield (t/ha) | | | Curcumin content (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011-12 | 2012-13 | 2013-14 | 2011-12 | 2012-13 | 2013-14 | 2011-12 | 2012-13 | 2013-14 |
| Straw mulch (t/ha) | | | | | | | | | |
| 0.0 | 181.8 | 224.7 | - | 14.03 | 17.05 | - | 2.9 | 3.1 | - |
| 6.25 | 311.6 | 352.4 | - | 25.96 | 23.01 | - | 3.3 | 3.3 | - |
| CD (p = 0.05) | 36.7 | 29.7 | | 2.62 | 2.07 | | NS | NS | |
| Nitrogen combination | | | | | | | | | |
| N1: Whole N organic (eqv. to 125 kg/ha) | 271.4 | 330.0 | 354.8 | 20.60 | 22.36 | 23.17 | 3.4 | 3.4 | 3.8 |
| N2:1/3 N organic (41.5 kg/ha) + 2/3 N inorganic (83.5 kg/ha) | 236.0 | 261.0 | 239.8 | 19.79 | 17.92 | 18.51 | 2.9 | 3.1 | 3.2 |
| N3:1/2 N organic (62.5 kg/ha) + 1/2 N inorganic (62.5 kg/ha) | 245.3 | 284.7 | 328.7 | 20.28 | 21.44 | 21.95 | 3.1 | 3.2 | 3.5 |
| N4:2/3 N organic (83.5 kg/ha) + 1/3 N inorganic (41.5 kg/ha) | 260.1 | 299.3 | 337.5 | 20.32 | 21.73 | 22.49 | 3.2 | 3.3 | 3.7 |
| N5*: 1/4 N organic (31 kg/ha) + 3/4 N inorganic (94 kg/ha) N5**: Whole N chemical fertilizer | 220.6 | 268.0 | 241.8 | 18.99 | 16.70 | 15.73 | 2.8 | 3.0 | 3.4 |
| CD (p = 0.05) | NS | 46.0 | 29.2 | NS | 3.27 | 2.38 | NS | NS | NS |

NS = Non significant, Interaction = NS, N*: 2011-12, N**: 2012-13 and 2013-14

yield of turmeric irrespective of N sources and their integrations.

Paddy straw mulching (6.25 t/ha) proved beneficial in turmeric cultivation as it enhanced the growth, rhizome yield and quality. Besides, it has also the potential to conserve the soil moisture and reduce the weed infestation. Integration of nitrogen sources to manage the nitrogen requirement of turmeric is also important as availability of organic sources is a big constraint, whereas total dependence on chemical fertilizers alone caused negative impact on the productivity. This study has shown that RDN in turmeric can be managed by integrating 50% each through chemical fertilizer, *i.e.* urea and FYM without any adverse effect on growth and yield of turmeric. Therefore, applying only half of RDN through FYM (instead of 100% RDN through FYM) and rest of the N through urea will not only sustain the turmeric productivity but also encourage area expansion under turmeric with the available quantity of FYM.

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# Establishment of *in vitro* propagation protocol for Hybrid Tea rose cv. Raktagandha

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## ABSTRACT

**An efficient protocol for *in vitro* multiplication of hybrid tea rose cv. Raktagandha was developed using axillary bud segments. Out of different pre-treatments of explants tried, the highest explant survival (78.05%) was obtained with carbendazim (0.2%) + diathane M-45 (0.2%) + 8-HQC (200 mg/l) for 3 h on a horizontal shaker (120 rpm). Sucrose concentration of 30 g/l in the medium seems to be optimal for *in vitro* shoot multiplication. Murashige and Skoog medium supplemented with 3.5 mg/l BAP + 0.2 mg/l NAA + 0.5 mg/l $GA_3$ was found most effective for culture establishment and shoot proliferation with highest number of micro-shoots (4.62 shoots / explant). Rooting of micro-shoots was induced on half-strength MS basal medium supplemented with NAA (0.5 mg/l) + IBA (0.5 mg/l). The regenerated plantlets were efficiently hardened in glass jars filled with vermiculite + agropeat (1:2) moistened with half-strength MS medium salts and covered with polypropylene lids, thereafter plants were successfully transferred to the glasshouse with good survival.**

**Key words:** Hybrid tea, micropropagation, protocol, rose.

## INTRODUCTION

Rose "Queen of Flowers" is an important horticultural and most popular ornamental plant in the World (Ozel *et al.*, 11). Traditionally, hybrid-tea roses (*Rosa hybrida* L.) have been considered to be one of the most prized flowers of the world because of their high ornamental value. Their importance has grown over the years with the emergence of a global cut flower market. It is admired for their perfect blooms, exquisite colour and unique fragrance. As cut flower, it occupies top position in acreage, production and consumption. Roses are generally multiplied vegetatively by grafting and budding that are very slow and time consuming methods. Moreover, diseases and environmental hazards make the cultivar degenerate gradually. Micropropagation procedures have facilitated mass production of good quality plantlets giving a boost to rose floriculture industry. This technique allow producing roses with higher quality under a virus indexing programme, attending in this way the market demand. Keeping this in view, the present investigation was carried out to establish an efficient and reproducible protocol for rapid and large scale propagation of 'Raktagandha' an important cut rose cultivar.

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## MATERIALS AND METHODS

The present study was carried out at the Central Tissue Culture Laboratory, L.B.S. Centre, IARI, New Delhi during 2010-2012. Rose cultivar Raktagandha maintained at Centre for Protected Cultivation Technology, ICAR-IARI, New Delhi was used for this experiment. The budsticks having 3 to 4 matured axillary buds were selected from the middle portion of current season flowering shoots. With secateurs they were excised during morning hours and cut into individual axillary bud segments (≥ 1.5 cm). The explants were washed with Teepol® (0.1%) solution for 5 min. followed by washing under running tap water for 15 min. The nodal segments were then treated with different pre-treatments such as. (i) carbendazim (0.2%) + 8-HQC (200 mg/l), (ii) carbendazim (0.2%) + diathane M-45 (0.2%) + 8-HQC (200 mg/l) along with control (distilled water) for 3 h on horizontal shaker (120 rpm). The pre-treated explants were then surface-sterilized with 0.1% mercuric chloride for 5 min. followed by two-three rinsings with autoclaved distilled water. The surface sterilized explants were cultured on MS medium supplemented with different concentrations of BAP (3.0, 3.5 and 4.0 mg/l), NAA (0.1 and 0.2 mg/l), and $GA_3$ (0.3 and 0.5 mg/l) to find out the best treatment combination for culture establishment. The surface sterilized explants were cultured on media containing MS + BAP (3.5 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) with five different concentrations of sucrose; *i.e.*, 20, 30, 40, 50 and 60 g/l. After four weeks, individual micro-shoots

(about 1 cm) were separated from the bunch and transferred onto fresh medium with the similar hormonal combination and a specific concentration of sucrose.

The sprouted shoots were then sub-cultured onto MS medium supplemented with different concentrations of BAP (3.0, 3.5 and 4.0 mg/l), NAA (0.1 and 0.2 mg/l), and $GA_3$ (0.3 and 0.5 mg/l) to find out the best treatment combination for shoot proliferation. The multiplied shoots on proliferation media were separated and individual micro-shoots were transferred onto elongation media comprising basal MS medium supplemented with various concentration of $GA_3$ (0.25, 0.5, and 1.0 mg/l) to standardize its optimum dose for micro-shoots elongation. Elongated shoots were then transferred individually in cultured vessels containing full-and half-strength of MS medium fortified with different concentrations of auxins like NAA and IBA individually or in combination for rooting. A dose of 40 g/l sucrose was added for culture establishment and shoot proliferation and 60 g/l was added in rooting medium. The *in vitro* rooted plantlets were removed from flasks, washed thoroughly with autoclaved distilled water to remove the sticking agar-agar to roots. The roots were then dipped in carbendazim (0.1%) for 10 sec. The plantlets were then acclimatized in glass jars filled with vermiculite + agro peat (1:2) moistened with half-strength MS medium salts (macro + micro) and covered with polypropylene lids. The plantlets were kept in culture room (15 days) before transferring to greenhouse. For culture initiation, 20-25 explants were inoculated per treatment in three replications. The cultures were maintained at 25±1°C under fluorescent white light (47 µmol $m^{-2}$ $s^{-1}$) at a photoperiod of 16/8 h light and dark cycles. The data was analyzed employing completely randomized design (CRD) and percent data was subjected to Arc Sin √% transformation before ANOVA.

## RESULTS AND DISCUSSION

Pre-treatment of explants with different fungicidal and bactericidal treatments had significant effect on survival of explants, microbial contamination, bud sprouting and days to bud sprouting (Fig. 1). The treatment ($T_3$) comprising carbendazim (0.2%) + diathane M-45 Indofil® (0.2%) + 8-hydroxy quinnoline citrate (200 mg/l) for 3 h agitation gave the highest explant survival and bud sprouting, which were significantly superior compared to the other treatments whereas, the minimum explant survival and bud sprouting were recorded with distilled water control ($T_1$). The pre-treatment of axillary bud explants with treatment ($T_3$) comprising carbendazim (0.2%) + diathane M-45 (0.2%) + 8-HQC (200 mg/l) for 3 h minimized microbial contamination significantly as compared to control. This pre-treatment also gave the earliest bud sprouting as compared to control. It is obvious that the fungicides used had both systemic and contact fungicides, thus gave efficient control of microbial infection. Similarly, 8-HQC was effective due to its bactericidal activities. Efficacy of these compounds has earlier been demonstrated by Machado *et al*. (10), and Bharadwaj *et al*. (3) in rose.

The maximum explant survival (79.55%) and bud sprouting (73.60%) were recorded for the treatment MS + BAP (4.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) (Table 1; Fig. 3a). The minimum response was noted with hormone-free medium. The above treatment also gave the earliest (6.65 days) bud sprouting when comparing with other treatments, which was maximum delayed (11.13) in control. The efficacy of BAP in stimulating shoot proliferation has earlier been reported

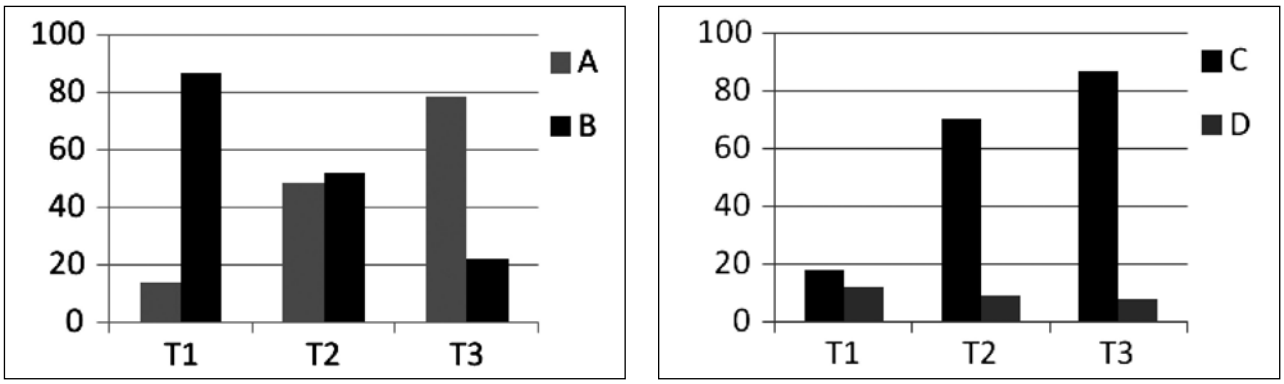


**Fig. 1.** Effect of different pre-treatments on *in vitro* culture initiation in hybrid tea rose cv. Raktagandha. T1 = control (distilled water) for 3 h, T2 = carbendazim (0.2%) + mancozeb-45 (0.2%) + 8-HQC (200 mg/l) for 3 h, T3 = carbendazim (0.2%) + mancozeb-45 (0.2%) + 8-HQC (200 mg/l) for 3 h; A = explant survival (%), B = microbial contamination (%), C = bud sprouting (%), and D = days to bud sprouting.

**Table 1.** Effect of growth regulators on *in vitro* explant survival and bud sprouting in Hybrid Tea rose cv. Raktagandha.

| Treatment | Explant survival (%) | Bud sprouting (%) | Days to bud sprouting |
|---|---|---|---|
| MS + No hormone (control) | 37.32 (37.67) | 31.97 (34.45) | 11.13 |
| MS + BAP (3.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.3 mg/l) | 61.38 (51.60) | 54.27 (47.48) | 9.12 |
| MS + BAP (3.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) | 71.66 (57.86) | 63.73 (53.00) | 7.41 |
| MS + BAP (3.5 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.3 mg/l) | 71.11 (57.52) | 72.40 (58.34) | 6.79 |
| MS + BAP (4.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) | 79.55 (63.15) | 73.60 (59.1) | 6.65 |
| CD at 5% | 2.72 | 1.21 | 0.51 |

The value given in parentheses denote the Arc Sin √% values

by and Kumar and Prateesh (8). Earlier, Douglas *et al*. (5) and Arnold *et al*. (1) reported the efficacy of cytokinins in combination with an auxin or together with GA.

The maximum number of shoots sprouted was noted with treatment MS + BAP (4.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) (Table 2; Fig. 3b). After first sub-culture maximum shoots per explant (3.25) was noted with the same treatment. The least number of shoots per explant (1.64) were recorded under control, *i.e.*, MS without any hormone. Shoot multiplication rate in third sub-culture revealed that maximum number of shoots per explant (4.62) as also recorded in the treatment MS + BAP (4.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) compared to least (3.10) in control. Growth regulators at an optimum dose leads to good shoot proliferation and the same was observed in each sub-culture. The favourable influence of BAP and NAA in different metabolic processes (Kulaeva, 7) is known to effect plant metabolism. The better results regarding shoot proliferation in tissue culture might be due to the role of optimum dose of BAP, which enhances axillary branching and multiple shoot formation. Superiority of BAP in shoot multiplication has earlier been shown by Scotti Compos and Pais (12). It is also opined that in multiple shoots a proliferation may be due to loss of apical dominance (Madhu Bala *et al*., 2; Bressan *et al*., 4; Douglas *et al*., 5; Singh and Syamal, 14).

Besides hormonal regime, other media components are also known to influence the *in vitro* growth response of rose cultures (Short and Roberts, 13). Concentration of sucrose in the medium has been found to affect photosynthetic potential (Langford and Wainwright, 9) and rate of shoot multiplication. Sucrose concentration of the medium showed a distinct influence on the rate of shoot proliferation in cultures. Proliferation rate depicted by mean number of nascent shoots produced per culture in four weeks was highest in 40 g/l sucrose, intermediate in 30 and 50 g/l and lowest in 20 and 60 g/l (Fig. 2). Sucrose facilitates the induction of vascular tissue differentiation in cultures. Without exogenous sucrose, the formation of tracheary elements will be greatly reduced or absent. Hyndman *et al.* (6) and Langford and Wainwright (9) reported that sucrose concentration of the medium incrementally influences the photosynthetic ability of *in vitro* growing shoots up to a certain level; but higher concentrations suppress the activity. This could be the reason for inferior growth response in 60 g/l sucrose as compared to 40 g/l as noted in the present study.

The data presented in Table 3 depicts the effect of basal medium strength and auxin on days to root

**Table 2.** Effect of growth regulators on *in vitro* shoot proliferation in Hybrid Tea rose cv. Raktagandha.

| Treatment | No. of shoots proliferated per explant | | |
|---|---|---|---|
| | After first sub-culture | After second sub-culture | After third sub-culture |
| MS + No hormone (control) | 1.64 | 2.45 | 3.10 |
| MS + BAP (3.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.3 mg/l) | 2.40 | 3.29 | 3.48 |
| MS + BAP (3.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) | 2.59 | 3.56 | 3.47 |
| MS + BAP (3.5 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.3 mg/l) | 2.66 | 3.67 | 4.17 |
| MS + BAP (3.5 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l) | 3.25 | 3.90 | 4.62 |
| CD at 5% | 0.407 | 0.345 | 0.350 |

**Table 3.** Effect of basal medium strength and auxins on rooting of microshoots in Hybrid Tea rose cv. Raktagandha.

| Treatment | Days to root initiation | Rooting (%) | Root length (cm) | Root quality | Plantlets growth |
|---|---|---|---|---|---|
| MS + No hormone (control) | 30.693 | 14.17 (22.12) | 1.207 | St | Poor |
| MS + NAA (0.5 mg/l) | 20.267 | 20.77 (25.70) | 1.330 | S, Th, St | Good |
| MS + IBA (0.5 mg/l) | 22.620 | 24.17 (29.46) | 2.307 | Th, S | Good |
| ½ MS + NAA (0.5 mg/l) + IBA (0.5 mg/l) | 16.873 | 82.50 (65.30) | 3.733 | T, L | V. Good |
| ½ MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) | 16.060 | 78.33 (62.29) | 3.917 | Th, L | V. Good |
| CD at 5% | 0.905 | 3.607 | 0.431 | | |

The value given in parantheses denote the Arc Sin √% values; T = thin, Th = thick, L = long, M = medium strength, St = stunted, S = small

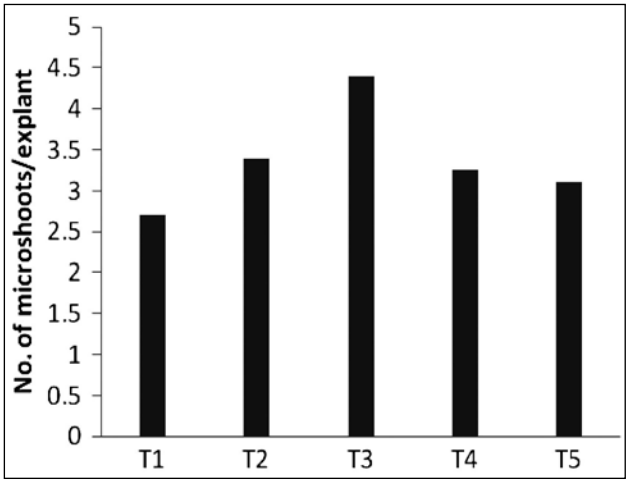


**Fig. 2.** Effect of different sucrose levels on shoot multiplication. T1 = 20 g/ l, T2 = 30 g/ l, T3 = 40 g/ l, T4 = 50 g/ l, T5 = 60 g/ l.

initiation, rooting, number of roots per shoot, root length and root quality. The earliest root initiation (16.0 days) was noted on half-strength MS + NAA (0.5 mg/l) + IBA (0.5 mg/l) followed by half-strength MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) (16.9 days) (Fig. 3e). The time taken for root initiation was most delayed in control (30.7 days). The highest rooting was observed for the treatment half-strength MS + NAA (0.5 mg/l) + IBA (0.5 mg/l). The rooting on reduced basal salt strength medium was significantly higher as compared to full-strength medium. The rooting percentage was maximum (82.5) on half-strength MS + NAA (0.5 mg/l) + IBA (0.5 mg/l) followed by half-strength MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) (78.33) as compared to minimum in control (14.17). The longest root length (3.73 cm) was induced with half-strength MS + NAA (0.5 mg/l) + IBA (0.5 mg/l). The qualitative data suggest that roots were not only few but stunted in medium devoid of auxins. When NAA was supplemented individually, roots were small stunted and thick while those cultured on medium supplemented with IBA had thin and long roots. Interestingly, roots on half-strength medium supplemented with the dual auxins were of medium length and thin. It is evident from the study that there has been synergistic effect when the two auxins were employed together. Optimum role of two auxins has been reported earlier also by Singh and Syamal (14), and Bharadwaj *et al*. (3). The highest plantlet survival (86.24%), the plant height (8.12 cm) with good number of leaves (4.82 per plant) were recorded for the treatment where plantlets were acclimatized in glass jars filled with vermiculite + agropeat supplemented with half strength MS medium (macro + micro) and covered with polypropylene lids as compared with, the plantlet survival in plastic pots covered with polythene bags (Table 4). The better results obtained in glass jars might be due to less open space but appropriate relative humidity as compared to those hardened in plastic pots covered with polythene bags. Efficacy of glass jars for *Rosa hybrida* has been earlier reported by Madhu Bala *et al*. (2), Singh and Syamal (14), and Bharadwaj *et al*. (3). The results of the present investigation demonstrate that rose cultivar Raktagandha can be multiplied *in vitro* employing the above protocol.

**Table 4.** Effect of different acclimatization strategies on survival of *in vitro* raised plantlets.

| Treatment | Survival (%) | Plantlet height (cm) | No. of leaves per plantlet |
|---|---|---|---|
| Plastic pot with polythene cover | 74.26 (59.52)* | 6.27 | 3.90 |
| Plantlet in glass jar with polypropylene lid | 86.24 (68.40) | 8.12 | 4.82 |
| CD at 5% | 4.81 | 1.92 | 0.02 |

*Value given in parantheses denotes the Arc Sin √% values

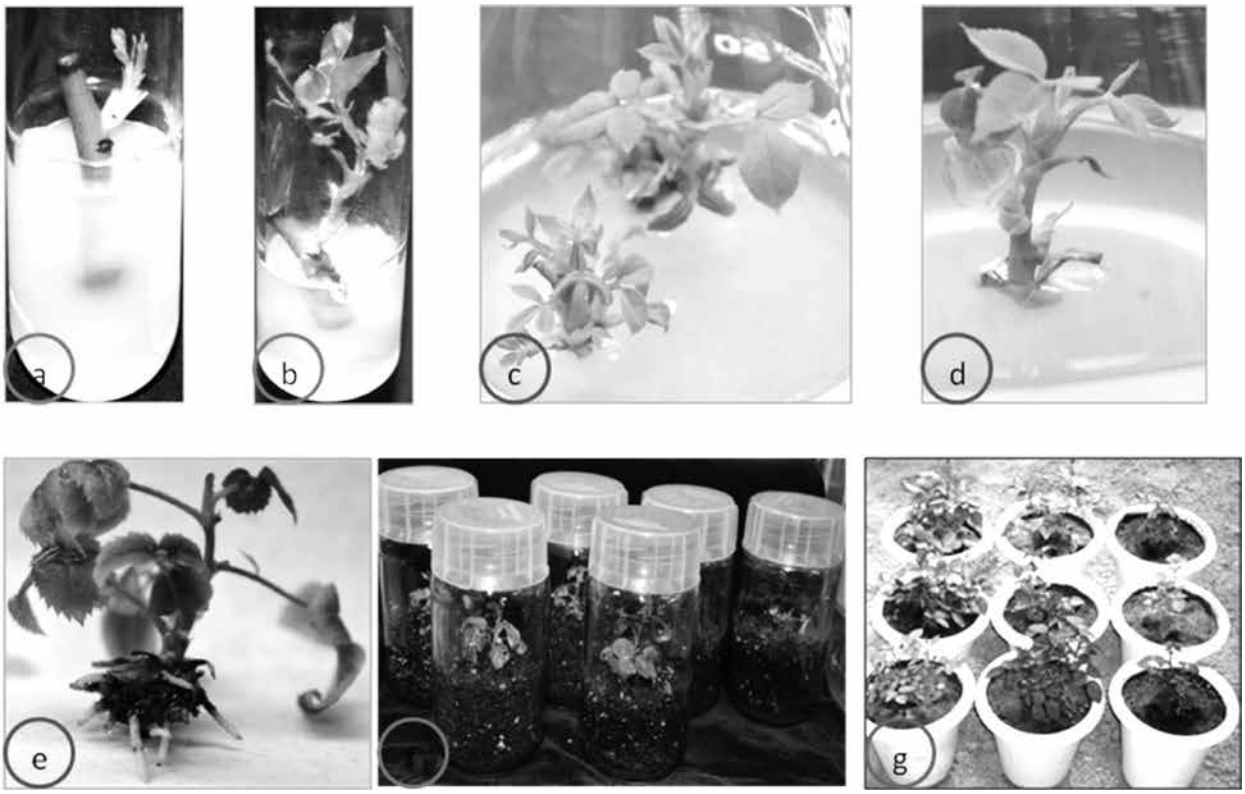


**Fig. 3.** *In vitro* plant regeneration in Hybrid Tea rose cv. Raktagandha (a, b, c) Bud sprouting, Sprouting of shoots and shoot proliferation on MS + BAP (4.0 mg/l) + NAA (0.1 mg/l) + $GA_3$ (0.5 mg/l), (d) Elongation of shoots on MS + $GA_3$ (0.5 mg/l), (e) *In vitro* rooting on ½ MS + NAA (0.5 mg/l) + IBA (0.5 mg/l), (f) Gradual hardening in glass jar, (g) Acclimatized plants in soil.

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# Screening of tulip (*Tulipa gesneriana* L.) germplasm for quality cut flower and bulb production

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## ABSTRACT

**A study was conducted to screen tulip genotypes for quality cut flower and bulb production under Kashmir agro-climatic conditions. Twenty tulip genotypes were evaluated. Highest scape length was recorded in Oxford Wonder (35.47 cm) which was at par with Blessing Lady (34.88 cm) and Apeldoorn Elite (33.24 cm). Wide variation was observed in flowering duration (15.25 to 27.24 days) in different genotypes and longest flowering duration was recorded in Purissima Yellow (27.24 days) followed by Apeldoorn Elite (25.20 days), American Dream (24.67 days), Blessing Lady (24.46 days) and Golden Oxford (23.66 days). Genotype Blessing Lady exhibited maximum vase-life 11.67 days, which was at par with Daydream (11.50 days) followed by Purissima Yellow (11.00 days), Golden Oxford (10.67 days) and American Dream (10.50 days). Blessing Lady (3.10), Purissima Yellow (2.80), American Dream (2.72) and Negrita Favorite (2.67) produced higher number of bulbs per plant as compared to lowest in Horizon (1.50). Propagation coefficient was found significantly high in Blessing Lady (354.11%) followed by Purissima White (314.13%), American Dream (308.47%), Apeldoorn Elite (303.12%), Purissima Yellow (297.61%) and Daydream (291.50%) as against lower propagation coefficient (< 200%) in Horizon, Hamilton, Character and Cassini. Based on overall performance several genotypes Blessing Lady, Daydream, Purissima Yellow, American Dream, Apeldoorn Elite and Golden Oxford were found promising in vegetative, floral and bulb production attributes and can be utilized commercially.**

**Key words:** Tulip, flowering duration, vase-life, water relation, propagation coefficient.

## INTRODUCTION

Tulip (*Tulipa gesneriana* L.) is an important temperate bulbous flower crop grown in beds, borders and pots for landscaping of gardens and cut flower for commercial purpose. It is originated in Turkey and Central Asia; Holland is known as home of tulip. It is known as queen of bulbs owing to its wide range of genotypes of attractive colours and shapes. It occupies 3rd position in international floriculture trade (Anon, 2). In India, tulips are grown successfully in cold regions of Jammu and Kashmir, Himachal Pradesh and Uttrakhand. The North Western Himalayas is the richest gene centre with about 50 species out of total 100-150 species (Jhon and Neelofer, 8). Its genotypes differ widely in form, size, shape, colour and flowering habit. It needs 17 to 20ºC temperature for flower formation (Ahmed and Khurshid, 1), while less than 5ºC temperature for 10 to 12 weeks is required for forcing. Tulip has been recently introduced in Kashmir Valley, India from Holland and becoming popular in owing to congenial agro-climatic conditions for cut flower and bulb production with low labour cost. In tulip growing, long and strong stem, enhanced flowering duration and vase-life, high multiplication rate of bulbs are major decisive factors. Under Kashmir conditions, flowering initiates during March end and last for 15 to 25 days depending upon genotypes with production of 1 to 2 flowering size bulbs. It has not been growing commercially due lack of quality planting material of suitable genotypes, short flowering duration and low multiplication rate. Quality of bulb is important criteria, other inputs rendered ineffective if the quality of bulb is poor (Jhon *et al*., 10). However, information on suitability of tulip genotypes for cut flower and bulb production are scanty under Kashmir agro-climatic conditions. Therefore, the present study was undertaken.

## MATERIALS AND METHODS

The present study was carried out at research field of ICAR-Central Institute of Temperate Horticulture, Srinagar during 2010-14 using 20 tulip genotypes introduced from Holland. The bulbs were procured from Department of Plant Introduction, Directorate of Floriculture, Kashmir, Srinagar, India. The experimental field is situated at about 33°59' N latitude and 74°46' E longitude and 1674.88 m elevation above mean sea level. The soil characteristics of experimental field were clay loam to silt clay, pH 6.83 and EC 0.38 dS $m^{-1}$

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with adequate drainage and water holding capacity. Healthy and uniform bulbs of 8 to 12 cm circumference were used as planting material. Prior to planting bulbs were treated with 0.05% carbendazim to avoid bulb rot. The experiment was laid out in randomized block design with three replications and 20 bulbs per treatment were planted during first week of November at the spacing of 20 cm × 20 cm and at a depth of 8 to 10 cm. Uniform intercultural operations were practiced for growing the crop.

The data were recorded on different parameters pertaining to growth, flowering and bulb production of different genotypes. The plant propagation coefficient (%) illustrates bulb multiplication rate, which is the ratio of the total weight of bulbs and daughter bulbs produced and the weight of bulb planted, multiplied by 100 (Kumar *et al*., 13). In vase-life study, tulip scapes were harvested at bud colour break stage in the morning during first week of April. The flowers were precooled at 5°C for about one hour by keeping in modular cold rooms to remove field heat. Then scapes were sorted to uniform length in respective genotypes and lower leaves removed to prevent them touching the preservative solution. After recording initial weight, scapes were placed in conical flask (250 ml) containing vase solution of 8-HQS 300 ppm (Kumar *et al*., 12). 8-HQS dealys the senescence of cut tulip due to cytokinin-like activity and inhibition of ethylene production, which maintains better water balance leading to improved vase-life. The experiment was laid out in completely randomized design (CRD) with three replications. The flasks were plugged with cotton to prevent loss of water due to evaporation. The experiment was conducted at temperature of 14 ± 3°C with relative humidity 70 ± 5% under natural light. Data were recorded on water uptake, water loss, water balance and stem bending incidence on 10$^{th}$ day when more than 90% flower wilted. Data were analyzed statistically as per the methods suggested by Gomez and Gomez (5). The critical difference (CD) value was used to determine difference between treatments worked out at 5% level of significance (Chandel, 3).

## RESULTS AND DISCUSSION

The tulip genotypes exhibited wide variation for vegetative, floral and bulb attributes. The data presented in Table 1 revealed that there were significant variations among different genotypes with respect to vegetative attributes. Among all the

**Table 1.** Vegetative attributes of tulip genotypes under of Kashmir agro-climatic conditions.

| Genotype | Days to sprouting | Sprouting per cent | Plant height (cm) | Leaf No. /plant | Wrapper leaf area (cm$^2$) | Field life (days) |
|---|---|---|---|---|---|---|
| Purissima White | 73.15 | 93.00 | 29.67 | 3.25 | 121.25 | 192.25 |
| Negrita Favorite | 87.33 | 86.00 | 32.16 | 4.60 | 93.67 | 185.33 |
| Kungfu | 72.67 | 84.33 | 30.47 | 3.75 | 87.00 | 179.36 |
| Ali Bi | 83.00 | 80.25 | 28.50 | 4.10 | 120.00 | 184.22 |
| Purissima Yellow | 72.35 | 92.45 | 26.25 | 4.00 | 110.33 | 187.67 |
| Banja Luka | 88.25 | 87.50 | 34.47 | 4.55 | 108.00 | 187.33 |
| Daydream | 76.21 | 92.00 | 37.08 | 4.60 | 120.24 | 184.12 |
| Oxford Wonder | 72.50 | 85.00 | 42.96 | 3.70 | 123.14 | 182.26 |
| Blessing Lady | 95.45 | 100.00 | 42.60 | 3.33 | 125.46 | 195.79 |
| Hamilton | 92.71 | 74.00 | 26.34 | 3.50 | 90.27 | 176.55 |
| Apeldoorn | 85.00 | 98.00 | 32.00 | 3.45 | 124.00 | 181.17 |
| Lle de France | 84.13 | 76.00 | 34.50 | 3.60 | 90.17 | 186.23 |
| American Dream | 88.25 | 81.00 | 33.24 | 3.45 | 120.10 | 190.00 |
| Apeldoorn Elite | 90.55 | 93.00 | 38.71 | 3.67 | 112.45 | 188.50 |
| Horizon | 91.14 | 72.00 | 22.40 | 3.00 | 70.00 | 175.34 |
| Cassini | 82.47 | 75.23 | 23.70 | 4.00 | 100.90 | 177.42 |
| Orange Emperor | 75.15 | 76.00 | 29.78 | 4.00 | 117.48 | 180.30 |
| Golden Oxford | 89.45 | 80.00 | 26.80 | 3.50 | 127.00 | 187.95 |
| Character | 83.32 | 72.00 | 33.00 | 3.00 | 82.67 | 177.35 |
| Leen van der Mark | 71.78 | 77.33 | 20.10 | 3.00 | 86.21 | 186.22 |
| CD at 5% | 4.31 | 5.20 | 3.75 | 0.59 | 10.78 | 6.95 |

genotypes earliest sprouting was recorded in Leen van der Mark (71.78 days) followed by Purissima Yellow (72.35 days), Oxford Wonder (72.50 days) while delayed sprouting was observed in Blessing Lady (95.45 days) and Hamilton (92.71 days). Sprouting percentage was varied significantly among the genotypes and recorded maximum 100% in Blessing Lady followed by 98% in Apeldoorn as compared to minimum (72%) in both Horizon and Character. Plant height was recorded highest in Oxford Wonder (42.96 cm) followed by Blessing Lady (42.60 cm), whereas it was recorded minimum in Leen van der Mark (20.10 cm) and Horizon (22.40 cm). Leaf number per plant varied from 3.00 to 4.60 among different genotypes. The leaves are already developed inside the bulbs their expression may possibly depend upon bulb health, genetic makeup of plant and environmental characteristic. Leaves are major site of photosynthesis and more the leaf area higher the photosynthesis, which ultimately improves flower and bulb size. Maximum wrapper leaf area was recorded (127.00 $cm^2$) in Golden Oxford followed by Blessing lady (125.46 $cm^2$), Apeldoorn (124.00 $cm^2$) and Oxford Wonder (123.14 $cm^2$). Number of days from planting of bulbs to drying of plants was mentioned as field life and plays important role in bulb development. It ranged from minimum 175.34 days in Horizon to maximum 195.79 days in Blessing Lady. Similar type of variation for different vegetative attributes was also reported by Jhon and Khan (7) in tulip.

In tulip cut flower production scape length, straightness and flower size are important criteria along with prolonged flowering duration and vase life. Data presented in Table 2 divulged that floral attributes significantly influenced by different genotypes. Earliest flower bud appearance was observed in Purissima White (128.22 days) followed by Oxford Wonder (129.07 days) and Purissima Yellow (129.25 days). Earliest flowering was recorded in Purissima White (135.50 days) followed by 137.00 days in both Purissima Yellow and Oxford Wonder, while delayed flowering was recorded in Blessing Lady (160.42 days) and Horizon (158.38 days). Wide variation was observed with respect to flowering duration and genotypes, which flowered up to end of March, first fortnight of April and second fortnight of April are categorized as early, mid and late flowering,

**Table 2.** Floral attributes of different tulip genotypes under Kashmir agro-climatic conditions.

| Genotype | Days to bud appearance | Days to flowering | Scape length (cm) | Scape thickness (mm) | Flower size (cm) | Flowering duration (days) |
|---|---|---|---|---|---|---|
| Purissima White | 128.22 | 135.50 | 26.00 | 6.94 | 7.45 | 15.25 |
| Negrita Favorite | 143.11 | 151.46 | 29.23 | 6.05 | 5.20 | 20.14 |
| Kungfu | 130.14 | 138.25 | 26.58 | 6.46 | 5.81 | 22.04 |
| Ali Bi | 141.36 | 148.50 | 23.50 | 6.55 | 5.69 | 22.00 |
| Purissima Yellow | 129.25 | 137.00 | 22.11 | 7.25 | 5.78 | 27.24 |
| Banja Luka | 146.50 | 154.33 | 31.57 | 7.22 | 5.81 | 19.47 |
| Daydream | 134.05 | 143.25 | 32.05 | 7.33 | 6.30 | 20.29 |
| Oxford Wonder | 129.07 | 137.00 | 35.47 | 9.02 | 8.10 | 19.50 |
| Blessing Lady | 151.67 | 160.42 | 34.88 | 8.64 | 6.25 | 24.46 |
| Hamilton | 152.33 | 157.24 | 22.27 | 6.23 | 6.89 | 19.00 |
| Apeldoorn | 143.25 | 151.00 | 28.60 | 6.95 | 6.12 | 23.33 |
| Lle de France | 142.05 | 150.50 | 30.20 | 6.22 | 7.04 | 20.25 |
| American Dream | 147.22 | 155.26 | 30.10 | 8.90 | 5.83 | 24.67 |
| Apeldoorn Elite | 144.10 | 153.24 | 33.24 | 7.17 | 7.35 | 25.20 |
| Horizon | 149.07 | 158.38 | 20.06 | 6.23 | 7.00 | 22.17 |
| Cassini | 132.75 | 139.00 | 21.27 | 6.46 | 6.81 | 20.06 |
| Orange Emperor | 133.96 | 140.00 | 25.61 | 5.62 | 5.98 | 22.47 |
| Golden Oxford | 147.33 | 155.23 | 24.10 | 8.80 | 7.20 | 23.66 |
| Character | 138.38 | 147.00 | 30.07 | 6.21 | 6.00 | 20.24 |
| Leen van der Mark | 129.52 | 138.33 | 19.00 | 6.59 | 5.15 | 21.06 |
| CD at 5% | 6.07 | 4.24 | 3.09 | 0.98 | 0.35 | 2.91 |

respectively. Genotypes Purissima White, Purissima Yellow, Kungfu, Oxford Wonder, Cassini, Orange Emperor and Leen van der Mark are early flowering, whereas Hamilton, Blessing Lady, Horizon, American Dream and Golden Oxford comes under late category and others Negrita Favorite, Ali Bi, Banja Luka, Daydream, Apeldoorn, Lle de France, Apeldoorn Elite and Character are mid flowering genotypes. Hence, these variations can be utilized for extending flowering duration in tulip. Early, mid and late flowering in tulip might be due different genetic makeup of genotypes and these findings are in conformity with the results of John and Khan (7).

Scape length significantly influenced by different genotypes and longest scape was recorded in Oxford Wonder (35.47 cm), which was at par with Blessing Lady (34.88 cm), while it was recorded shortest (19.00 and 21.27 cm) in Leen van der Mark and Cassini, respectively. It may be possibly due to varied bulb size and genetic differences among genotypes. Scape thickness was recorded maximum in Oxford Wonder (9.02 mm) followed by American Dream (8.90 mm), Golden Oxford (8.80 mm) and Blessing Lady (8.64 mm) as compared to minimum in Orange Emperor (5.62 mm). Oxford Wonder (8.10 cm) and Purissima White (7.45 cm) produced largest size flowers as against smallest in Leen van der Mark (5.15 cm) and Negrita Favorite (5.20 cm). Similar differences in floral attributes of tulip were obtained by John and Khan (7); and Ahmed and Khurshid (1). Flowering duration extended from 15.25 to 27.24 days in different genotypes and genotypes Purissima Yellow (27.24 days), Apeldoorn Elite (25.20 days), American Dream (24.67 days) and Blessing Lady (24.46 days) were found superior over others with longer flowering duration.

In vase-life study, significant variations recorded among the genotypes with respect to flower fresh weight and water relation parameters (Table 3). Flower fresh weight varied from 10.60 to 26.38 g among different genotypes, which is due to diverse scape length and genetic difference. Highest flower fresh weight was recorded in Blessing Lady (26.38 g), which was at par with Oxford Wonder (24.69 g) and Daydream (24.57 g). Water uptake per scape was found higher in Blessing Lady (25.69 g), Oxford

**Table 3.** Vase-life study on different tulip genotypes.

| Genotype | Flower fresh wt. (g) | Water relation parameter (g/ scape) | | | Bending incidence (%) | | |
|---|---|---|---|---|---|---|---|
| | | Water uptake | Water loss | Water balance | 0-30° | 30-60° | >60° |
| Purissima White | 17.00 | 13.30 | 15.03 | -1.73 | 30 | 60 | 10 |
| Negrita Favorite | 10.60 | 12.74 | 12.75 | -0.01 | 10 | 30 | 60 |
| Kungfu | 12.60 | 18.92 | 19.85 | -0.93 | 70 | 30 | 0 |
| Ali Bi | 15.40 | 18.64 | 19.74 | -1.10 | 80 | 10 | 10 |
| Purissima Yellow | 16.72 | 23.54 | 21.94 | 1.60 | 90 | 10 | 10 |
| Banja Luka | 21.35 | 22.67 | 23.00 | -0.33 | 60 | 40 | 0 |
| Daydream | 24.57 | 23.41 | 21.46 | 1.95 | 60 | 30 | 10 |
| Oxford Wonder | 24.69 | 24.80 | 25.85 | -1.05 | 80 | 20 | 0 |
| Blessing Lady | 26.38 | 25.69 | 23.10 | 2.59 | 50 | 40 | 10 |
| Hamilton | 16.33 | 20.05 | 20.80 | -0.75 | 30 | 70 | 0 |
| Apeldoorn | 18.80 | 22.07 | 22.49 | -0.42 | 20 | 60 | 20 |
| Lle de France | 17.12 | 22.81 | 23.31 | -0.50 | 30 | 40 | 30 |
| American Dream | 18.49 | 23.74 | 22.72 | 1.02 | 70 | 30 | 0 |
| Apeldoorn Elite | 20.26 | 22.00 | 21.46 | 0.54 | 90 | 10 | 0 |
| Horizon | 13.95 | 16.59 | 17.98 | -1.39 | 20 | 60 | 20 |
| Cassini | 13.80 | 17.34 | 18.84 | -1.50 | 10 | 70 | 20 |
| Orange Emperor | 16.78 | 19.68 | 20.50 | -0.82 | 10 | 30 | 60 |
| Golden Oxford | 21.92 | 18.64 | 18.44 | 0.20 | 60 | 40 | 0 |
| Character | 14.53 | 16.50 | 18.17 | -1.67 | 30 | 50 | 20 |
| Leen van der Mark | 12.77 | 13.08 | 13.15 | -0.07 | 30 | 60 | 10 |
| CD at 5% | 2.87 | 1.87 | 2.02 | 0.23 | | | |

Wonder (24.80 g) and American Dream (23.74 g), while water loss per scape was recorded highest in Oxford Wonder (25.85 g) followed by Lle de France (23.31 g) and Blessing Lady (23.10 g). Genotypes Blessing Lady (2.59 g), Daydream (1.95 g), Purissima Yellow (1.60 g), American Dream (1.02 g), Apeldoorn Elite (0.54 g) and Golden Oxford (0.20 g) expressed positive and maximum water balance at the end of vase life. Most of scapes from Purissima Yellow (90%), Apeldoorn Elite (90%), Oxford Wonder (80%), Ali Bi (80%), American Dream (70%) and Kungfu (70%) expressed low stem bending incidence (0-30°). Perusal of data divulged that stem bending incidence is genotype dependent and varies from genotype to genotype, these findings corroborated the earlier work of Kim *et al*. (11) in tulip and Ferrante *et al.* (4) in gerbera. Longest vase-life (Fig. 1) was recorded 11.67 days in Blessing Lady followed by Daydream (11.50 days), Purissima Yellow (11.00 days), Golden Oxford (10.67 days), American Dream (10.50 days) and Apeldoorn Elite (10.25 days) as against shortest in Purissima White (6.50 days). The variation in vase-life among genotypes may be due to inherent traits (Gondhali *et al*., 6), which resulted in varied water relation parameters and disturbed water balance.

In successful tulip cultivation, production of flower grade bulb is priority criteria due to low multiplication rate. It may be possibly due to lack of adaptable genotypes, improper forcing/ growing techniques, bulb rot problem and improper bulb storage. Bulbs of more than 10 to 12 cm circumference can produce flowers and termed as flower grade bulbs. Number of bulbs per plant significantly affected by different genotypes (Table 4). Genotype Blessing Lady (3.10) produced highest number of bulbs per plant, which was at par with Purissima Yellow (2.80) and American Dream (2.72), while lowest was in Horizon (1.50). These results are in conformity with findings of John and Khan (7) and Jhon *et al.* (9). Bulb size found maximum in Oxford Wonder (13.07 cm), Purissima White (12.91 cm), Apeldoorn Elite (12.67 cm), Purissima Yellow (12.36 cm) and Blessing Lady (12.27 cm), which were statistically superior over other genotypes, whereas Oxford Wonder (20.20 g), Purissima White (20.11 g) and Banja Luka (19.23 g) found better over others with respect to bulb weight. Lowest bulb weight was recorded in Hamilton (9.22 g), Character (10.11 g) and Horizon (10.23 g).

Number of daughter bulbs per plant was recorded significantly high in Apeldoorn Elite (2.50), Blessing Lady (2.49), Oxford Wonder (2.37) and Daydream (2.25) over others genotypes. Daughter bulbs weight per plant was found highest 7.23 g in Blessing Lady and was at par with Golden Oxford (6.71 g). Similar type of variation in bulbs and bulblets production was also noticed by Jhon *et al.* (9) in tulip. Total bulb yield (t/ ha) differed significantly among different genotypes and Blessing Lady was found significantly superior over other genotypes with highest total bulb yield of 15.93 t/ha followed by 13.39 t/ha in Purissima Yellow, 13.14 t/ha in Oxford Wonder. Lowest total bulb yield was recorded 4.62, 5.00 and 5.32 t/ha in Horizon, Character and Hamilton, respectively. Propagation coefficient recorded highest 354.11% in Blessing Lady, which is significantly superior (Fig. 2) over other genotypes. Genotypes Purissima White (314.13%), American Dream (308.47%), Apeldoorn Elite (303.12%) and Purissima Yellow (297.61%) were also found promising with high propagation coefficient. This may be due to enhanced leaf area

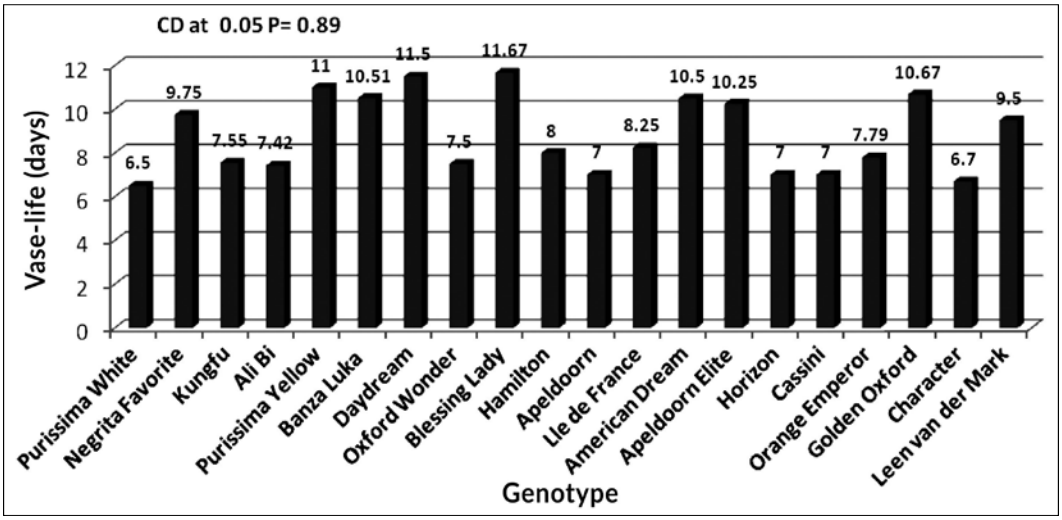


**Fig. 1.** Vase-life of different tulip genotypes.

**Table 4.** Bulb attributes of different tulip genotypes under Kashmir agro-climatic conditions.

| Genotype | Bulb No./ plant | Bulb size (cm) | Bulb wt. (g) | No. of daughter bulbs/ plant | Daughter bulbs wt./ plant (g) | Total bulb yield (t/ha) |
|---|---|---|---|---|---|---|
| Purissima White | 2.17 | 12.91 | 20.11 | 1.23 | 3.49 | 11.78 |
| Negrita Favorite | 2.67 | 11.17 | 12.82 | 1.00 | 3.11 | 9.33 |
| Kungfu | 2.00 | 11.05 | 14.11 | 1.21 | 3.07 | 7.82 |
| Ali Bi | 2.50 | 11.10 | 12.17 | 1.37 | 3.25 | 8.41 |
| Purissima Yellow | 2.80 | 12.36 | 17.50 | 2.13 | 4.57 | 13.39 |
| Banja Luka | 2.01 | 12.15 | 19.23 | 1.12 | 3.62 | 10.56 |
| Daydream | 2.60 | 11.87 | 18.00 | 2.25 | 5.67 | 13.11 |
| Oxford Wonder | 2.30 | 13.07 | 20.20 | 2.37 | 6.12 | 13.14 |
| Blessing Lady | 3.10 | 12.27 | 18.23 | 2.49 | 7.23 | 15.93 |
| Hamilton | 1.97 | 9.23 | 9.22 | 1.67 | 3.12 | 5.32 |
| Apeldoorn | 2.17 | 11.67 | 15.12 | 1.96 | 3.65 | 8.61 |
| Lle de France | 2.21 | 10.11 | 12.34 | 1.12 | 3.11 | 7.59 |
| American Dream | 2.72 | 11.27 | 17.33 | 2.17 | 6.17 | 13.11 |
| Apeldoorn Elite | 2.60 | 12.67 | 16.26 | 2.50 | 6.23 | 12.12 |
| Horizon | 1.50 | 9.02 | 10.23 | 1.62 | 3.14 | 4.62 |
| Cassini | 2.00 | 8.12 | 11.14 | 1.12 | 3.37 | 6.41 |
| Orange Emperor | 1.85 | 9.87 | 13.18 | 1.53 | 4.12 | 7.12 |
| Golden Oxford | 2.33 | 12.17 | 13.17 | 2.17 | 6.71 | 9.34 |
| Character | 1.67 | 10.33 | 10.11 | 1.54 | 3.13 | 5.00 |
| Leen van der Mark | 2.34 | 9.67 | 11.25 | 1.67 | 4.17 | 7.62 |
| CD at 5% | 0.40 | 1.57 | 2.93 | 0.48 | 0.78 | 1.24 |

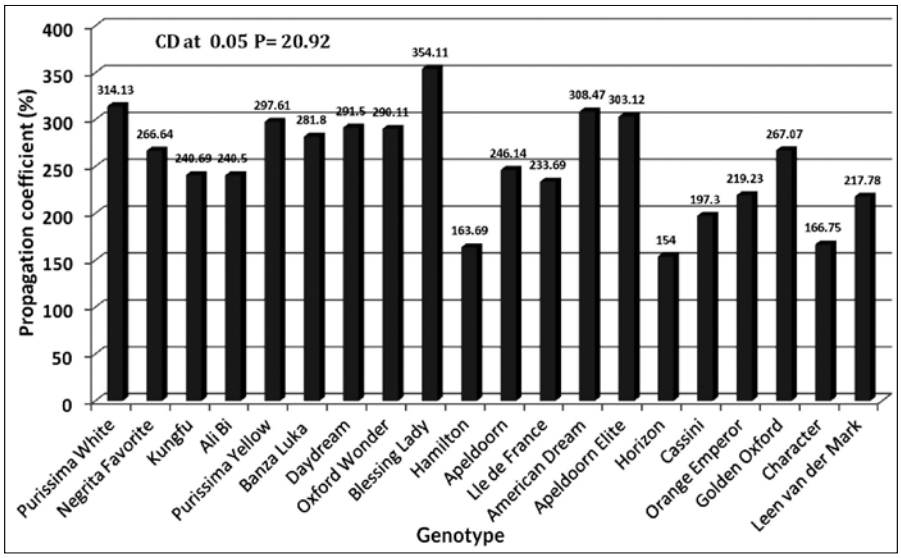


**Fig. 2.** Propagation coefficient of tulip genotypes.

and extended field life. It was recorded lowest in genotypes Horizon (154.00%), Hamilton (163.69%), Character (166.75%) and Cassini (197.30%) owing to low bulb multiplication rate.

Tulip is gaining popularity among the flower growers in Kashmir Valley because of increasing demand of its cut flowers and bulbs owing to highly attractive, dazzling flowers of uniform shape and size

with huge variation in colour and form. Its cultivation is a lucrative enterprise and flowers are sold at Rs. 20 to 30 per cut stem, while bulbs fetch Rs. 10 to 20 per bulb both in national and international markets. Based on performance genotypes Blessing Lady, Daydream, Purissima Yellow, American Dream, Apeldoorn Elite and Golden Oxford were found promising and can be utilized commercially for quality cut flower and bulb production.

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# Influence of packaging material along with wet refrigerated storage conditions on post-harvest life of cut chrysanthemum cv. Reagan White

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## ABSTRACT

**An experiment was carried out to investigate the effect of different packaging films comprising of LDPE (100 and 200 gauge), polypropylene, polyethylene, micro-perforated polyethylene and cellophane bags on low temperature storage of cut chrysanthemum flowers cv. Reagan White. The results obtained showed that the LDPE was most effective film in enhancing the vase-life and quality under both refrigerated wet storage and normal ambient conditions. The LDPE 100 gauge packed flower stems resulted in enhanced vase-life compared to other treatments by maintaining flower and leaf membrane stability index coupled with lower physiological loss in weight. The percent decline in total soluble sugars and reducing sugar contents in leaf and ray floret tissues was less in LDPE packaged flowers as compared to other treatments and control. The wrapped flowers stems in refrigerated wet storage after 15 days at 4°C were held in vase at ambient conditions. The maximum vase-life (12.67 days) was found with LDPE 100 gauge packaged flower stems. The higher $CO_2$ and lower $O_2$ concentrations were recorded in LDPE packed flower stems, which were attributed to prevention of oxidative stress. There was marked favourable biochemical changes in the cut flower tissue, *viz.* total soluble sugars, reducing sugar, leaf chlorophyll content, MDA content, and superoxide dismutase activity in vase under ambient conditions.**

**Key words**: Chrysanthemum, biochemical changes, packaging materials, refrigerated wet storage, vase-life.

## INTRODUCTION

Floriculture is an emerging and fast expanding market in India. In 2013-14, flowers are grown in area of 2.55 lakh ha, producing 17.54 lakh tonnes of loose flowers and 5.42 lakh tonnes of cut flowers. This large production of flowers at the same time brings glut in market, as flower is highly perishable commodity. Farmers are not getting even the actual cost of production. Sometimes, it becomes unacceptable by consumers due to its rapid deterioration in quality causing huge losses to growers. These losses to farmers or retailer can be avoided by using effective use of either of preservatives, packaging materials, storage at low temperature *etc*. The vase-life of cut flowers is highly related with the turgidity of petals and stem cells. The turgidity of flowers is dependent on the balance between continuous water utilization and its supply. Total soluble sugars and reducing sugar maintain turgidity of flowers as they are the source of carbohydrate that improves quality and facilitates of slow opening of buds. Chrysanthemum occupies the third rank in term of production after rose and marigold. Spray chrysanthemums are more popular and traded than standard. These cut flowers are normally packaged in different wrapping materials and transported. Packaging of produce in polyfilms at low temperature creates modified atmospheric conditions having high $CO_2$ and less $O_2$. Storage of chrysanthemum cut stems in different packaging materials showed increase in vase-life, limited fresh weight loss, increased carotenoids content and slow increase in malondialdehyde content and others super radicals (Datta *et al*., 3). The use of refrigerated wet storage of flowers reduces water loss, decreases in the metabolic activity of stems and bacterial growth in vase, thus improves the shelf-life for longer duration (Nowak and Rudnick, 9). The present investigation was undertaken to standardize the best packaging materials suited for refrigerated wet storage of chrysanthemum to manage glut situations.

## MATERIALS AND METHODS

Experiments were carried out in the Research Farm of the Directorate of Floricultural Research, IARI, New Delhi, during 2013-2014. Chrysanthemum cv. Reagan White flowers were harvested at maturity and with stem length of 50 cm. The cut spray were packaged in different packaging materials, *i.e*. $T_1$ = LDPE 200 gauge, $T_2$ = LDPE 100 gauge, $T_3$ = Polypropylene, $T_4$ = Polyethylene, $T_5$ = Microperforated polyethylene, and $T_6$ = Cellophane. The flowers without packaging were taken as control ($T_0$). The five flower stems were wrapped in above mentioned materials and control were transferred in flask containing double-distilled water (500 ml) and kept at 4°C. The day of transferring

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flower stem in double-distilled water, wrapped with respective packaging materials was considered as zero day. Five stems in three replications for seven different treatments were conducted. The experiment was conducted at temp 4°-6°C and relative humidity of 65%, PPF 16/8 h of 350-400 µmol $m^{-2}s^{-1}$. A separate set of treatment combinations were kept for destructive sample analysis. Observations were recorded on 3rd, 15th, 27th, 39th upto 51st days.

In another experiment, effort was made to examine the vase-life by transferring the wrapped flowers after 15 days of storing at 4°C from refrigerator to normal ambient conditions. Here 15th day was considered as zero day. Flower stem weight was calculated on percentage basis by taking initial minus fresh weight. The water uptake by cut stem was recorded. Membrane stability index of leaf and ray florets was estimated according to Bailey *et al.* (1). The chlorophyll content of leaves were measured by DMSO method (Hiscox and Israelstam, 6), tissue total soluble solids (TSS) and reducing sugar of floret and leaf were estimated by Nelson's arsenomolydate method. Superoxide dismutase activity was estimated by monitoring the inhibition of photochemical reduction of nitro-blue tetrazolium as described by Bayer and Fridovich (2). Lipid peroxidation was determined by the thiobarbituric acid reaction as described by Heath and Packer (7). The termination of vase-life was determined as that time when pollens busted from outer part of disc florets or flower showing. The oxygen and carbon dioxide concentration in packaging material was measured using gas sensor till the symptoms of floret wilting appeared. The experiment was conducted in factorial randomized block design having seven treatments including control with single flower stem as one unit and three replications using statistical analysis system software.

## RESULTS AND DISCUSSION

It was evident that all the six packaging materials extended the vase-life of chrysanthemum flower stems as compared to control. The effect of different packaging materials on membrane stability of ray floret and leaf tissue significantly differed as compared to control in refrigerated storage conditions. Amongst the treatment $T_2$ (71.04) recorded the maximum MSI of ray florets followed significantly by $T_1$ (64.70) and $T_6$ (55.16) but treatments $T_3$, $T_4$ and $T_5$ did not differ significantly differed as compared to control. MSI of leaf was also non-significantly differed in $T_1$ (67.83) and $T_2$ (69.23) but significantly differed with other treatments and control. The MSI of leaf was lower to ray-florets. Among the duration, the maximum MSI was recorded at initials days of storage and afterward it was declined slowly during storage but significantly differed with other treatments (Table 1).

There was a significant slight increase in physiological fresh weight of flower stems in all treatments and control with respect to days of increasing storage period. Among the treatments, there was 20 and 13.81% change in fresh weight in $T_1$ and $T_2$ as compared to control. There was continuous increase in the fresh weight in $T_2$ and $T_1$ upto 39 days, in T3, T4, T5 and T6 upto 27 days; and control upto

**Table 1.** Effect of packaging materials on the membrane stability index of ray florets and leaf tissues during wet refrigerated storage of chrysanthemum cut-flowers.

| Treatment\ Day | Ray floret | | | | | | Leaf | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 15 | 27 | 39 | 51 | Mean | 3 | 15 | 27 | 39 | 51 | Mean |
| $T_0$ | 84.49 | 65.54 | 44.50 | 29.46 | 17.76 | 48.35 | 84.73 | 58.25 | 35.19 | 25.06 | 15.63 | 43.77 |
| $T_1$ | 83.74 | 74.11 | 63.85 | 57.36 | 44.42 | 64.70 | 84.21 | 77.92 | 70.13 | 60.66 | 46.23 | 67.83 |
| $T_2$ | 84.77 | 81.55 | 67.36 | 60.94 | 60.56 | 71.04 | 83.16 | 74.06 | 68.22 | 63.36 | 57.36 | 69.23 |
| $T_3$ | 73.32 | 65.19 | 51.33 | 46.25 | 25.07 | 52.23 | 74.87 | 70.65 | 55.88 | 41.23 | 23.7 | 53.26 |
| $T_4$ | 72.02 | 64.70 | 53.98 | 46.47 | 27.97 | 53.03 | 72.85 | 50.00 | 35.27 | 30.32 | 24.67 | 42.62 |
| $T_5$ | 77.25 | 68.06 | 56.27 | 45.98 | 21.36 | 53.79 | 75.73 | 42.18 | 35.32 | 30.38 | 23.87 | 41.49 |
| $T_6$ | 76.29 | 68.72 | 60.50 | 49.81 | 20.50 | 55.16 | 76.19 | 51.87 | 37.51 | 30.26 | 24.70 | 44.11 |
| Mean | 78.84 | 69.70 | 56.83 | 48.04 | 31.09 | | 78.82 | 60.71 | 48.22 | 40.18 | 30.89 | |
| CD at 5% | | | | | | | | | | | | |
| Treat. (T) | | | | | | 5.23 | | | | | | 5.78 |
| Dur. (D) | | | | | | 7.58 | | | | | | 7.60 |
| T × D | | | | | | 8.98 | | | | | | 9.12 |

D = Days after treatment

15 days of refrigerated wet storage. Maximum fresh weight was found at 27 days of storage, which declined significantly thereafter (Table 4). Lower reduction in fresh weight could be attributed to low respiration rate at low temperature and packaging materials that maintain high humidity inside it (Varu *et al*., 12).

Our results showed that there was continuous decline in water uptake in all treatments, but maximum uptake was recorded at initial days, which were non-significantly differed among the treatments as well as durations of storage. The leaf total soluble sugars (TSS) were maximum in leaf tissue in $T_2$, which was significantly different from $T_5$, $T_6$ and control but was non-significantly different with other treatments. Among the duration leaf TSS was non-significantly differ upto 15 days of storage afterwards it was significantly differed at 27, 39 and 51 days of storage. Findings showed leaf RS was at par with leaf TSS in all treatments. Whereas, leaf RS was found to significant upto 15 days of refrigerated wet storage afterwards it was non-significantly at respective durations. Sugars (TSS & RS) were found maximum in initial day of storage, which declined progressively. While reducing sugars in leaf was maximum in $T_3$ that was highly significantly compared to control, but was non-significantly different with other treatments (Table 2). It was evident that the TSS and reducing sugar contents in leaf declined with increment of duration. In ray florets, the maximum TSS was estimated in $T_2$ (31.49 mg/g FW) followed by $T_4$ (31.05 mg/g FW), which was non-significantly different with other treatments but significantly differed with control (22.40 mg/g FW). Among the sampling interval in vase, the maximum TSS was observed at 3 day (40.89 mg/g FW) that was significantly higher compared to 27, 39 and 51 days. While maximum reducing sugar was found in $T_2$ (31.49 mg/g FW) followed by $T_4$ (31.05 mg/g FW), which was non-significantly different with control. The results showed among the treatment has not much significant differed in TSS and RS of leaf and ray florets but highly significantly differed with control (Table 3). However, amongst the packaging material, LDPE was found to be having good performances.

In the sub-experiment, some packs flowers wrapped with packaging materials were transferred from refrigerator to normal ambient conditions after 15 days of storage, and the different parameters were recorded. The maximum vase-life (12.67 days) was observed in flowers wrapped with $T_2$ non-significantly followed by $T_1$ (11.23 days) but significantly followed by other treatments as well as control. The maximum water uptake was observed 73.49 ml in $T_2$ LPDE significantly followed by 65.40 ml in $T_1$ LDPE (200 gauge) and 61.16 ml in polypropylene. While, minimum water uptake 19.33 ml was recorded in control with minimum vase-life of 4.24 days. The minimum percentage loss in fresh wt. (5.52%) was recorded in $T_2$ LPDE (100 gauge) significantly followed with other treatments. This is highly correlated with water uptake (Table 5). Similar findings were reported by Roychowdhury (11) in tuberose spikes wrapped with polyethylene (PE), which exhibited least loss in fresh weight (0.69%). The $CO_2$ concentration was found higher inside the packaging and differed significantly with control. The $O_2$ concentration was found lower inside the packaging. The balance of gaseous concentration

**Table 2.** Effect of packaging materials on leaf total soluble sugars and reducing sugar contents in chrysanthemum cut flowers under refrigerated wet storage.

| Treatment\ Day | TSS (mg/ g FW) | | | | | | RS (mg/ g FW) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 15 | 27 | 39 | 51 | Mean | 3 | 15 | 27 | 39 | 51 | Mean |
| $T_0$ | 40.22 | 36.56 | 23.56 | 17.67 | 11.67 | 25.94 | 23.41 | 17.72 | 13.67 | 11.62 | 8.34 | 14.96 |
| $T_1$ | 41.48 | 38.67 | 30.12 | 26.67 | 18.34 | 31.06 | 22.45 | 19.21 | 16.42 | 14.23 | 12.23 | 16.91 |
| $T_2$ | 40.54 | 39.54 | 32.76 | 29.12 | 22.51 | 32.89 | 21.37 | 19.68 | 17.31 | 16.11 | 14.44 | 17.79 |
| $T_3$ | 41.67 | 38.12 | 31.23 | 25.72 | 16.54 | 30.66 | 23.56 | 19.21 | 16.11 | 14.32 | 12.11 | 17.06 |
| $T_4$ | 42.32 | 37.87 | 30.36 | 24.84 | 17.74 | 30.63 | 22.71 | 18.72 | 16.56 | 14.62 | 12.78 | 17.08 |
| $T_5$ | 40.11 | 37.54 | 26.12 | 19.72 | 12.56 | 27.21 | 21.65 | 18.11 | 14.45 | 12.51 | 10.32 | 15.41 |
| $T_6$ | 41.46 | 37.41 | 27.56 | 20.67 | 14.86 | 28.39 | 22.54 | 19.04 | 14.76 | 12.72 | 11.42 | 16.09 |
| Mean | 41.12 | 37.96 | 28.82 | 23.48 | 16.32 | | 22.53 | 18.82 | 15.61 | 13.73 | 11.66 | |
| CD at 5% | | | | | | | | | | | | |
| Treat. (T) | | | | | | 2.89 | | | | | | 1.85 |
| Dur. (D) | | | | | | 5.46 | | | | | | 3.62 |
| T × D | | | | | | 6.07 | | | | | | 4.28 |

**Table 3.** Effect of refrigerated wet storage and packaging materials on ray florets total soluble solids and reducing sugar contents in chrysanthemum.

| Treatment\ Day | Total soluble sugars (mg/g FW) | | | | | | Reducing sugar (mg/g FW) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 15 | 27 | 39 | 51 | Mean | 3 | 15 | 27 | 39 | 51 | Mean |
| $T_0$ | 39.05 | 34.78 | 18.87 | 10.56 | 8.76 | 22.40 | 27.14 | 20.54 | 18.45 | 11.65 | 7.86 | 17.13 |
| $T_1$ | 41.65 | 37.87 | 31.67 | 25.65 | 18.43 | 31.05 | 26.74 | 23.52 | 22.45 | 18.56 | 15.87 | 21.43 |
| $T_2$ | 39.67 | 37.87 | 31.75 | 27.84 | 20.34 | 31.49 | 27.34 | 23.58 | 21.67 | 20.67 | 17.42 | 22.14 |
| $T_3$ | 40.56 | 36.87 | 28.90 | 22.64 | 16.70 | 29.13 | 25.70 | 21.62 | 18.43 | 14.87 | 10.43 | 18.21 |
| $T_4$ | 38.75 | 35.34 | 30.70 | 21.60 | 17.57 | 28.79 | 27.45 | 26.48 | 22.65 | 15.67 | 13.78 | 21.21 |
| $T_5$ | 42.67 | 39.65 | 28.76 | 16.34 | 13.21 | 28.12 | 26.73 | 22.85 | 17.56 | 12.56 | 9.56 | 17.85 |
| $T_6$ | 43.89 | 38.50 | 30.50 | 20.33 | 14.36 | 29.51 | 26.43 | 24.76 | 20.75 | 13.78 | 11.50 | 19.44 |
| Mean | 40.89 | 37.26 | 28.74 | 20.71 | 15.62 | | 26.79 | 23.34 | 20.28 | 15.39 | 12.35 | |
| CD at 5% | | | | | | | | | | | | |
| Treat. (T) | | | | | | 3.31 | | | | | | 2.46 |
| Dur. (D) | | | | | | 5.52 | | | | | | 4.17 |
| T × Dur. | | | | | | 6.51 | | | | | | 4.86 |

**Table 4.** Effect of refrigerated wet storage and packaging materials on stem fresh weight and water uptake in chrysanthemum cv. Reagen White.

| Treatment\ Day | Stem FW (g) | | | | | | Water uptake (ml/ day) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 15 | 27 | 39 | 51 | Mean | 3 | 15 | 27 | 39 | 51 | Mean |
| $T_0$ | 23.63 | 25.05 | 24.67 | 17.47 | 13.03 | 20.77 | 25.13 | 15.53 | 11.34 | 8.54 | 4.49 | 13.01 |
| $T_1$ | 21.35 | 24.44 | 25.24 | 25.59 | 21.60 | 23.64 | 27.00 | 20.2 | 16.2 | 13.14 | 9.60 | 17.23 |
| $T_2$ | 23.33 | 26.33 | 26.56 | 27.10 | 21.53 | 24.97 | 27.86 | 21.13 | 16.27 | 14.54 | 10.66 | 18.09 |
| $T_3$ | 21.58 | 24.36 | 25.04 | 25.09 | 17.20 | 22.65 | 27.06 | 20.00 | 16.73 | 11.00 | 8.47 | 16.65 |
| $T_4$ | 22.30 | 24.49 | 25.13 | 21.90 | 14.67 | 21.69 | 27.82 | 19.93 | 15.45 | 16.11 | 7.34 | 17.33 |
| $T_5$ | 24.64 | 26.48 | 26.65 | 22.40 | 15.13 | 23.06 | 28.46 | 18.94 | 13.74 | 9.20 | 7.60 | 15.58 |
| $T_6$ | 21.80 | 24.51 | 25.28 | 24.83 | 18.13 | 22.91 | 26.74 | 17.94 | 12.87 | 7.74 | 5.87 | 14.23 |
| Mean | 22.66 | 25.09 | 25.51 | 23.48 | 17.33 | | 27.15 | 19.09 | 14.65 | 11.45 | 7.72 | |
| CD at 5% | | | | | | | | | | | | |
| Treat. (T) | | | | | | 2.25 | | | | | | 2.48 |
| Dur. (D) | | | | | | 3.14 | | | | | | 4.84 |
| T × Dur. | | | | | | 4.12 | | | | | | 5.42 |

inside the packaging depends on permeation of packing materials. High concentration of $CO_2$ (5-10%) has been reported to retard senescence, due to its ethylene inhibitory activity and through its effect in maintaining high levels of polyamines in tissues (Philosoph-Hadas *et al*., 10). This $CO_2$-senescence retarding effect on vegetative organs may also affect, in turn, florets' opening, since green leaves and bracts may serve as possible sources for assimilate import to the floret sink during opening after storage (Meir *et al*., 8).

Having higher chlorophyll content of wrapped flowers and control was observed at 0 days, degradation of chlorophyll content was observed in wrapped and open sets with the advancement of senescence. Here slowest degradation of chlorophyll was noticed in LPDE (100 gauge) and LDPE (200 gauge) and it differed significantly differed with other treatments and control for all durations (Fig. 1). The leaf and ray floret total soluble sugars were found continuously decreasing as senescence of flower stem proceeded. The leaf and ray florets, sugar levels differed significantly among the durations (Fig. 2).

Different components of antioxidant enzyme activities were measured at 0, 4, 8 and 12 day interval. The activity of the SOD enzyme was found continuously increasing as senescence proceeds. The

**Table 5.** Effect of packaging materials after 15 days of removal from refrigeration in chrysanthemum cv. Reagan White.

| Treatment\ Day | Vase-life (days) | Total water uptake (ml) | % loss in cut flower FW (g) | Floret tissue $CO_2$ conc. (%) | Floret tissue $O_2$ conc. (%) |
|---|---|---|---|---|---|
| $T_0$ | $4.24^{e} \pm 0.58$ | $19.33^{e} \pm 1.45$ | $37.45^{a} \pm 1.39$ | $0.33^{d} \pm 0.003$ | $78^{a} \pm 0.030$ |
| $T_1$ | $11.37^{ab} \pm 0.67$ | $65.40^{b} \pm 1.66$ | $7.95^{b} \pm 0.46$ | $5.45^{a} \pm 0.145$ | $13.46^{f} \pm 0.392$ |
| $T_2$ | $12.67^{a} \pm 0.34$ | $73.49^{a} \pm 2.68$ | $5.52^{c} \pm 0.184$ | $5.83^{a} \pm 0.050$ | $12.11^{f} \pm 0.322$ |
| $T_3$ | $10.33^{b} \pm 0.38$ | $61.16^{b} \pm 1.51$ | $10.67^{c} \pm 1.02$ | $4.12^{b} \pm 0.308$ | $15.63^{d} \pm 0.119$ |
| $T_4$ | $9.27^{c} \pm 0.42$ | $49.19^{c} \pm 1.89$ | $14.21^{d} \pm 0.92$ | $3.45^{c} \pm 0.160$ | $14.32^{d} \pm 0.103$ |
| $T_5$ | $7.00^{d} \pm 0.54$ | $41.35^{d} \pm 1.44$ | $22.29^{e} \pm 0.88$ | $4.56^{b} \pm 0.081$ | $17.24^{b} \pm 0.154$ |
| $T_6$ | $8.43^{c} \pm 0.57$ | $52.62^{c} \pm 2.32$ | $13.43^{e} \pm 0.60$ | $3.12^{c} \pm 0.132$ | $16.31^{c} \pm 0.201$ |

maximum SOD enzyme activity was found in control as compared to wrapped flowers stem, which may be due to the accumulation of superoxide radicals in the floret tissue, caused by storage stress and disturbances in antioxidant balance. The MDA content was found significantly lower in packaged flower stems as compared to control. As day proceeded, the MDA content was found to increase significantly. MDA is a decomposition product of polyunsaturated fatty acid that may be probably due to oxidative stress. Here, a positive relation was found between open and packaged materials in terms of SOD activity and MDA contents (Fig. 3). Earlier, Ezhilmathi *et al*. (4) also reported petal senescence to be associated with increase in hydrolytic enzymes, degradation of macromolecules and an increase in respiratory activity.

The low density polyethylene and other packaging materials had modified the $O_2$ and $CO_2$ levels within the package atmosphere due to its permeation. That resulted in increased $CO_2$ concentration, humidity and slowing down of transpiration. This lead to slow down in respiration rate leading to slow conversion of sugar, which maintains higher turgidity and freshness of flowers and leaf, thus improving the quality of flower

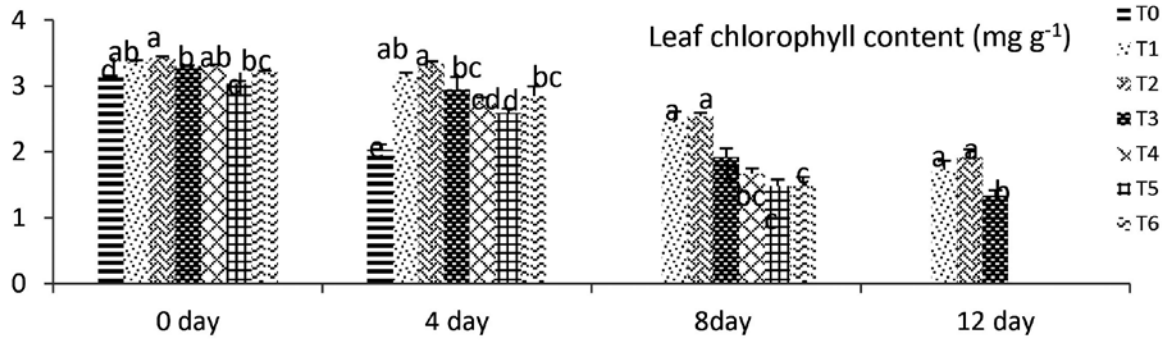


**Fig. 1.** Leaf chlorophyll content in vase after 15 day wet stored cut chrysanthemum flowers.

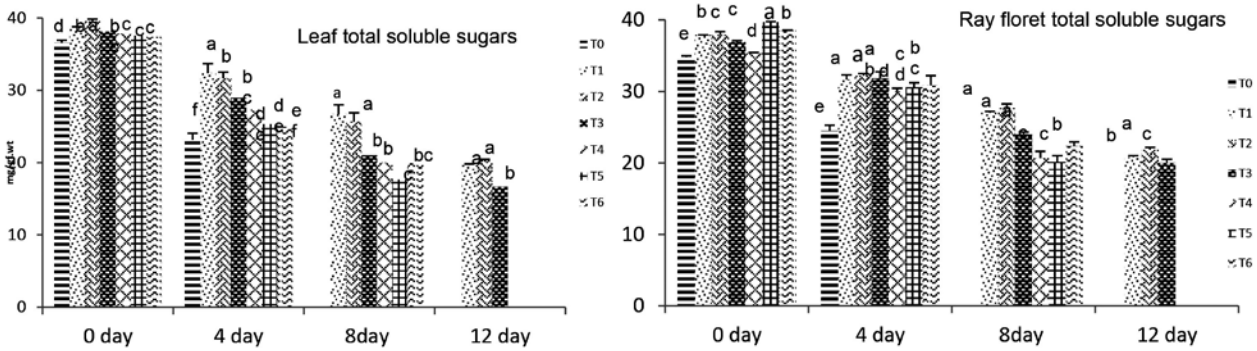


**Fig. 2.** Leaf and ray floret total soluble sugars of the wet refrigerated cut chrysanthemum flowers in vase.

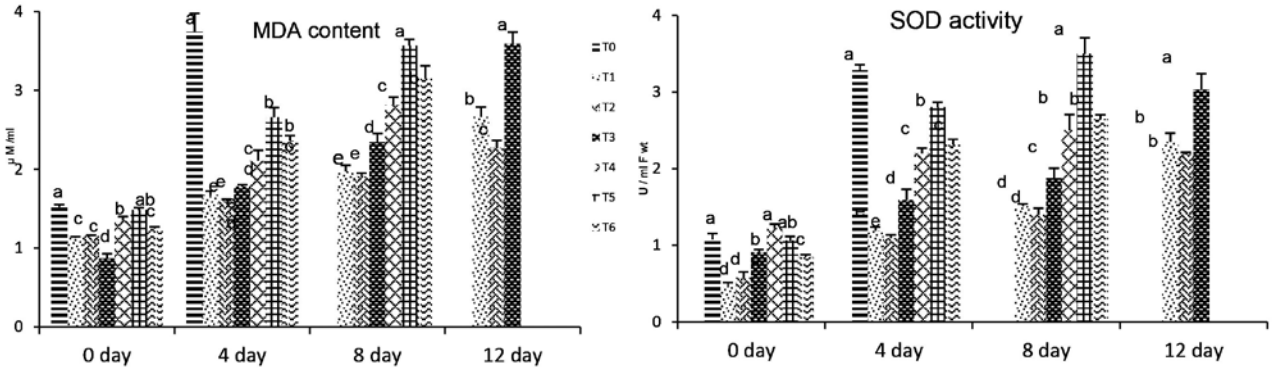


**Fig. 3.** Changes in MDA content and SOD activity of the wet refrigerated cut chrysanthemum flowers in vase.

stems. The polyethylene packaging reduced the permeability to moisture and air, thereby reducing the weight loss probably due to a reduction in moisture loss, respiration and cell division processes. From the study it was concluded that use of LDPE (100 gauge) packaging for chrysanthemum cut flowers in refrigerated wet storage conditions or normal ambient conditions was most effective in prolonging the vase-life and also improving the quality.

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# Multivariate analysis of yield associated traits in *Safed musli* (*Chlorophytum borivilianum*) genotypes under semi-arid conditions

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## ABSTRACT

**The study on the genetic variability, association between traits and direct and indirect effects of different traits on root yield of *safed musli* is required for the development of high yielding varieties. Hence, the interrelationship of 17 quantitative traits (leaf width, leaf length, No. of leaves/ plant, leaf area, No. of capsules/inflorescence, No. of seeds, No. of inflorescences/ tuber, length of inflorescences, size of seeds, No. of flowers/ inflorescence, floral width, No. of tubers, tuber length, tuber girth, fresh weight of tubers) in 52 *safed musli* genotypes of were evaluated at ICAR-DMAPR during 2015 and 2016. Standard deviation and analysis of variance revealed high genetic variation among studied genotypes for all traits in which coefficient of variation ranged from 205.52 (leaf area) to 19.57 (floral width). Based on mean performance DCB-48 (129 g), DCB-17 (110.2 g), DCB-18 (108 g), DCB-5 (107.6 g), DCB- 37 (105 g) were the top five genotypes for fresh tuber yield per plant. Pearson correlation coefficient showed the positive and significant relation of number of tubers per plant (0.83), and tuber length (0.77) with yield (tuber FW). According to path analysis, number of tubers (0.84) possessed the highest positive direct effect followed by leaf width (0.14) and size of seeds (0.11 mm) on dependent variable yield (tuber fresh weight) of *safed musli*. The result of stepwise regression analysis revealed that tuber length and tuber girth has considerable effects on tuber yield.**

**Key words:** Genetic variability, path analysis, *Safed musli*, root yield, trait association.

## INTRODUCTION

*Chlorophytum borivilianum* popular as *Safed musli* is known for aphrodisiac potential with no side effects and prescribe for enhancing male potency and overcoming signs of fatigue (Joshi *et al*., 10). The species originated from the southern part of India belongs to family Liliaceae and reported to be a cross-pollinated with tetraploid chromosome number 2n = 4x = 28 (Geetha and Maiti, 7). Among the 215 species, *C. borivilianum* yields highest steroidal saponins, known as borivilianosides as the main bioactive compounds present in its root (Bordia *et al*., 4). *Safed musli* is distributed in the forest area of tropical and sub-tropical region with altitude of 1500 m and cultivated mainly in Southern Rajasthan, Western Madhya Pradesh, North Gujarat and few parts of Karnataka. At present, the estimated global market demand and production is approximately 35,000 t/annum and 5000 t/annum respectively which fulfill less than 15% of the required demand (Kothari and Singh, 11). Its high economic value and unsustainable collection from the natural habitat has resumed the attention to develop high root yielding varieties with desirable quantity and quality of saponin. Determination of correlation coefficients is an important statistical procedure to evaluate breeding programs for high yield as well as to examine direct and indirect variables contributions to yield (Sadat *et al*., 13).

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## MATERIALS AND METHODS

A total of 52 germplasm accessions (Table 1) of *Safed musli* (Fig. 1) were evaluated in randomized block design with three replication at the experimental farm of ICAR-Directorate of Medicinal and Aromatic Plant Research, Anand, Gujarat for two years 2015 and 2016. The experimental field was located at 19°35 north, longitude 40°51 east altitude 1,000 m above the sea level, soil with sandy loam texture and an average annual precipitation greater than 174 mm. Fasciculated roots of *Safed musli* were planted in last week of June, 2015-16 on ridges of 15-20 cm height in single row plot of 4 m length, keeping row to row and plant to plant spacing of 45 and 30 cm, respectively. Crop management undertaken to maintain a healthy crop.

Data were collected on 17 traits (quantitative and qualitative) in all replications on 10 randomly selected normal plants per plot. The two year data (2015 & 2016) were combined and simple phenotypic correlation coefficient among all observed components. Correlation coefficients between traits were computed based on Pearson`s method and later separated into direct and indirect effects *via* path

**Table 1.** Studied *Safed musli* genotypes and their place of collection.

| Sl. No. | Genotype | Place of collection | Sl. No. | Genotype | Place of collection |
|---|---|---|---|---|---|
| 1. | DCB 1 | Anand, Gujarat | 27. | DCB 27 | Rajasthan |
| 2. | DCB 2 | Anand, Gujarat | 28. | DCB 28 | Rajasthan |
| 3. | DCB 3 | Valsad, Gujarat | 29. | DCB 29 | Rajasthan |
| 4. | DCB 4 | Vasidanta Gujarat | 30. | DCB 30 | Rajasthan |
| 5. | DCB 5 | Akola, Maharashtra | 31. | DCB 31 | Rajasthan |
| 6. | DCB 6 | Rajasthan | 32. | DCB 32 | Akola, Maharashtra |
| 7. | DCB 7 | Anand, Gujarat | 33. | DCB 33 | Akola, Maharashtra |
| 8. | DCB 8 | Dang, Gujarat | 34. | DCB 34 | Akola, Maharashtra |
| 9. | DCB 9 | Dang, Gujarat | 35. | DCB 35 | Akola, Maharashtra |
| 10. | DCB 10 | Jabalpur, MP | 36. | DCB 36 | Akola, Maharashtra |
| 11. | DCB 11 | Jabalpur, MP | 37. | DCB 37 | Akola, Maharashtra |
| 12 | DCB 12 | Mandsaur, MP | 38. | DCB 38 | Akola, Maharashtra |
| 13. | DCB 13 | Mandsaur, MP | 39. | DCB 39 | Akola, Maharashtra |
| 14. | DCB 14 | Mandsaur, MP | 40. | DCB 40 | Akola, Maharashtra |
| 15. | DCB 15 | Anand, Gujarat | 41. | DCB 41 | Akola, Maharashtra |
| 16. | DCB 16 | Anand, Gujarat | 42. | DCB 42 | Akola, Maharashtra |
| 17. | DCB 17 | Anand, Gujarat | 43. | DCB 43 | Akola, Maharashtra |
| 18. | DCB 18 | Dang, Gujarat | 44. | DCB 44 | Akola, Maharashtra |
| 19. | DCB 19 | Dang, Gujarat | 45. | DCB 45 | Akola, Maharashtra |
| 20. | DCB 20 | Mandsaur, MP | 46. | DCB 46 | Akola, Maharashtra |
| 21. | DCB 21 | Mandsaur, MP | 47. | DCB 47 | Anand, Gujarat |
| 22. | DCB 22 | Mandsaur, MP | 48. | DCB 48 | Anand, Gujarat |
| 23. | DCB 23 | Mandsaur, MP | 49. | DCB 49 | Anand, Gujarat |
| 24. | DCB 24 | Mandsaur, MP | 50. | DCB 50 | Mandsaur, MP |
| 25. | DCB 25 | Mandsaur, MP | 51. | DCB 51 | Mandsaur, MP |
| 26. | DCB 26 | Mandsaur, MP | 52. | DCB 52 | Mandsaur, MP |

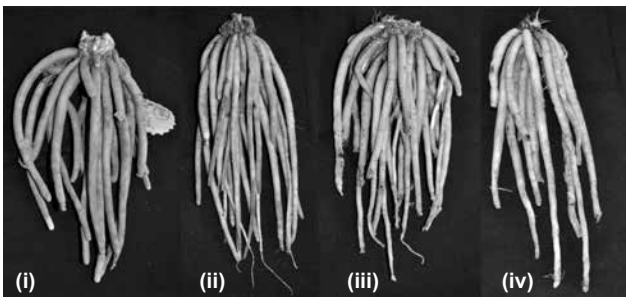


**Fig. 1.** Variation in root length, number of fingers and girth in studied genotypes. (i) DCB-48; (ii) DCB-35; (iii) DCB-7; and (iv) DCB-26.

coefficient analysis based on the procedure of Ahmed *et al*. (1) for determination of the direct and indirect effects of the traits on yield of tubers. Stepwise multiple regression analysis was carried out using SAS version 9.3 statistical programme by assessing the cumulative effect of yield components on tubers yield, taking number of tubers per plant as the dependent variable and other traits as independent variables. Biplot graphical display was performed based on principal component analysis in order to identify best performing germplasm and a cluster was used for classification of variable genotypes.

## RESULTS AND DISCUSSION

The variability prevalent among the germplasm lines of *C. borivilianum* has been well described by several authors (Jat, 8; Bordia *et al.*, 4; Jat and Sharma, 9; Kothari and Singh, 11; Geetha and Maiti, 6; Bhagat and Jadeja, 2). In Tables 2-4, correlation analysis showed that the root yield per plant have positive and highly significant correlation with leaf width (0.17), leaf length (0.36), number of fingers

**Table 2.** Analysis of descriptive statistics of evaluated traits in 52 genotypes of *Safed musli.*

| Trait | Range | Mean ± SE (m) | SD | CV | Student`s test |
|---|---|---|---|---|---|
| Leaf width (cm) | 0.08 - 1.84 | 1.11 ± 0.04 | 0.33 | 29.76 | 24.22 |
| Leaf length (cm) | 2.12 - 21.62 | 13.38 ± 0.56 | 4.04 | 30.25 | 23.83 |
| No. of leaves/ plant | 1.40 - 8.20 | 5.66 ± 0.22 | 1.62 | 28.72 | 25.10 |
| Leaf area ($m^2$) | 37.49 - 2284.38 | 147.44 ± 42.02 | 303.03 | 205.52 | 3.50 |
| No. of capsules/ inflorescence | 0.0 - 21.00 | 7.65 ± 0.69 | 5.01 | 65.54 | 11.00 |
| No. of seed/ capsules | 0.0 - 11.00 | 3.71 ± 0.38 | 2.79 | 75.13 | 9.59 |
| No. of inflorescence/ tuber | 0.40 - 4.40 | 1.95 ± 0.14 | 1.04 | 53.34 | 13.51 |
| Inflorescence length (cm) | 2.14 - 37.04 | 23.46 ± 1.20 | 8.68 | 37.01 | 19.48 |
| Size of capsule (mm) | 0.0 - 6.47 | 3.25 ± 0.27 | 1.97 | 60.69 | 11.88 |
| Length of flower spikes (cm) | 1.08 - 18.00 | 9.18 ± 0.50 | 3.61 | 39.30 | 18.34 |
| Size of seeds (mm) | 0.0 - 2.76 | 1.38 ± 0.11 | 0.79 | 57.29 | 12.58 |
| No. of flowers/ inflorescence | 3.20 - 29.80 | 14.83 ± 0.69 | 5.01 | 33.76 | 21.35 |
| Floral width (cm) | 0.64 - 3.28 | 2.54 ± 0.06 | 0.49 | 19.57 | 36.84 |
| No. of tubers | 6.20 - 69.40 | 26.7 ± 1.98 | 14.29 | 53.52 | 13.47 |
| Tuber length (cm) | 3.48 - 18.71 | 12.88 ± 0.46 | 3.32 | 25.83 | 27.90 |
| Tuber girth (mm) | 1.26 - 7.27 | 5.65 ± 0.16 | 1.16 | 20.60 | 34.99 |
| Tuber fresh weight (g) | 9.60 - 110.20 | 51.91 ± 3.83 | 27.67 | 53.31 | 13.52 |

SD = Standard deviation; CV = Coefficient of variation

per root (0.83) and root girth (0.77). Kumar *et al*. (12) also reported that increase of leaf length, and width is a sign of positive correlation with root yield as the spreading of canopy provide large photosynthetic efficiency to plant. Plant population had positive and significant correlation with fresh root yield (Chandra *et al*., 5). The negative correlation of number of capsules per plant (e) and number of seeds per capsules (f) (r = -0.04, -0.03, respectively) showed that these two variables (e) and (f) associated with a decrease in fresh root yield (q). A negative correlation demonstrates a connection between two variables in the same way a positive correlation coefficient does, and the relative strengths are the same. The reason for low negative value of these variables probably due to the nature of cross-pollination with vegetative propagation of crop as well as poor seed germination showed no meaningful relationship between variables and yield, may lead to some undesirable selection based on these characters. To improve the yield components that have negative association with one another, suitable recombinants may be obtained through biparental mating, mutation breeding or diallel selective mating by breaking undesirable linkages.

Path and regression analysis with standardized variables determined relationships among the traits and the relative importance of their direct and indirect effects on yield, and the correlation coefficients to be segregated to the direct and indirect effects (Bhatt, 3). The highest positive direct effects on grain yield per plant were exhibited by number of fingers per plant (0.84) followed by length of inflorescence (0.18) and leaf width (0.14), while leaf length, number of capsules per inflorescence, size of capsules, length of flower spike, floral width, had negative but non-significant direct effects on fresh weight of roots (yield) with a value of -0.19, -0.04, -0.08, -0.15 and -0.11, respectively (Table 5). Highest positive indirect effects on yield were observed for root length (0.45) and root girth (0.33) and these traits caused increasing of root yield indirectly. High values of indirect effects *via* tuber length and tuber girth suggested that indirect selection for root girth may also increase the yield of roots (Table 3). Biplot display based on the plot of Principle component 2 on Principle component 1

**Table 3.** Result of stepwise regression analysis of studied traits for *Safed musli* yield.

| Variable | CV | R-square | Adj R-square | MSE | F value |
|---|---|---|---|---|---|
| o | 20.505 | 0.7856 | 0.6847 | 6.973 | 7.79 |
| p | 24.713 | 0.9790 | 0.9692 | 2.9791 | 99.24 |
| q | 26.237 | 0.894 | 0.848 | 150.07 | 19.68 |

**Table 4.** Pearson correlation coefficients between fresh root weight and other related traits in *safed musli.*

| Trait | a | b | c | d | e | f | g | h | i | j | k | l | m | n | o | p | q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 1.00 | | | | | | | | | | | | | | | | |
| b | 0.52* | 1.00 | | | | | | | | | | | | | | | |
| c | -0.14 | -0.12 | 1.00 | | | | | | | | | | | | | | |
| d | 0.04 | 0.01 | -0.01 | 1.00 | | | | | | | | | | | | | |
| e | 0.33 | 0.29 | -0.04 | 0.20 | 1.00 | | | | | | | | | | | | |
| f | 0.32 | 0.27 | -0.03 | 0.08 | 0.61* | 1.00 | | | | | | | | | | | |
| g | 0.40 | 0.20 | -0.04 | -0.06 | 0.27 | 0.56* | 1.00 | | | | | | | | | | |
| h | 0.52* | 0.41 | -0.07 | 0.10 | 0.58* | 0.64* | 0.63* | 1.00 | | | | | | | | | |
| i | 0.40 | 0.33 | -0.03 | 0.14 | 0.67* | 0.87* | 0.67* | 0.67* | 1.00 | | | | | | | | |
| j | 0.44 | 0.33 | -0.08 | 0.15 | 0.63* | 0.50 | 0.55* | 0.83* | 0.63* | 1.00 | | | | | | | |
| k | 0.35 | 0.35 | -0.04 | 0.10 | 0.67* | 0.79* | 0.63* | 0.64* | 0.96* | 0.63* | 1.00 | | | | | | |
| l | 0.30 | 0.08 | -0.12 | 0.03 | 0.20 | 0.2 | 0.38 | 0.51* | 0.23 | 0.55* | 0.30 | 1.00 | | | | | |
| m | -0.22 | -0.26 | 0.96* | -0.01 | -0.02 | -0.02 | -0.04 | -0.05 | -0.02 | -0.04 | -0.02 | -0.05 | 1.00 | | | | |
| n | 0.01 | 0.23 | -0.10 | -0.14 | -0.09 | -0.01 | 0.01 | -0.09 | -0.01 | -0.03 | 0.03 | -0.14 | -0.14 | 1.00 | | | |
| o | 0.37 | 0.54* | -0.09 | 0.07 | 0.19 | 0.33 | 0.37 | 0.35 | 0.38 | 0.37 | 0.38 | 0.19 | -0.15 | 0.56* | 1.00 | | |
| p | -0.24 | -0.24 | 0.97* | -0.02 | -0.08 | -0.05 | -0.09 | -0.15 | -0.07 | -0.14 | -0.07 | -0.15 | 0.97* | -0.07 | -0.10 | 1.00 | |
| q | 0.17 | 0.36 | -0.15 | -0.08 | 0.10 | 0.25 | 0.30 | 0.22 | 0.32 | 0.21 | 0.35 | 0.04 | -0.18 | 0.83* | 0.77* | -0.14 | 1.00 |

*Significant at P< 0.01. (a) Leaf width (cm), (b) Leaf length (cm), (c) No. of leaves/plant, (d) Leaf area (cm²), (e) No. of capsules/ inflorescence, (f) No. of seeds/capsule, (g) No. of inflorescences/plant, (h) Length of inflorescence (cm), (i) Size of capsule (mm), (j) Length of flower spike (cm), (k)Size of seed (mm), (l) No. of flowers/inflorescence, (m) Floral width (cm), (n) No. of tubers, (o)Tuber length (cm), ( p) Tuber girth (cm) and (q) Fresh weight of tubers (gm).

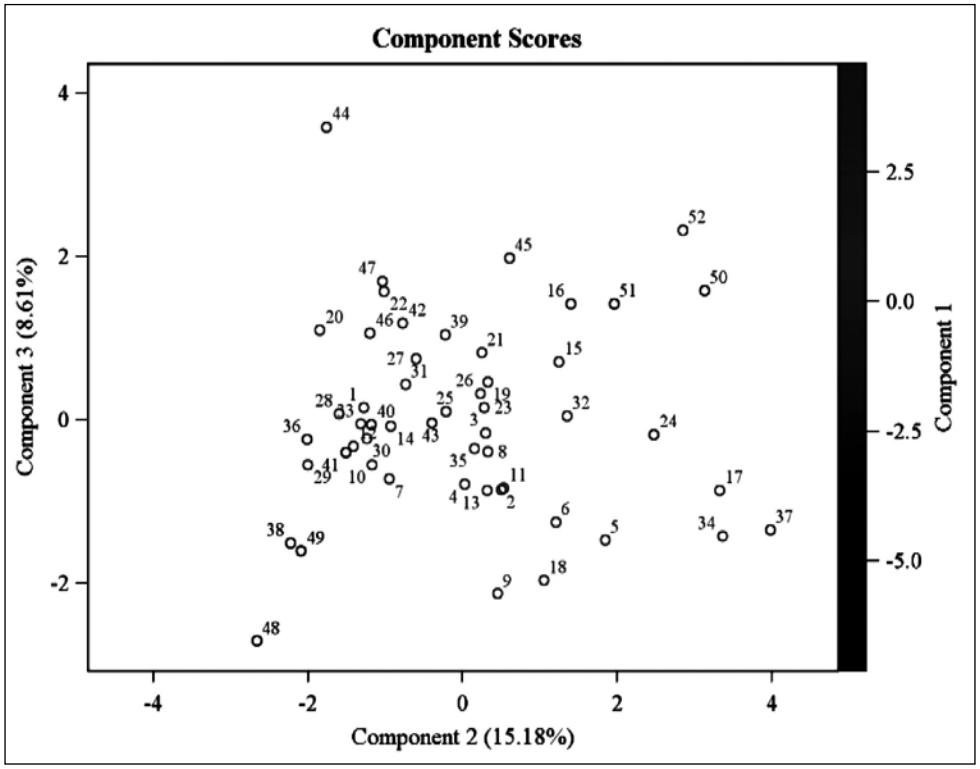


**Fig. 2.** Biplot display of 52 *Safed musli* genotypes based on principal component analysis.

**Table 5.** Direct and indirect effects of morphological traits and yield components on tubers yields using path analysis.

| Trait | a | b | c | d | e | f | g | h | i | j | k | l | m | n | o | p | Genotypic corr. with q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 0.14* | -0.14 | 0.01 | -0.27 | -0.02 | 0.01 | 0.04 | 0.11 | -0.05 | -0.08 | 0.06 | 0.01 | -0.06 | 0.19 | 0.04 | 0.04 | 0.35 |
| b | 0.10 | -0.19 | 0.01 | -0.19 | -0.01 | 0.08 | 0.02 | 0.08 | -0.02 | -0.05 | 0.03 | 0.05 | -0.04 | 0.29 | 0.02 | 0.03 | 0.3 |
| c | 0.09 | -0.14 | 0.01 | -0.14 | -0.01 | 0.01 | 0.04 | 0.09 | -0.03 | -0.07 | 0.05 | 0.02 | -0.04 | 0.19 | 0.03 | 0.03 | 0.32 |
| d | 0.01 | 0.02 | -0.21 | 0.02 | -0.07 | -0.016 | -0.08 | 0.02 | -0.44 | -0.08 | -0.05 | -0.01 | -0.01 | -0.12 | 0.02 | 0.01 | -0.09 |
| e | 0.06 | -0.04 | -0.92 | -0.03 | -0.04 | 0.02 | 0.02 | 0.13 | -0.06 | -0.11 | 0.08 | 0.03 | -0.06 | -0.05 | 0.01 | 0.02 | 0.08 |
| f | 0.06 | -0.05 | -0.12 | -0.12 | -0.03 | 0.03 | 0.03 | 0.13 | -0.07 | -0.1 | 0.1 | 0.02 | -0.05 | 0.12 | 0.02 | 0.02 | 0.3 |
| g | 0.07 | -0.04 | -0.2 | -0.23 | -0.01 | 0.01 | 0.08 | 0.12 | -0.05 | -0.08 | 0.07 | 0.03 | -0.04 | 0.18 | 0.03 | 0.02 | 0.46 |
| h | 0.08 | -0.08 | -0.18 | -0.27 | -0.03 | 0.02 | 0.05 | 0.18 | -0.06 | -0.13 | 0.09 | 0.04 | -0.08 | 0.1 | 0.03 | 0.03 | 0.32 |
| i | 0.08 | -0.06 | -0.17 | -0.01 | -0.03 | 0.02 | 0.05 | 0.14 | -0.08 | -0.11 | 0.11 | 0.03 | -0.06 | 0.15 | 0.03 | 0.03 | 0.4 |
| j | 0.07 | -0.07 | -0.16 | 0.02 | -0.03 | 0.02 | 0.04 | 0.16 | -0.06 | -0.15 | 0.08 | 0.04 | -0.08 | 0.06 | 0.02 | 0.03 | 0.22 |
| k | 0.08 | -0.06 | -0.16 | -0.01 | -0.03 | 0.02 | 0.05 | 0.14 | -0.08 | -0.11 | 0.11 | 0.03 | -0.06 | 0.13 | 0.03 | 0.03 | 0.38 |
| l | 0.02 | -0.01 | -0.78 | -0.52 | -0.02 | 0.01 | 0.04 | 0.1 | -0.03 | -0.08 | 0.05 | 0.08 | -0.05 | -0.03 | 0.01 | 0.01 | 0.12 |
| m | 0.07 | -0.07 | -0.14 | 0.04 | -0.02 | 0.01 | 0.03 | 0.13 | -0.04 | -0.1 | 0.06 | 0.03 | -0.11 | 0.12 | 0.02 | 0.04 | 0.24 |
| n | 0.03 | -0.06 | -0.86 | -0.03 | 0.03 | 0.04 | 0.02 | 0.02 | -0.01 | -0.01 | 0.01 | -0.03 | -0.02 | 0.84* | 0.036 | 0.02 | 0.92 |
| o | 0.08 | -0.08 | -0.2 | -0.09 | -0.01 | 0.02 | 0.04 | 0.08 | -0.03 | -0.04 | 0.05 | 0.09 | -0.04 | 0.45 | 0.07 | 0.04 | 0.67 |
| p | 0.08 | -0.1 | -0.21 | -0.04 | -0.01 | 0.01 | 0.03 | 0.1 | -0.03 | -0.07 | 0.05 | 0.02 | -0.07 | 0.33 | 0.04 | 0.06 | 0.5 |

*Significant at P<0.01. (a) Leaf width (cm), (b) Leaf length (cm), (c) No. of leaves/ plant, (d) Leaf area (cm²), (e) No. of capsules/ inflorescence, (f) No. of seeds/ capsule, (g) No. of inflorescences/ plant, (h) Length of inflorescence (cm), (i) Size of capsule (mm), (j) Length of flower spike (cm), (k) Size of seed (mm), (l) No. of flowers/ inflorescence, (m) Floral width (cm), (n) No. of tubers, (o) Tuber length (cm), ( p) Tuber girth (cm) and (q) Fresh weight of tubers (g).

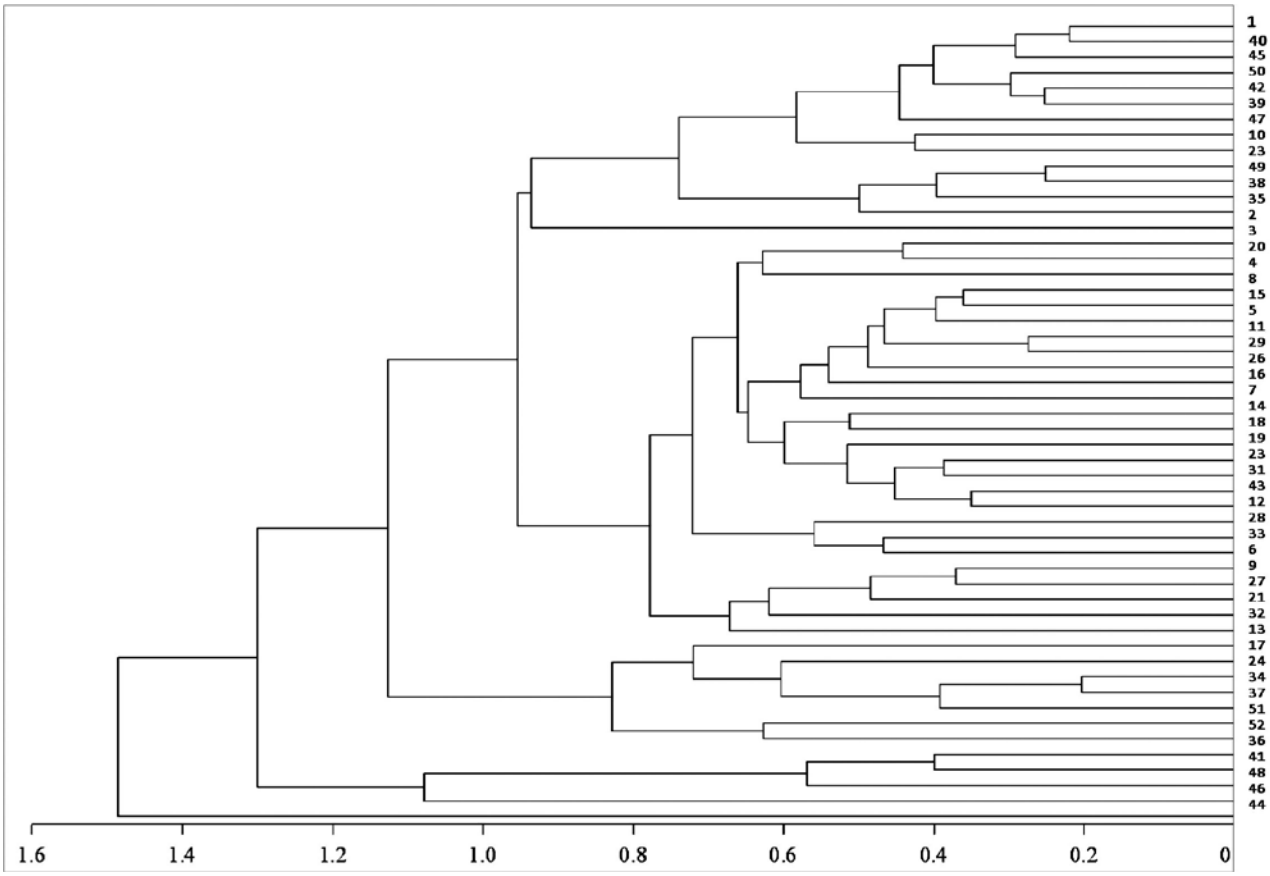


**Fig. 3.** Phenotypic-based cluster analysis to classify variables based on Ward`s method.

classified the genotypes in four groups (Fig. 2 & 3) that one of them comprises genotypes having the more number of roots and girth ability for fresh root yield production (DCB- 44, 45, 52, 50, 51, 47, 42, 26, 24, 32, 39, and 42). These genotypes were selected as the suitable population for breeding programmes and improvement of important traits.

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# Effect of edible surface coatings on the storability of pear fruits

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## ABSTRACT

**Punjab Beauty is leading variety of semi-soft pear and commercially grown in Punjab. Fruits mature in third week of July, when temperature and humidity are very high, which reduces the shelf-life. An experiment was conducted to extend the post-harvest life of pear fruits using surface coatings such as carboxymethyl cellulose (CMC) @ 0.25%, *Aloe vera* gel (AVG) @ 0.25% and chitosan @ 0.25%. The control fruits were kept uncoated. Coated and control fruits were packed in CFB boxes and kept at 0-1°C and 90-95% relative humidity. Fruits were analysed for various physico-chemical characteristics, *viz*., PLW, palatability, TSS, acidity, total sugars, total phenolics and pectin methyl esterase activity after 30, 45, 60, 67 and 74 days of storage. Results revealed that all edible coatings had significant effect on quality parameters of fruits during storage period. After 67 days of storage, minimum PLW (5.16%) and highest palatability rating (7.10), total sugars (8.67%), total phenolics (60.3 mg/100 g FW) and PME activity (1.60 ml of 0.02 N NaOH) were recorded in carboxymethyl cellulose (CMC) @ 0.25% coated pear fruits. However, after 74 days of storage, fruits from all the treatments were of unacceptable quality. Carboxymethyl cellulose @ 0.25% was found to be the best coating to extend the post-harvest life of pear fruits up to 67 days under cold storage conditions.**

**Key words:** Pear, storage, surface coatings, fruit quality, post-harvest.

## INTRODUCTION

Low chill varieties of pear are being cultivated successfully under sub-tropics of northern India. In Punjab, several semi-soft varieties of pear have been recommended for cultivation, but Punjab Beauty is most popular among the growers. Fruits of this variety mature in the third week of July, when the temperature and humidity are very high, which reduces the shelf-life of fruits. Modified atmosphere packaging has been used in the recent past to extend the storage life of fruits (Nath *et al*., 8). However, non-biodegradable nature of polyethylene films and even high carbon dioxide injury, ethanol production and off-flavour development due to anaerobic respiration, poses several problems. Keeping this in view, fruit coatings can be considered as eco-friendly alternate to the polyethylene films. Semi-permeable coatings can create a modified atmosphere similar to CA storage, which is less expensive. The atmosphere created by various surface coatings depends on the prevailing environmental conditions. Coatings are also used to extend the shelf-life of fruits and to improve appearance. Edible coatings are conventionally used to improve and maintain the fruit appearance due to their eco-friendly nature (Petersen *et al*., 9). Coatings act as barrier to moisture loss and gaseous exchange during handling and storage, retards food deterioration and enhances its safety (Cha and Chinnan, 3). Thus, the present study was conducted to evaluate the efficacy of different coatings for extending the storage life of pear cv. Punjab Beauty under low temperature storage conditions.

## MATERIALS AND METHODS

The present investigation was carried out during 2014 in Post-harvest Laboratory, Department of Fruit Science, PAU, Ludhiana. Physiologically mature fruits of pear cv. Punjab Beauty were harvested from pear orchard at Fruit Research Farm during morning hours. Fruits showing deformities were discarded and only healthy and uniform fruits were selected for the experiment. Fruits were washed, air-dried and subjected to various coatings, *viz*., Carboxymethyl cellulose (CMC) @ 0.25%, *Aloe vera* gel (AVG) @ 0.25%, Chitosan® @ 0.25%. An aqueous solution of *Aloe vera* gel was prepared by diluting 2.5 g *Aloe vera gel* freeze dried powder to 1 l with distilled water and 0.05% Tween 20 as a surfactant. To prepare 1 l solution of chitosan® (0.25%), 2.5 g of chitosan® was dissolved in 900 ml distilled water containing 1% acetic acid, then the pH of the solution was maintained at 5.0 with 2 mol/l NaOH and made upto 1 l. The control fruits were given water dip only. Twenty fruits were taken in each replication of each treatment. After coatings, fruits were dried in air and packed in CFB boxes with paper lining and paper cuttings as cushioning material and were kept at 0-1°C and 90-95% relative

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humidity. The data on physiological loss in weight (PLW), palatability rating, TSS, acidity, total sugars, total phenolics and pectin methyl esterase (PME) activity were recorded after 30, 45, 60, 67 & 74 days of storage. The PLW of fruits was calculated on initial weight basis. The per cent loss in weight after each storage interval was calculated by subtracting final weight from the initial weight of the fruits and then converted into percentage value. Experimental fruits were evaluated for sensory quality (palatability) by a panel of five judges. A nine point 'Hedonic scale' was used for its inference (Amerine *et al*., 2). Total soluble solids (TSS) were determined with the help of hand refractometer (Erma, Japan) and expressed in per cent. The readings were corrected with the help of temperature correction chart at 20°C temperature. Acidity was determined by titrating 2 ml of strained juice of fruits against 0.1 N NaOH solution using phenolphthalein as an indicator. The titratable acidity was calculated and expressed in terms of percent maleic acid. Total sugars were estimated by following the AOAC (1) method. Phenolics were estimated as total tannins after developing colour with Folin-Denis's reagent (AOAC, 1) method and expressed in mg/100 g fresh weight (FW). For estimation of Pectin methyl esterase activity, enzyme extract was prepared by taking 20 g fruit pulp which was blended in 60-100 ml NaCl solution (0.15 ml), filtered through two layers of cheese cloth, centrifuged at 2,000 rpm for 30 min. at 4°C. The supernatant was used as an enzyme source (Mahadev and Sridhar, 7).

The experiment was laid out in completely randomized block design (Factorial) and data were analyzed for analysis of variance using statistical software SAS 9.3 (SAS Institute Inc., Cary, NC, USA).The means were compared using LSD test at significance level of 0.05.

## RESULTS AND DISCUSSION

Physiological loss in weight of fruit contributes toward the post-harvest losses. The rate of water loss from the fruits affects its post-harvest life. Various fruit coatings showed significant differences in PLW of pear during storage as compared to control (Fig. 1a). PLW increased linearly with the advancement of storage period, but after 74 days maximum loss in weight was recorded, while minimum was recorded after 30 days of storage period. After 67 days of storage, maximum (6.87%) PLW was found in control, while the minimum (5.16%) was found in CMC @ 0.25% treated fruits. Similar results were also reported in pear and peach fruits (Togrul and Arslan, 13). Climacteric mature fruits when detached from tree undergoes a series of metabolic processes, which ultimately results in loss of weight (Wills *et al*., 15).

Palatability rating (PR) depicts the consumer acceptability of the fruit. PR increased gradually up to 45 days of storage, but thereafter a decline was observed in all the treatments (Fig. 1b). Fruits treated with CMC @ 0.25% were found in moderately acceptable condition after 67 days of storage with 7.10 palatability rating score whereas, control fruits registered the minimum (3.25) palatability rating. The retention of high palatability in coated fruits may be due to lower physiological loss in weight, maintenance of higher fruit firmness, TSS and acid content.

Total soluble solids of the stored fruits showed a significant difference with storage time and treatments (Fig. 1c). An increase in TSS was observed with the advancement of storage period in all the treatments but this increase was registered up to 45 and 67 days of storage in control and CMC @ 0.25% treated fruits, respectively. Similar to this increase in TSS with storage period has been reported by Singh and Janes (12). Effective changes in TSS are naturally occurring phenomenon and might be correlated with the hydrolytic changes in starch concentration during post-harvest storage period as reported by Wills *et al*. (14). After 74 days of storage CMC @ 0.25% coated fruits retained the highest (12.70%) level of TSS and lowest (12.3%) was recorded in control. Similarly, the fine coating of sago (10%) increased shelf-life of custard apple fruits with high total soluble solids in zero energy cool chamber (Jholgiker and Reddy, 5).

Acidity of pear fruits experienced a decrease, followed by an increase with the advancement of storage period (Fig. 1d). Decline in acidity was recorded up to 60 days of storage in all the treatments except CMC @ 0.25% treatment where this decrease was recorded up to 67 days. With the utilization of organic acid in pyruvate decarboxylation reaction during the ripening process of fruits a decrease in titratable acids during storage occurs as also suggested by Rhodes *et al.* (11). An increase in acidity at the end of storage indicates the deterioration of fruit quality.

Total sugars of the stored fruits increased during the initial periods of storage, but thereafter a decline in total sugars was recorded in all the treatments (Fig. 2a). After 30 days of storage, highest total sugars were recorded in untreated fruits, but after 45 days of storage a decline was observed. In coated fruits increase in total sugars was observed upto 60 days of storage except CMC @ 0.25% coated fruits, where this increase was observed upto 67 days of storage. Decline in sugar content at the end of storage depicts the deterioration of fruit quality. At the end of storage, fruits coated with CMC @ 0.25% retained the highest (8.54%) total sugars content as compared to other treatments. The increase in sugars during storage

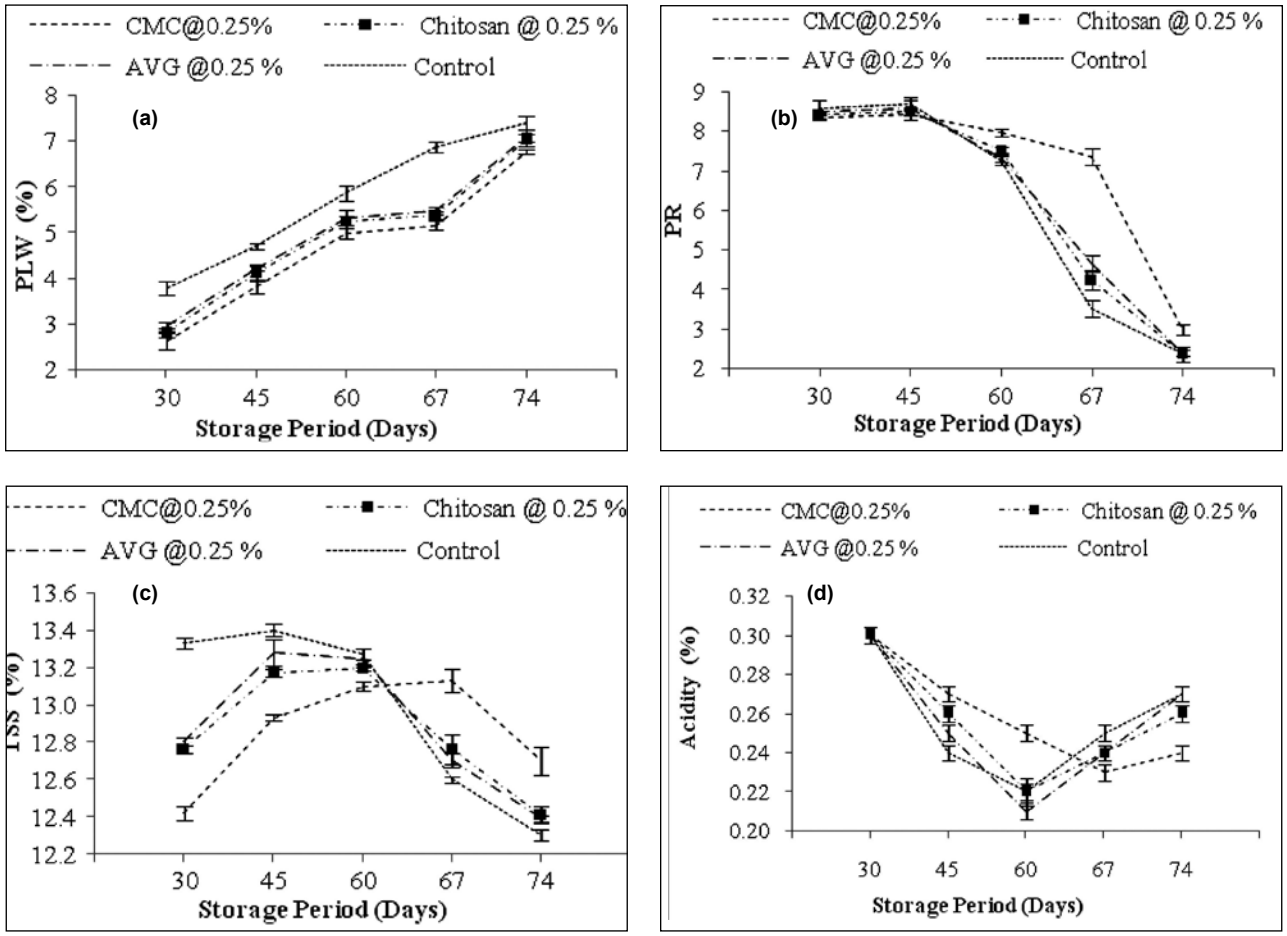


**Fig. 1.** Effect of post-harvest treatments on (a) PLW, (b) PR, (c) TSS and (d) acidity of pear fruits during storage. Vertical bars represent ± SE of mean.

may possibly be due to breakdown of complex organic metabolites into simple molecules or due to hydrolysis of starch into sugars. The decline in the sugar content at the later stages of storage may be due to the utilization of sugars in metabolic processes of the fruit. Similar results were also reported by Kaur *et al.* (6). Phenols are the important antioxidants and their content gradually declines with the ripening of fruits. In all the treatments total phenolics content declined with the extension in storage period (Fig. 2b), but this decline was slow in coated fruits as compared to untreated (control) fruits. It may be due to the low activity of polyphenol oxidase (PPO) enzyme in coated fruits. Similarly, reduction in total phenols during storage of peach fruits was observed by Jawandha *et al.* (4). During the entire storage period, highest total phenolics were recorded in fruits coated with CMC @ 0.25%. At the end of storage maximum (48.2 mg/100 g FW) total phenols were registered in CMC @ 0.25% treated fruits, whereas minimum (44.1 mg/100 g FW) were found in control fruits followed by fruits coated with AVG @ 0.25%. High retention of phenols in coated fruits may be due to the reduction in rate of phenols oxidation by various coatings.

Pectin methyl esterase activity affects the fruit texture and rigidity of cell wall. An increase in PME activity was recorded in all the treatments during the early periods of storage, but on the later stages of storage a decline in PME activity was recorded (Fig. 2c). All the treatments showed an increase in PME activity up to 60 days of storage except control and CMC @ 0.25% treatment, where this increase was recorded up to 45 days and 67 days of storage, respectively. At the end of storage, highest (1.28 ml of 0.02 N NaOH) PME activity was recorded in CMC @ 0.25% coated fruits, it may be due to the availability of more substrate (pectin) for enzyme activity in CMC @ 0.25% coated fruits, which was exhausted at earlier stages of storage in other treatments. Similar results were reported in *ber* fruits by Randhawa *et al*. (10).

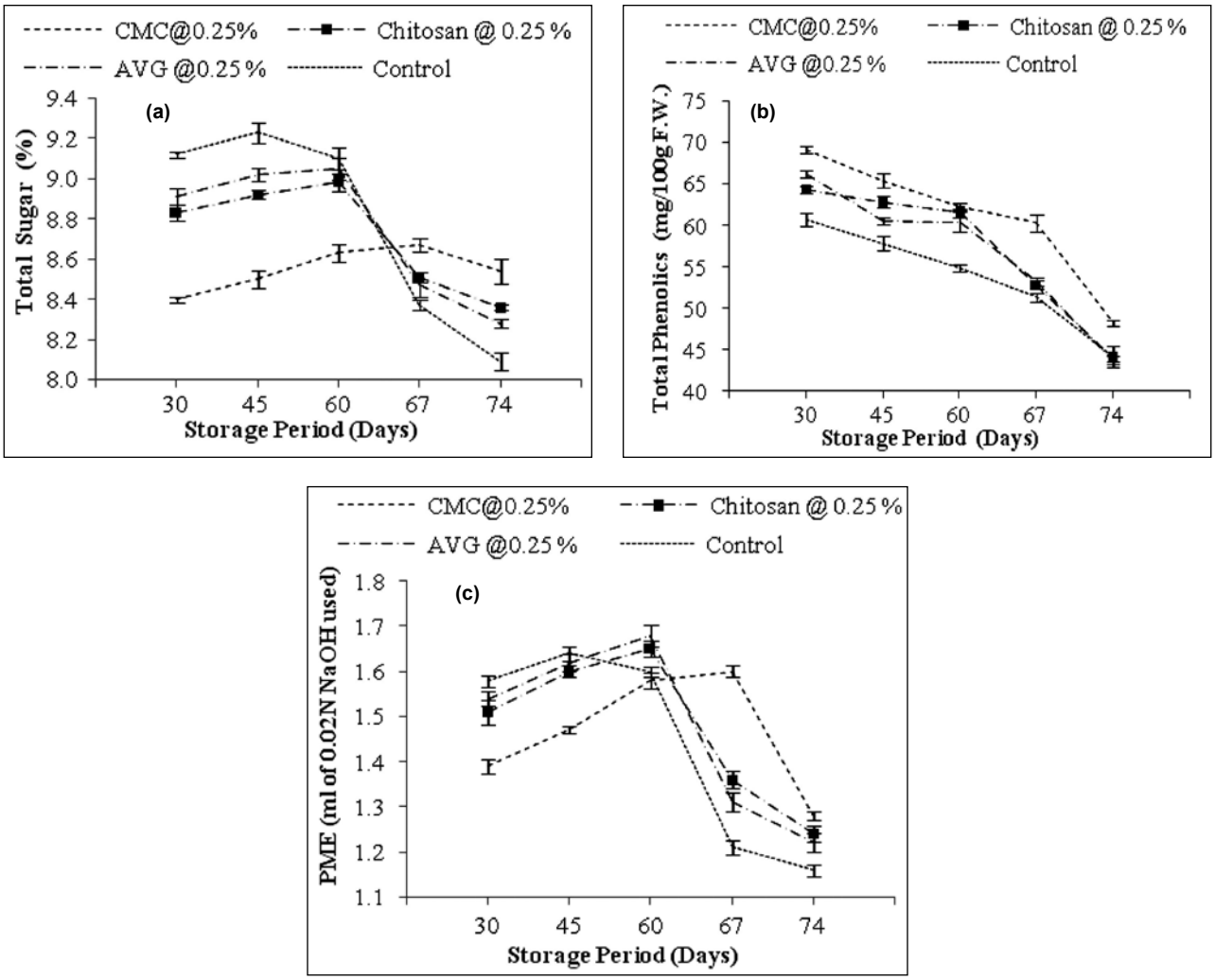


**Fig. 2.** Effect of post-harvest treatments on (a) total sugars, (b) total phenolics and (c) PME of stored pear fruits. Vertical bars represent ± SE of mean.

Results from this research showed that pear fruits coated with carboxymethyl cellulose (CMC) @ 0.25% can be stored for 67 days with moderately acceptable quality under cold storage conditions.

## ACKNOWLEDGEMENT

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***Short communication***

# Ascertaining physico-mechanical properties of *Prunus nepalensis* Steud fruit and seed using image processing and experimental methods

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## ABSTRACT

**The physico-mechanical properties of *Prunus nepalensis* fruit and seed were determined at moisture content of 88.50 and 15.62%, respectively. Image processing technique was also used to measure major dimensions and data was compared and correlated with experimental data. Significant correlation was observed between length and width of fruit and seed measured by experimental and image processing technique. The true density, bulk density and porosity of fruit were measured as 1077.41; 598.08 kg $m^{-3}$; and 43.42%, respectively whereas, for seed these values were observed to be 1178.84; 508.80 kg $m^{-3}$; and 57.94%. The angle of repose of fruit and seed was found to be 26.43 and 22.13°, respectively. Frictional coefficient was found lower in aluminium sheet than other surfaces. The obtained properties can be helpful for designing of processing equipment for this fruit.**

**Key words**: *Prunus nepalensis*, physical and mechanical properties, image processing.

*Prunus nepalensis* fruits, belong to family Rosaceae, is an important indigenous and nutritionally rich underutilized fruit of North Eastern Region of India and locally known as *Sohiong* in *Khasi*. The fruit is rich in β-carotene (257.1 µg %), vitamin C (608.9 mg %), antioxidant and minerals (Agrahar and Subbulakshmi, 1; Seal, 11). Fruits are drupe, fleshy, dark purple in colour at ripening and green to pinkish colour in beginning stage and contain round shape hard stone with smooth surface (Shankar and Synrem, 13). It is found in *Khasi* and Jaintia hills of Meghalaya between 1,500 to 2,000 m altitude. Fruit is eaten fresh by local people and fruit juice and pulp are used for preparation of squash, jam, RTS (ready-to-serve) and cheery wine.

Physico-mechanical properties of *P. nepalensis* fruits and seeds are essential in designing machines and equipment for post-harvest processing (Aviara *et al*., 2). Size and shape are important in the development of cleaning and grading machinery, bulk density and porosity in designing of drying and aeration system, angle of repose and coefficient of friction are important for mass flow and storage structures design (Kaleemullaha and Gunasekar, 4). Digital image analysis technique is a faster, non-destructive alternative to the traditional equipment currently used in the grain industry to determine the physical dimensions of seeds and grains (Mandal *et al*., 6; Razavi *et al*., 9). Therefore, the present investigation was undertaken to determine the physico-mechanical properties of *P. nepalensis* fruits and seeds by experimental as well as image processing technique and compare their results.

The work was carried out at the Division of Agricultural Engineering, ICAR Research Complex for NEH Region, Meghalaya. Matured ripen *P. nepalensis* fruits were procured from market at Shillong. Seeds were extracted manually by squeezing the fruits and cleaned thoroughly, and allowed to dry naturally for two days. Moisture content of the fruits and seeds were determined by using standard hot air oven method at 105 ± 5°C for 24 h and found to be 88.50 and 15.62%, respectively.

Length, width and thickness of the fruit were measured by a Vernier calipers with an accuracy of 0.02 mm. Average diameter of fruit and seed was calculated by using the arithmetic mean and geometric mean of length, width and thickness. Arithmetic mean diameter, geometric mean diameter and sphericity and surface area were calculated by using standard relationships. Unit mass of fruit and seed were determined by a digital electronic balance having accuracy of 0.01 g (Mettler Toledo, Switzerland, PB3002-SDR). Average mass of fruit and seed was calculated by weighing 100 numbers of randomly selected from a lot of 1000 fruits. Volume, true density and bulk density were determined by pycnometric method (Mohsenin, 7). Toluene was used instead of water because toluene has the advantages of little tendency to soak into fruits and

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seeds and a low surface tension thus enabling it to flow smoothly over the surface of fruits and seeds (Razavi *et al*., 10).

Porosity was calculated from bulk and true densities using the relationship given by Mohsenin (7). Angle of repose was determined using a hollow cylinder of 0.15 m diameter and 0.5 m height following the method described elsewhere (Razavi *et al*., 10). Static coefficient of friction was determined on three different frictional surfaces, aluminium, galvanized iron (GI) sheet and plywood using the method of tilting platform (Pradhan *et al*., 8). Texture characteristic of *P. nepalensis* fruits in terms of hardness was measured using a Stable Micro System TA-XT2 texture analyzer (Texture Technologies Corp., UK). Hardness value was considered as mean peak cutting force and expressed in kgf. The studies were conducted at a pre-test speed of 1.0 mm $s^{-1}$, test speed of 0.5 mm $s^{-1}$, distance of 2 mm, and load cell of 50.0 kg (Sirisomboon *et al*., 14).

An HP scanjet (Hewlett-Packard model # C7716A) document scanner was used to take images of the fruits and seeds and the images were analysed using the MATLAB 7.8.0 (The Math Works, Inc., Natick, MA, USA) software. The dimensions were determined using the procedure described by Mandal *et al*. (6). The scanned and analyzed images of *P. nepalensis* fruits and seeds are shown in Fig. 1. Sphericity and roundness were calculated using standard formula (Razavi *et al*., 10). The results of the image analysis were compared to the data obtained by experimental method by using SPSS statistical software (IBM, version 20.0.0).

## RESULTS AND DISCUSSION

The mean and standard deviation values of all physico-mechanical properties of *P. nepalensis* fruits and seeds measured experimentally and by image processing technique are summarized in Tables 1 and 2, respectively. Length of fruit in experimental method ranged from 17.41 to 22.62 mm, whereas, in image analysis it ranged from 15.83 to 21.20 mm. Length of *P. nepalensis* seed in experimental method was found in the range of 13.70 to 18.72 mm. By image analysis technique, seed length was found in the range of 14.21 to 18.24 mm. Width of *P. nepalensis* fruits and

**Table 1.** Physico-mechanical properties of *Prunus nepalensis* fruit at 88.50% moisture content.

| Trait | Experimental method | | | Image analysis method | |
|---|---|---|---|---|---|
| | No. of obs. | Mean | SD | Mean | SD |
| Length (mm) | 100 | 19.82 | 1.09 | 18.87 | 1.37 |
| Width (mm) | 100 | 19.53 | 1.14 | 17.03 | 0.88 |
| Thickness (mm) | 100 | 18.20 | 1.30 | | |
| Surface area ($mm^2$) | 100 | 1156.61 | 127.92 | | |
| Projected area ($mm^2$) | 100 | - | - | 252.33 | 26.47 |
| Geometric mean dia. (mm) | 100 | 19.16 | 1.05 | | |
| Arithmetic mean dia. (mm) | 100 | 19.18 | 1.05 | | |
| Sphericity | 100 | 0.97 | 0.02 | 0.85 | 0.03 |
| Roundness | 100 | - | - | 0.90 | 0.06 |
| Unit mass (g) | 100 | 5.09 | 0.77 | | |
| Mass of 1000-seed (g) | 5 | 5141.14 | 163.96 | | |
| Volume (cc) | 10 | 4.89 | 1.14 | | |
| True density (kg $m^{-3}$) | 10 | 1057.68 | 29.24 | | |
| Bulk density (kg $m^{-3}$) | 10 | 598.08 | 13.21 | | |
| Porosity (%) | 10 | 43.42 | 2.02 | | |
| Angle of repose (°) | 5 | 26.43 | 2.08 | | |
| Static coefficient of friction on | | | | | |
| Aluminium | 5 | 0.421 | 0.004 | | |
| Plywood | 5 | 0.434 | 0.004 | | |
| Galvanized iron | 5 | 0.477 | 0.012 | | |
| Hardness (kg) | 9 | 0.966 | 0.089 | | |

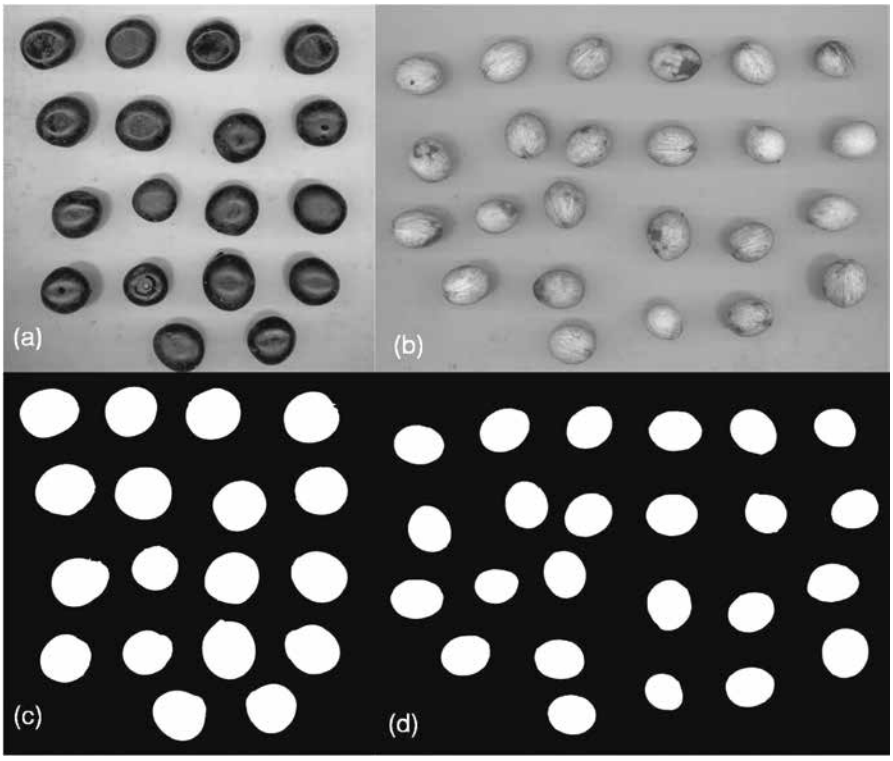


**Fig. 1.** Scanned and processed binary images of *P. nepalensis* fruits (a, c) and seeds (b, d).

**Table 2.** Physico-mechanical properties of *Prunus nepalensis* seed at 15.62% moisture content.

| Trait | Experimental method | | | Image analysis method | |
|---|---|---|---|---|---|
| | No. of obs. | Mean | SD | Mean | SD |
| Length (mm) | 100 | 17.09 | 1.23 | 16.57 | 1.03 |
| Width (mm) | 100 | 14.01 | 0.97 | 13.96 | 0.75 |
| Thickness (mm) | 100 | 13.96 | 0.81 | | |
| Surface area ($mm^2$) | 100 | 701.17 | 53.80 | | |
| Projected area ($mm^2$) | 100 | - | - | 180.79 | 19.97 |
| Geometric mean dia. (mm) | 100 | 14.93 | 0.57 | | |
| Arithmetic mean dia. (mm) | 100 | 15.02 | 0.58 | | |
| Sphericity | 100 | 0.877 | 0.055 | 0.601 | 0.16 |
| Roundness | 100 | - | - | 0.837 | 0.03 |
| Unit mass (g) | 100 | 1.83 | 0.51 | | |
| Mass of 1000-seed (g) | 5 | 2050.40 | 183.24 | | |
| Volume (cc) | 10 | 1.62 | 0.42 | | |
| True density (kg $m^{-3}$) | 10 | 1178.84 | 109.25 | | |
| Bulk density (kg $m^{-3}$) | 10 | 508.80 | 13.82 | | |
| Porosity (%) | 10 | 57.94 | 3.41 | | |
| Angle of repose (°) | 5 | 22.13 | 1.47 | | |
| Static coefficient of friction on | | | | | |
| Aluminium | 5 | 0.369 | 0.025 | | |
| Plywood | 5 | 0.414 | 0.004 | | |
| Galvanized iron | 5 | 0.435 | 0.014 | | |

seeds ranged from 17.11- 22.02 mm and 12.01-17.62 mm, respectively in experimental method whereas, in image analysis these values were 15.20-18.80 mm and 11.92-15.22 mm, respectively for fruit and seed.

Regression between image processing and experimental method for length and width of fruit and seed are presented in Table 3. Length of fruit and seed in image processing technique was 4.79 and 3.04% lower, respectively than experimental method. Width of seed was lower by 0.36% in image analysis method; however, width of fruit was lower by 12.80%. The correlation between the methods for length and width of fruit was 0.943 and 0.805, whereas for seeds these values were 0.941 and 0.936, respectively. This shows that there is strong correlation between image processing technique and experimental method for length and width of *P. nepalensis* fruits and seeds. Similar findings were reported by previous researchers for various seeds (Keefe, 5; Mandal *et al*., 6; Shahin, 12; Tańska *et al*., 15).

Arithmetic mean diameter, geometric mean diameter, sphericity and surface area of fruits were calculated to be 19.18 mm, 19.16 mm, 0.97 and 1156.61 $mm^2$, respectively. In case of seeds, these values were found to be 15.02 mm, 14.93 mm, 0.877 and 701.17 $mm^2$, respectively. Both the fruits and seeds of *P. nepalensis* were found spherical as sphericity values were more than 0.80 and 0.70 (Dutta *et al*., 3). Roundness and projected area were determined using image analysis technique and found 0.904 and 252.33 $mm^2$ of *P. nepalensis* fruit and 0.837 and 180.79 $mm^2$ of seed, respectively. Unit mass and volume of *P. nepalensis* fruit ranged between 3.55-7.05 g and 3.6-6.5 $cm^3$, while that of seed between 0.99-2.25 g and 0.8-2.1 $cm^3$, respectively. Bulk density, true density and porosity of *P. nepalensis* fruit were 598.08 kg $m^{-3}$, 1057.68 kg $m^{-3}$ and 43.42%, respectively. Both bulk and true density values were lower than arecanut kernels (Kaleemullaha and Gunasekar, 4) with its highest moisture content. Average bulk density of *P. nepalensis* seed (508.80 kg $m^{-3}$) was lower than fruit but true density (1178.84 kg $m^{-3}$) and porosity (57.94%) were higher, which justify its less sphericity than fruit.

**Table 3.** The relationship between experimental (y, mm) and image processing data (x, mm) for length and width of *Prunus nepalensis* fruits and seeds.

| Dimension | Regression equation | $R^2$ |
|---|---|---|
| Fruit length | Y = 1.177x - 4.795 | 0.943 |
| Fruit width | Y = 0.869x - 0.427 | 0.805 |
| Seed length | Y = 1.116x - 1.365 | 0.941 |
| Seed width | Y = 0.911x + 1.254 | 0.936 |

The angle of repose of *P. nepalensis* fruit varied between 23.44° to 28.68° and of seed between 20.15° to 23.76°. GI sheet surface had the highest coefficient of friction (0.477 for fruit and 0.435 for seed), and it was found that the static coefficient of friction was lowest against aluminium (0.429 for fruit and 0.369 for seed) with both fruit and seed. This was due to the smoother and polished surface of aluminium sheet compared to other sheets used. Fruit had higher friction than seed due to higher moisture content. The hardness of *P. nepalensis* fruit was found to vary between 0.879 to 1.184 kg with a mean value of 0.966 kg, which was lower compared to other fruits reported. This suggests that separation of pulp from kernel will be easier when done mechanically (Pradhan *et al*., 8).

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***Short communication***

# Effect of inorganic and organic fertilizers along with *Azotobacter* on growth, yield and quality of Kinnow mandarin

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## ABSTRACT

**A study was conducted during 2013 and 2014 on the effect of integrated use of inorganic and organic fertilizers (vermicompost) along with bio-inoculants on plant growth, yield and quality of Kinnow mandarin. Vegetative growth parameters like plant height and canopy volume showed maximum increase with cent per cent nitrogen through urea augmented with *Azotobacter*. Replacing 25 per cent of nitrogen in the form of vermicompost resulted in maximum number of fruits, fruit weight, fruit volume, fruit length and width and fruit yield. TSS, Total sugars (reducing and non-reducing) and ascorbic acid contents of the fruits improved with the integrated application of vermicompost along with inorganic fertilizers and biofertilizers.**

**Key words**: Kinnow mandarin, vermicompost, *Azotobacter*, growth, quality.

Kinnow mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenore) occupies a discrete position among citrus fruits due to its high yield, fresh consumption and aromatic flavour. In recent years, the quick and substantial response to fruit production due to mineral fertilization eclipsed the use of organic manures; the inadequate supply of latter sources exacerbated this change. Integrated plant nutrient supply system encourages integration of different sources of nutrients such as organic, biological and inorganic fertilizers *etc*. Incorporation of inoculants like *Azotobacter* either sole or in combination with inorganic and organic fertilizers have shown to improve nitrogen nutrition of plants through biological nitrogen fixation and also secretion of some growth promoting substances, which affect the growth, nutrition and microbial activity in the rhizosphere.

Experiment was carried out at Research farm of Division of Fruit Science, Faculty of Agriculture, SKUAST-J, Udheywalla during 2013 and 2014 on seven-year-old Kinnow mandarin trees grafted on *Jhatti khatti* rootstock, having uniform size and vigour. Vermicompost (N: 1.78%, P: 2.93% and K: 1.25%) was used as organic manure along with inorganic fertilizers and biofertilizer (*Azotobacter*). The experiment was laid out in a randomized block design with three replications. The treatments consisted of $T_1$ (100% N as urea), $T_2$ (25% N as vermicompost and 75% N as urea), $T_3$ (50% N as vermicompost and 50% N as urea), $T_4$ (75% N as vermicompost and 25% N as urea), $T_5$ (*Azotobacter* + 100% N as urea), $T_6$ (*Azotobacter* + 25% N as vermicompost and 75% N as urea), $T_7$ (*Azotobacter* + 50% N as vermicompost and 50% N as urea), $T_8$ (*Azotobacter* + 75% N as vermicompost and 25% N as urea), $T_9$ (*Azotobacter* + 100% N as vermicompost), $T_{10}$ (100% N as vermicompost), $T_{11}$ (*Azotobacter* application @ 100 g per tree) and $T_{12}$ (control). Plant height was measured using graduated stick. Canopy volume was calculated as per Westwood *et al*. (7). The fruit quality parameters were analyzed following standard procedures as described by AOAC (1). The total phenols content in the fruit juice was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 6). Statistical analysis was performed on pooled data of two years using statistical analysis programme SPSS V.16.

Results revealed that maximum increase in plant height (13.49%) and canopy volume (38.97%) was recorded with the application of cent per cent nitrogen in the form of urea along with *Azotobacter* ($T_5$) whereas minimum increase in plant height (6.32%) and canopy volume (16.10%) was recorded under control ($T_{12}$). However, treatment $T_6$ was equally effective (Table 1). Application of nitrogen resulted in vigorous vegetative growth of the plants. This favoured photosynthetic activity of the plants and greater synthesis of carbohydrates, which was responsible for building up of new tissues and better development of plants as reported by Rao *et al.* (4).

Data regarding fruit length, fruit width, peel thickness and yield characteristics of Kinnow mandarin is presented in Table 1. Maximum fruit

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**Table 1.** Effect of vermicompost, urea and *Azotobacter* on plant height, canopy volume, fruit length, fruit width, peel thickness ands yield characteristics of Kinnow mandarin.

| Treatment | % increase in plant height | % increase in canopy volume | Fruit length (cm) | Fruit width (cm) | Peel thickness (cm) | No. of fruits/ tree | Yield (kg/ tree) | Yield (t/ ha) |
|---|---|---|---|---|---|---|---|---|
| $T_1$ | 12.15 ± 1.6$^{ef}$ | 36.71 ± 3.39$^{de}$ | 5.50 ± 0.01$^{f}$ | 6.13 ± 0.02$^{e}$ | 0.53 ± 0.005$^{e}$ | 121.83 ± 1.47e | 21.53 ± 0.31$^{f}$ | 5.96 ± 0.08$^{f}$ |
| $T_2$ | 10.74 ± 0.35$^{de}$ | 32.65 ± 3.33$^{cd}$ | 5.61 ± 0.04$^{h}$ | 6.26 ± 0.03$^{g}$ | 0.53 ± 0.007$^{de}$ | 124.83 ± 1.47e | 21.76 ± 0.20$^{f}$ | 6.03 ± 0.05$^{f}$ |
| $T_3$ | 10.58 ± 1.56$^{de}$ | 30.81 ± 7.85$^{c}$ | 5.49 ± 0.03$^{f}$ | 6.20 ± 0.04$^{f}$ | 0.53 ± 0.010$^{de}$ | 117.67 ± 2.65$^{d}$ | 19.90 ± 0.63$^{e}$ | 5.51 ± 0.17$^{e}$ |
| $T_4$ | 9.53 ± 1.48$^{bcd}$ | 23.79 ± 1.34$^{b}$ | 5.41 ± 0.02$^{d}$ | 5.97 ± 0.04$^{d}$ | 0.52 ± 0.006$^{d}$ | 112.33 ± 2.25$^{cd}$ | 18.31 ± 0.31$^{d}$ | 5.07 ± 0.08$^{d}$ |
| $T_5$ | 13.49 ± 2.03$^{f}$ | 38.97 ± 6.07$^{f}$ | 5.71 ± 0.03$^{i}$ | 6.39 ± 0.05$^{h}$ | 0.54 ± 0.005$^{de}$ | 151.67 ± 2.94$^{g}$ | 28.32 ± 0.58$^{h}$ | 7.84 ± 0.16$^{h}$ |
| $T_6$ | 13.10 ± 2.22$^{f}$ | 37.31 ± 2.69$^{de}$ | 5.75 ± 0.02$^{j}$ | 6.46 ± 0.05$^{i}$ | 0.52 ± 0.005$^{d}$ | 162.50 ± 3.72$^{h}$ | 30.45 ± 0.72$^{i}$ | 8.43 ±0.19$^{i}$ |
| $T_7$ | 11.97 ± 2.77$^{ef}$ | 33.87 ± 3.39$^{cde}$ | 5.68 ± 0.0$^{2}$i | 6.37 ± 0.02$^{h}$ | 0.52 ± 0.0$^{a}$ | 138.33 ± 5.88$^{f}$ | 24.08 ±1.31$^{g}$ | 6.67 ±0.36$^{g}$ |
| $T_8$ | 9.99 ± 1.18$^{cde}$ | 24.32 ± 1.47$^{b}$ | 5.54 ± 0.02$^{g}$ | 6.24 ± 0.02$^{fg}$ | 0.52 ± 0.007$^{d}$ | 118.33 ± 3.32$^{d}$ | 19.89 ± 0.60$^{e}$ | 5.51 ±0.17$^{e}$ |
| $T_9$ | 8.68 ± 0.95$^{bcd}$ | 23.09 ± 4.54$^{b}$ | 5.44 ± 0.03$^{e}$ | 6.14 ± 0.04$^{e}$ | 0.52 ± 0.006$^{d}$ | 114.00 ± 4.38$^{c}$ | 18.21 ± 0.81$^{d}$ | 5.04 ±0.22$^{d}$ |
| $T_{10}$ | 8.19 ± 1.99$^{abc}$ | 21.27 ± 2.44$^{ab}$ | 5.36 ± 0.04$^{c}$ | 5.87 ± 0.05$^{c}$ | 0.51 ± 0.007$^{c}$ | 112.33 ± 2.22$^{c}$ | 17.17 ± 0.43$^{c}$ | 4.76 ±0.12$^{c}$ |
| $T_{11}$ | 7.32 ± 0.99$^{ab}$ | 19.55 ± 5.36$^{ab}$ | 5.32 ± 0.03$^{b}$ | 5.62 ± 0.03$^{b}$ | 0.50 ± 0.005$^{b}$ | 110.33 ± 2.73$^{b}$ | 15.56 ± 0.40$^{b}$ | 4.31 ±0.11$^{b}$ |
| $T_{12}$ | 6.32 ± 0.84$^{a}$ | 16.10 ± 2.04$^{a}$ | 5.09 ± 0.02$^{a}$ | 5.29 ± 0.05$^{a}$ | 0.49 ± 0.008$^{b}$ | 100.00 ± 2.44$^{a}$ | 14.10 ± 0.48$^{a}$ | 3.91 ±0.13$^{a}$ |

Data are expressed as pooled mean ± standard deviation. Means with different letters, in the same column, indicate significant differences ($P \leq 0.05$)

length (5.75 cm), fruit width (6.46 cm), number of fruits (162.50), fruit yield (30.45 kg per plant) and per hectare yield (8.43 t/ha) was recorded under treatment $T_6$. However, results for these parameters were at par with treatment $T_5$. Different treatments had non-significant effect on peel thickness of Kinnow mandarin fruits. Application of inorganic fertilizers and organic fertilizers alongwith biofertilizer inoculation showed the highest response in respect of fruit attributes as compared to application of inorganic and organic manures without bio-fertilizers. The higher uptake and accumulation of nutrients in the tissues and fruits of Kinnow mandarin with recommended dose of NPK might have occurred due to stimulation of the rates of various physiological and metabolic processes resulting in better fruit yield. The results are in conformity with those of Perungkotturselvi and Koilraj (3) on acid lime.

Maximum fruit weight (187.37 g) and fruit volume (189.33 cc) as compared to other treatments was observed under treatment $T_6$ (Table 2). Specific gravity of Kinnow mandarin did not show any significant difference among treatments. Maximum juice recovery (53.91%) was recorded under $T_5$, whereas minimum juice content was recorded in control ($T_{12}$). The pH of fruit juice showed non-significant results among different treatments. The notable improvement with respect to fruit growth parameters with the combined use of organic, inorganic and bio-fertilizers may be attributed to sufficient availability of nitrogen, phosphorus, potassium and other essential nutrients. Results are in line with the findings of Ravishankar *et al*. (5). Highest total polyphenols content (61.26 mg GAE per 100 ml of juice) was recorded in fruits of plants receiving no fertilization ($T_{12}$). Plants under stress produced more secondary metabolites and phenols and under this experiment; while nitrogen was the limiting factor under the control treatment that led to increased production of phenols as compared to all other treatments. Similar findings have been reported by Wu *et al*. (8).

Table 3 reveals chemical characteristics of Kinnow fruits. Maximum TSS of 11.02°B was recorded under treatment $T_7$. Acidity of fruits did not show much variation among different treatments. Maximum ascorbic acid content (26.50 mg/100 g fruit) was recorded under $T_9$. Maximum total sugars (5.91%) were recorded under treatment $T_7$ and maximum reducing sugars were recorded under treatment $T_8$. However, maximum non-reducing sugars (2.96%) were recorded maximum with the application of 50 per cent nitrogen as vermicompost and 50 per cent as urea along with *Azotobacter* ($T_7$). Better physico-chemcal characteristics of

**Table 2.** Effect vermicompost, urea and *Azotobacter* on fruit weight, fruit volume, specific gravity, per cent juice recovery and total polyphenols content of Kinnow mandarin fruits.

| Treatment | Fruit wt. (g) | Fruit vol. (cc) | Specific gravity | Juice recovery (%) | pH | Total polyphenols (mg GAE/ ml of juice) |
|---|---|---|---|---|---|---|
| $T_1$ | 176.73 ± 1.99$^{h}$ | 179.83 ± 2.78$^{h}$ | 0.98 ± 0.008$^{b}$ | 53.26 ± 1.86$^{i}$ | 4.02 ± 0.78$^{f}$ | 54.85 ± 1.49$^{b}$ |
| $T_2$ | 174.28 ± 1.17$^{g}$ | 176.50 ± 1.04$^{g}$ | 0.99 ± 0.007$^{bc}$ | 52.08 ± 1.21$^{h}$ | 3.95 ± 0.68$^{e}$ | 55.82 ± 1.51$^{b}$ |
| $T_3$ | 169.10 ± 2.23$^{f}$ | 170.83 ± 2.78$^{f}$ | 0.99 ± 0.004$^{bcd}$ | 51.22 ± 1.98$^{f}$ | 3.90 ± 0.62$^{cde}$ | 59.52 ± 1.62$^{cd}$ |
| $T_4$ | 162.98 ± 1.99$^{e}$ | 163.50 ± 1.51$^{d}$ | 1.00 ± 0.005$^{de}$ | 49.93 ± 1.83$^{d}$ | 3.87 ± 0.59$^{abcd}$ | 60.35 ± 1.89$^{cd}$ |
| $T_5$ | 186.72 ± 0.41$^{i}$ | 188.67 ± 1.36$^{j}$ | 0.99 ± 0.006$^{bcd}$ | 53.91 ± 0.68$^{j}$ | 4.08 ± 0.81$^{a}$ | 52.24 ± 1.27$^{a}$ |
| $T_6$ | 187.37 ± 0.50$^{i}$ | 189.33 ± 1.03$^{i}$ | 0.99 ± 0.004$^{bc}$ | 53.83 ± 0.54$^{j}$ | 4.02 ± 0.80$^{abc}$ | 53.07 ± 1.39$^{a}$ |
| $T_7$ | 174.03 ± 3.01$^{g}$ | 173.83 ± 2.78$^{f}$ | 1.00 ± 0.000$^{e}$ | 51.68 ± 2.89$^{g}$ | 3.92 ± 0.77$^{g}$ | 55.13 ± 1.41$^{b}$ |
| $T_8$ | 168.10 ± 1.74$^{f}$ | 168.83 ± 1.32$^{e}$ | 1.00 ± 0.005$^{de}$ | 50.32 ± 1.83$^{e}$ | 3.88 ± 0.72$^{f}$ | 58.52 ± 1.44$^{c}$ |
| $T_9$ | 159.73 ± 2.11$^{d}$ | 163.83 ± 2.99$^{d}$ | 0.98 ± 0.005$^{a}$ | 48.28 ± 1.79$^{c}$ | 3.83 ± 0.69$^{de}$ | 59.43 ± 1.57$^{cd}$ |
| $T_{10}$ | 154.92 ± 1.46$^{c}$ | 156.00 ± 0.63$^{c}$ | 0.99 ± 0.005$^{cde}$ | 48.28 ± 1.54$^{c}$ | 3.82 ± 0.68$^{abcd}$ | 60.99 ± 1.82$^{d}$ |
| $T_{11}$ | 150.57 ± 2.06$^{b}$ | 152.00 ± 1.78$^{b}$ | 0.99 ± 0.008$^{cde}$ | 46.80 ± 1.89$^{b}$ | 3.85 ± 0.72$^{ab}$ | 61.13 ± 1.94$^{d}$ |
| $T_{12}$ | 140.98 ± 1.59$^{a}$ | 143.00 ± 1.54$^{a}$ | 0.99± 0.008$^{f}$ | 45.54 ± 1.62$^{a}$ | 3.88 ± 0.74$^{cd}$ | 61.26 ± 2.01$^{d}$ |

Data are expressed as pooled mean ± standard deviation. Means with different letters, in the same column, indicate significant differences ($P \leq 0.05$)

**Table 3.** Effect of vermicompost, urea and *Azotobacter* on total soluble solids (TSS), titratable acidity, ascorbic acid, total sugars, reducing sugars and non-reducing sugars of Kinnow mandarin.

| Treatment | TSS (°B) | Titratable acidity (%) | TSS: acid ratio | Ascorbic acid (mg/100 g) | Total sugars (%) | Reducing sugars (%) | Non-reducing sugars (%) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 10.62 ± 0.04$^{c}$ | 0.75 ± 0.005$^{b}$ | 14.25 ± 0.09$^{b}$ | 25.51 ± 0.12$^{i}$ | 5.65 ± 0.02$^{c}$ | 2.63 ±0.01$^{b}$ | 2.87 ± 0.02$^{de}$ |
| $T_2$ | 10.73 ± 0.05$^{d}$ | 0.74 ± 0.0$^{b}$ | 14.50 ± 0.06$^{c}$ | 25.30 ± 0.09$^{g}$ | 5.70 ± 0.02$^{de}$ | 2.77 ±0.02$^{f}$ | 2.78 ± 0.01$^{bc}$ |
| $T_3$ | 10.73 ± 0.05$^{d}$ | 0.74 ± 0.005$^{b}$ | 14.60 ± 0.12$^{d}$ | 25.21 ± 0.55$^{fg}$ | 5.72 ± 0.03$^{e}$ | 2.77 ±0.01$^{f}$ | 2.79 ± 0.02$^{c}$ |
| $T_4$ | 10.78 ± 0.04$^{d}$ | 0.73 ± 0.005$^{b}$ | 14.87 ± 0.14$^{e}$ | 24.49 ± 0.15$^{d}$ | 5.70 ± 0.02$^{de}$ | 2.80 ± 0.03$^{fg}$ | 2.75 ± 0.03$^{b}$ |
| $T_5$ | 10.78 ± 0.07$^{d}$ | 0.76 ± 0.005$^{b}$ | 14.28 ± 0.18$^{b}$ | 23.23 ± 0.17$^{g}$ | 5.68 ± 0.02$^{cd}$ | 2.67 ±0.03$^{c}$ | 2.87 ± 0.04$^{d}$ |
| $T_6$ | 10.90 ± 0.08$^{f}$ | 0.75 ± 0.005$^{b}$ | 14.63 ± 0.18$^{d}$ | 23.77 ± 0.16$^{h}$ | 5.84 ± 0.02$^{g}$ | 2.80 ±0.01$^{fg}$ | 2.89 ± 0.02$^{def}$ |
| $T_7$ | 11.02 ± 0.04$^{g}$ | 0.74 ± 0.005$^{b}$ | 14.99 ± 0.14$^{f}$ | 25.27 ± 0.11$^{i}$ | 5.91 ± 0.01$^{h}$ | 2.80 ±0.01$^{fg}$ | 2.96 ± 0.01$^{g}$ |
| $T_8$ | 10.87 ± 0.08$^{ef}$ | 0.74 ± 0.005$^{b}$ | 14.79 ± 0.19$^{e}$ | 26.24 ± 0.18$^{e}$ | 5.89 ± 0.02$^{h}$ | 2.82 ±0.01$^{g}$ | 2.92 ± 0.02$^{fg}$ |
| $T_9$ | 10.75 ± 0.05$^{d}$ | 0.74 ± 0.005$^{b}$ | 14.63 ± 0.18$^{d}$ | 26.50 ± 0.10$^{f}$ | 5.78 ± 0.03$^{f}$ | 2.76 ±0.02$^{e}$ | 2.87 ± 0.03$^{d}$ |
| $T_{10}$ | 10.80 ± 0.06$^{de}$ | 0.73 ± 0.0$^{b}$ | 14.79 ± 0.08$^{e}$ | 24.28 ± 0.44$^{c}$ | 5.76 ± 0.03$^{f}$ | 2.69 ±0.04$^{c}$ | 2.91 ± 0.03$^{ef}$ |
| $T_{11}$ | 10.53 ± 0.05$^{b}$ | 0.73 ± 0.0$^{b}$ | 14.43 ± 0.07$^{c}$ | 24.98 ± 0.09$^{b}$ | 5.57 ± 0.05$^{b}$ | 2.63 ±0.08$^{d}$ | 2.79 ± 0.03$^{c}$ |
| $T_{12}$ | 10.03 ± 0.08$^{a}$ | 0.72 ± 0.0$^{b}$ | 13.94 ± 0.11$^{a}$ | 21.42 ±0.12$^{a}$ | 5.32 ± 0.01$^{a}$ | 2.54 ±0.02$^{a}$ | 2.63 ± 0.04$^{a}$ |

Data are expressed as pooled mean ± standard deviation. Means with different letters, in the same column, indicate significant differences ($P \leq 0.05$)

fruits with the application of vermicompost along with biofertilizers may be attributed to adequate supply of nitrogen that stimulates the functioning of number of enzymes in the physiological process. Our results are in agreement with Kumar and Kumar (2) who reported that application of vermicompost alleviated the physico-chemical characteristics of mango cv. Dashehari. On the basis of the aforesaid findings, it can be concluded that among the different treatments, application of 25 per cent nitrogen as vermicompost along with 75 per cent nitrogen as urea augmented with *Azotobacter* recorded vegetative growth at par with $T_1$, recorded the highest physical and yield parameters and was found to be best on overall basis.

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***Short communication***

# Technique to minimize phenolics in walnut *in vitro* culture initiation

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## ABSTRACT

**Total phenol content of the walnut genotypes was determined to explore the relationship between total phenols and regeneration response for different walnut genotypes. Walnut leaves from ten genotypes (CITH-Walnut-I, CITH-Walnut-II, BBW-8, CITH-Walnut-IV, BP-3, SP-1, LG-11, Hamdan, Suleiman, and Opex Culchery) were used for extraction of total phenols through modified Folin-Ciocalteu method. Total leaf phenolic content ranges from 140 µg $g^{-1}$ (WGB-1) to 285 µg $g^{-1}$ (BBW-8). Phenolic interactions expressed as darkening of the explants lead to death. Among different antioxidants used ascorbic acid @ 350 mg/l was found best with almost no phenol exudation in the medium and shoot initiation occurred after 8 days of inoculation. The number of shoots was highest (10), followed by citric acid used @ 350 mg/l showing low degree of exudation where shoot initiation was noted in 15 days and with 15.0 shhots per explant.**

**Key words:** Walnut, phenolic content, antioxidants, *in vitro* culture, ascorbic acid.

Walnut tree (*Juglans regia* L.), is native to Eastern Europe and North Asia, but is also found throughout North, Central and South America. The tree has great socio-economic importance being frequently cultivated in temperate zones of the world mainly because of its edible seed, having oil which is rich in unsaturated fatty acids, phytosterols and tocopherols. For many years the trees are grown in orchards because of the delicious fruits, which can be eaten raw and are excellent for dessert as well as in baking and confectionery. Walnut is propagated through grafting and budding, but survival rate is very low. Micropropagation provides more efficient technique for large scale multiplication. Various attempts have been made using different types of explant, media, culture condition and rooting techniques, with promising results (McGranahan *et al.*, 1; Vahdati *et al*., 2). High phenolic content in explants effect the *in-vitro* multiplication of walnut. Phenolics are involved in growth and reproduction and provide plants with resistance to pathogens and predators. In tissue culture studies, phenolic substances, especially oxidized phenolics generally effect *in-vitro* proliferation negatively. Tissue browning and blackening are also one of the major problems for *in-vitro* culturing in many economically important plants. Oxidized phenolic compounds may inhibit enzyme activity and result in darkening of the culture medium and subsequent lethal browning of explant. The production of exudates from freshly cultured explants of walnut has also been a problem, solved by employing explant pre-soaks and transferring explants frequently to fresh medium. Frequent sub-culturing and use of some antioxidants such as citric acid, ascorbic acid, PVP (polyvinyl pyrrolidone) and activated charcoal, which can reduce phenolic oxidation and contribute to regeneration from explants (Toth *et al.*, 3). In this study we determined total phenol amount in leaves of different walnut genotypes and best media conditions (anti-oxidant supplement) was standardized for efficient regeneration and shoot multiplication of difficult to regenerate walnut genotype.

Material for analyses was taken from two-year-old ten walnut genotypes (CITH-Walnut-I, CITH-Walnut-II, BBW-8, CITH-Walnut-IV, BP-3, SP-1, LG-11, Hamdan, Suliman, and Opexculchery) maintained in polyhouse of ICAR-Central Institute of Temperate Horticulture, Srinagar, India. For each sample, about 0.5-1.0 g of leaf were manually collected and grinded into mortar and pestle in 80% ethanol. Total phenolic content was determined by using modified Folin-Ciocalteu method in the extracts. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu phenol reagent method. About 0.5 ml of Folin-Ciocalteu solution was added to each sample and allowed to stand for 3 min. in dark and then 2 ml of 20% $Na_2CO_3$ was added and mixed thoroughly. All the samples were kept in boiling water bath for 1 min. and absorbance was measured at 650 nm using UV double spectrophotometer. Experiments have been made with axillary buds and nodal segments of walnut. Juvenile material was taken from 1-2 year-old plantlets germinated under greenhouse conditions that had been kept under stringent plant protection conditions. The medium used was DKW

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medium (Driver and Kuniyuki, 4) supplemented with 3% sucrose, 0.7% agar-agar and BAP (2 mg/l), IBA (0.1 mg/l) and $GA_3$ (0.1 mg/l). The culture conditions were 16 h photoperiod at 25°C. To decrease explant exudation, sub-culturing was done regularly after 10-15 days. To prevent phenolic oxidation, antioxidants like ascorbic acid, citric acid, PVP and activated charcoal. Each treatment was replicated 4 times and observations in stages of development were recorded periodically. The data was analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using SPSS for windows (v. 15. SPSS Inc USA).

Total phenols expressed as percentage of dry weight of leaf material varied significantly among species. BBW-8 and Suleiman had the highest phenol concentrations (285 and 280 µg/g of leaf tissue respectively), whereas WGB-I and Opexcultury leaves had very low levels (140 and 155 µg $g^{-1}$ of leaf tissue respectively). Phenol contents were significantly different among the walnut genotypes (Table 1). Phenol exudation and tissue culture response was directly related to total phenol content. Degree of phenol exudation was again highest in BBW-8 and Suleiman, while as it was lowest in WGB-I (Table 1). Phenol exudation greatly influenced the tissue culture response and was found to be correlated with quality control (Joana *et al.*, 5). Exudation of phenolics has been reported to cause explants necrosis in *in-vitro* culture of many plant species (Standardi and Romani, 6). Negative effect of phenolics on *in-vitro* proliferation has also been revealed by other researchers (Ahmed *et al.*, 7). DKW medium was chosen for this study based on previous studies which revealed that DKW medium is suitable (and in many cases superior) for the culture of *J. regia* as well as other *Juglans* species (Driver and Kuniyuki, 4; Vahdati *et al.*, 2). Mature, dormant seeds of "Hamdan" variety were harvested from CITH, Farm and were stored at 5°C before being used. Seeds were then stratified in moist sand at 5°C in darkness for 90 to 120 days. After stratification, seeds were germinated in plastic pots containing a 1 peat: 1 perlite (v/v) medium. Seedlings were maintained in a temperature controlled polyhouses for 2 to 3 months. Stems from actively growing seedlings were stripped of leaflets and surface disinfested in 0.8% (v/v) sodium hypochlorite (15% Clorox™ bleach) for 15 to 20 min., followed by four rinses in sterile, deionized water. Nodal explants (1 to 2 cm long) were excised and placed upright in 25 × 95-mm culture vials containing 15 ml of DKW medium supplemented with 3% sucrose, 0.7% agar and BAP (2 mg/l), IBA (0.1 mg/l) and $GA_3$ (0.1 mg/l).

DKW medium gave good results with respect to shoot multiplication in walnut genotypes and the application of 0.1 mg/l $GA_3$ induced better shoot elongation (Vahdati *et al.*, 2; Ahmed *et al.*, 7). Application of different anti-oxidants to medium greatly influences the regeneration rate and survival of explants. Results showed that the number of axillary shoots arising from the micro-shoots was highest (7.0) on medium supplemented with 350 mg/l ascorbic acid, 5 on medium with 350 mg/l citric acid and 250 mg/l ascorbic acid compared to control (Table 2, Fig. 1). Application of antioxidants also influenced the rate of shoot initiation. Media supplemented with 350 mg/l ascorbic acid took only few days (8) for shoot initiation followed by 13 days on medium with 250 mg/l ascorbic acid (Table 2). Role of antioxidants in improving tissue culture response has been reported by number of workers (Vahdati *et al.*, 2; Ahmed *et al.*, 7). The addition of ascorbic acid

**Table 1.** Differential response of walnut genotypes in tissue culture with respect to different phenolic levels.

| Genotype | Total phenolics (µg $g^{-1}$) | Degree of phenolic exudation |
|---|---|---|
| Sulieman | $280^{f}$ ± 0.82 | ++++ |
| Hamdan | $215^{d}$ ± 2.04 | ++++ |
| LG-5 | $165^{c}$ ± 2.04 | +++ |
| LG-9 | $170^{c}$ ± 2.04 | +++ |
| SP-3 | $235^{e}$ ± 5.40 | ++++ |
| BP-4 | $167^{c}$ ± 1.91 | +++ |
| KPT-5 | $230^{e}$ ± 2.04 | ++++ |
| WGB-1 | $140^{a}$ ± 2.04 | ++ |
| Opex Culchery | $155^{b}$ ± 2.04 | +++ |
| BBW-8 | $285^{f}$ ± 3.54 | ++++ |

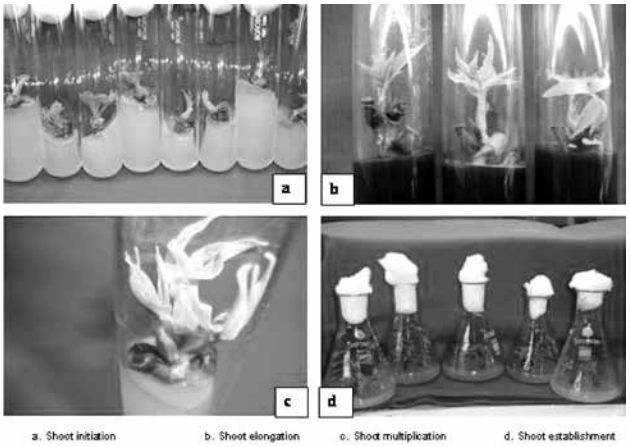


**Fig. 1.** *In-vitro* multiplication of *Juglans regia*.

**Table 2.** Effect of different antioxidants on *in vitro* multiplication of walnut genotype "Hamdan".

| Antioxidant | Conc. of antioxidant (mg /l) | Degree of phenolic exudation | Days to shoot initiation | No of shoots/ explant |
|---|---|---|---|---|
| Control | 0.0 | ++++ | $27^{h} \pm 0.71$ | $2^{a} \pm 0.41$ |
| Ascorbic acid | 150 | +++ | $17^{de} \pm 0.41$ | $4^{ab} \pm 0.71$ |
| | 250 | ++ | $13^{b} \pm 0.41$ | $7^{c} \pm 0.41$ |
| | 350 | - | $8^{a} \pm 0.41$ | $10^{d} \pm 1.08$ |
| Citric acid | 150 | +++ | $22^{g} \pm 0.82$ | $4^{ab} \pm 0.41$ |
| | 250 | ++ | $18^{ef} \pm 1.41$ | $4^{ab} \pm 0.82$ |
| | 350 | + | $15^{bcd} \pm 0.41$ | $8^{cd} \pm 0.71$ |
| PVP | 0.1% | +++ | $20^{f} \pm 0.82$ | $3^{a} \pm 0.41$ |
| | 0.2% | +++ | $17^{de} \pm 0.41$ | $3^{a} \pm 0.71$ |
| | 0.3% | ++ | $14^{bc} \pm 0.41$ | $6^{bc} \pm 1.08$ |
| Activated charcoal | 150 | +++ | $23^{g} \pm 0.82$ | $3^{a} \pm 0.41$ |
| | 250 | +++ | $18^{ef} \pm 0.82$ | $4^{ab} \pm 0.71$ |
| | 350 | ++ | $16^{cde} \pm 0.41$ | $7^{c} \pm 1.08$ |

to the culture medium reduced phenolic browning and prolonged survival and frequent sub-culturing was also practiced to overcome the effects of phenolic compounds (McGranahan *et al.*, 1). McGranahan *et al.* (1) also suggested that frequent sub-culturing of the explants on to fresh medium improved the quality of cultures and increased the survival rate.

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***Short communication***

# Validation of potato cyst nematode resistant genotypes through molecular markers

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## ABSTRACT

**Forty-two potato genotypes exhibiting phenotypic resistance against Potato Cyst Nematode (PCN) species *Globodera rostochiensis (*golden) and *Globodera pallida* (white) were screened for molecular validation using tightly linked genetic DNA markers. *H1* and *GroV1 gene* conferring resistance against golden nematodes *were* validated by TG 689 & 57R and X02 markers, respectively. Similarly, resistance against *Globodera pallida* governed by *QTL GpaVvrn* and *QTLs Gpa5, Gpa6*, was confirmed by corresponding markers HC and SPUD1636. On the basis of phenotypic and genotypic confirmation, this study identifies CP 1843, 1879 and JEX/A 267 as elite potato genotypes that can be utilized as parental lines for introgression of resistant genes against both PCN species.**

**Key words:** Genotypes, potato cyst nematode, marker-assisted breeding, *QTLs,* markers.

Potato cyst nematode (*Globodera* spp.) is a global pest. The annual yield loss due to this pest range from 20% to 70% (Oerke *et al*., 6). Resistance against Potato cyst nematode (PCN) is mainly derived from *Solanum tuberosum* ssp. *andigena*, *Solanum vernei* and *Solanum spegazzini* (Milczarek *et al*., 5). In India, Potato cyst nematode was first reported in year 1961 from Nilgiri hills of Tamil Nadu (Jones, 2). Considering its economic importance domestic quarantine (embargo) was imposed. At present the pest is restricted to the areas of Tamil Nadu and Kerala (Krishna Prasad, 4). Recent surveys in the Nilgiris revealed PCN presence is in high intensities (>51 cysts/100 cc soil). In India both species are prevalent and the pathotypes available are *Ro1*, *Ro2* and *Ro5* of *Globodera rostochiensis* and *Pa1*, *Pa2* and *Pa3* of *Globodera pallida* (Krishna Prasad, 3). Being a quarantined pest and difficulty in management, resistance breeding is the most sustainable strategy. In India, the first potato cyst nematode resistant variety bred through conventional breeding was Kufri Swarna released in 1985 with *Solanum vernei* as the source of resistance. It has resistance against pathotypes *Ro1* and *Pa2*. However, continuous cultivation of specific resistant varieties leads to build up of other virulent pathotypes. Recently, another *S. vernei* derived resistant hybrid 'OS/93-D-204' has been bred and released as a variety 'Kufri Neelima' for Nilgiri hills. It is resistant to pathotypes *Ro1* and *Pa2*. Development of resistant varieties through conventional breeding is laborious and time consuming. In order to hasten the resistance breeding programme, interventions of molecular markers have a vital role. It provides information about the prevalence of resistance genes in population and facilitates combining/ pyramiding of multiple resistance genes. Keeping this in view the aim of present study was to evaluate the usefulness of different PCR based markers linked to PCN resistance genes for rapid screening of Indian potato genotypes (cultivars and breeding lines).

The PCN resistant potato cultivars and breeding lines are maintained under tissue culture of National Active Germplasm Repository of CPRI, Shimla. A total of 42 breeding lines of potato including 8 exotic varieties were grouped according to phenotypic traits into three categories, *i.e.* group I including genotypes resistant against *G. rostochiensis*, group II for *G. pallida* resistant genotypes and group III of combined resistance. The total genomic DNA was extracted from tissue culture raised plantlets by extraction kit (Sigma Aldrich) and was amplified in DNA thermal cycler (Applied Biosystems) using markers TG689 and 57 R (*H1* gene, chromosome V); Gro 1.4.1 (*Gro 1.4* gene, chromosome III); X02 (*GroVI* gene, chromosome V); SPUD 1636 (*QTLs Gpa5* and *Gpa6,* chromosome V) and HC (*GpaVvrn,* chromosome V). The components of the PCR reaction mixture are: 1X Tris buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* polymerase, 1 µM primer and 20 ng template DNA for each reaction with final volume of 20 µl. The PCR conditions for the different markers are followed as per protocol given by Milczarek *et al*. (5), Asano *et al.* (1) and Schultz *et al*. (7). The annealing temperature of each marker was standardized by gradient PCR

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(Biorad 100). The amplified products were resolved on a 2.0% agarose gel at a constant voltage of 60 V for 150 min. using a horizontal gel electrophoresis system.

Several major genes are known to be associated with PCN resistance in both wild and cultivated *Solanum* species. Out of which five major genes/ QTLs have been validated in the present study by a set of linked molecular markers in known phenotypically resistant genotypes. The result depicted that the markers TG689, 57R and X02 for *G. rostochiensis* did not amplify in any of the *G. pallida* resistant genotypes. Similarly, the markers HC and SPUD1636 for *G. pallida* resistance did amplify in any of the *rostochiensis* resistant genotypes as expected (Table 1).

*H1* gene for *G. rostochiensis* resistance was validated in all the genotypes screened by marker 57R while the marker TG 689 for the same gene amplified in 28 out of 33 genotypes (Table 1, Fig. 1&2). *GroV1* gene was present in 15 genotypes depicting that the source of resistance is derived from *S. vernei*. Genotypes CP 1598, 1669, 1670, 1671, 1720, 1843, 2290, 2329, 2339, 3209, JEX/A 164, 413, 622, 780 and 911 contain both *H1* and *GroV1* genes. Gene *Gro 1.4* derived from *S. spegazzinii* was present in none of the genotypes. Our results are in accordance to Schultz *et al*. (7) who reported that marker 57R is the most common in *G. rostochiensis* resistant genotypes. The marker TG 689 also holds promise for selection of *G. rostochiensis* resistant genotypes. However, this marker did not amplified in all resistant genotypes which were similar to Milzareck *et al*. (5) results of occurrence of resistant and “marker-negative” individuals. *Gro 1.4* gene did not amplify in any of the genotypes, necessitating validation of this marker in wider germplasm pool for broadening the genetic base.

**Table 1.** Genotypic profile of potato genotypes resistant against *G. rostochiensis* (Group I), *G. pallida* (Group II) and both species (Group III).

| Group | Genotype | Genes/ QTLs | | | | | |
|---|---|---|---|---|---|---|---|
| | | *H1* | | *GroVI* | *GpaVvrn* | *Gpa5, Gpa6* | *Gro1-4* |
| Group I | *Solanum tuberosum* ssp. *tuberosum* | TG 689 | 57R | X02 | HC | SPUD1636 | Gro 1-4-1 |
| | CP 1598 | + | + | + | - | - | - |
| | CP 1669 | + | + | + | - | - | - |
| | CP 1670 | + | + | + | - | - | - |
| | CP 1671 | + | + | + | - | - | - |
| | CP 1720 | + | + | + | - | - | - |
| | *Solanum tuberosum* ssp. *andigena* | | | | | | |
| | JEX/A 132 | + | + | - | - | - | - |
| | JEX/A 164 | + | + | + | - | - | - |
| | JEX/A 413 | - | + | + | - | - | - |
| | JEX/A 622 | + | + | + | - | - | - |
| | JEX/A 780 | + | + | + | - | - | - |
| | JEX/A 911 | - | + | + | - | - | - |
| | Exotic varieties | | | | | | |
| | Reba/ CP 4362 | + | + | - | - | - | - |
| | Pike/ CP 4364 | + | + | - | - | - | - |
| | Andover/ CP 4367 | + | + | - | - | - | - |
| | Salem/ CP 4363 | + | + | - | - | - | - |
| | Keuka Gold/ CP 4365 | + | + | - | - | - | - |
| | Eva/ CP 4366 | - | + | - | - | - | - |
| | Lehigh/ CP 4360 | + | + | - | - | - | - |
| | Marcy/ CP 4361 | + | + | - | - | - | - |
| | Genotypes showing positive results | 16/19 | 19/19 | 10/19 | - | - | - |

*Contd...*

*Table 1 Contd...*

| Group | Genotype | Genes/ QTLs | | | | | |
|---|---|---|---|---|---|---|---|
| | | *H1* | | *GroVI* | *GpaVvrn* | *Gpa5, Gpa6* | *Gro1-4* |
| Group II | *Solanum tuberosum* ssp. *tuberosum* | | | | | | |
| | CP 3303 | - | - | - | - | - | - |
| | CP 3305 | - | - | - | - | - | - |
| | CP 3306 | - | - | - | - | - | - |
| | CP 3307 | - | - | - | - | - | - |
| | CP 3308 | - | - | - | - | - | - |
| | *Solanum tuberosum* ssp. *andigena* | | | | | | |
| | JEX/A 79 | - | - | - | - | - | - |
| | JEX/A 506 | - | - | - | - | - | - |
| | JEX/A 712 | - | - | - | - | - | - |
| | JEX/A 877 | - | - | - | - | - | - |
| | Genotypes showing positive results | - | - | - | 0/9 | 0/9 | - |
| Group III | *Solanum tuberosum* ssp. *tuberosum* | | | | | | |
| | CP 1843 | + | + | + | - | + | - |
| | CP 1879 | + | + | - | + | - | - |
| | CP 2059 | + | + | - | - | - | - |
| | CP 2290 | + | + | + | - | - | - |
| | CP 2329 | + | + | + | - | - | - |
| | CP 2339 | + | + | + | - | - | - |
| | CP 2417 | + | + | - | - | - | - |
| | CP 3181 | + | + | - | - | - | - |
| | CP 3209 | + | + | + | - | - | - |
| | CP 3534 | + | + | - | - | - | - |
| | *Solanum tuberosum* ssp. *andigena* | | | | | | |
| | JEX/A 121 | + | + | - | - | - | - |
| | JEX/A 216 | - | + | - | - | - | - |
| | JEX/A 267 | + | + | - | + | - | - |
| | JEX/A 708 | - | + | - | - | - | - |
| | Genotypes showing positive results | 12/14 | 14/14 | 5/14 | 2/14 | 1/14 | 0/14 |

+ = Presence of gene; - = Absence of gene

For *G. pallida* resistance, *QTL GpaVvrn* was present in two genotypes, while *QTLs Gpa5* and *Gpa6* were present in only one genotype (Table 1), which was validated by markers HC and SPUD1636, respectively. The other resistant genotypes could not amplify the markers confirming presence of resistance source other than *S. vernei.* Genotypes CP 1843, 1879 and JEX/A 267 contain resistance genes against both *G. rostochiensis* and *G. pallida*. Thus, these genotypes serve as valuable source of breeding material for pyramiding PCN resistance genes.

In conclusion, detection of resistance genes by DNA based markers may complement the phenotypic evaluation. 57R is the most robust marker for identification of *G. rostochiensis* resistant genotypes. Furthermore, there is a need for validation of these tightly linked markers in wider population and development of new markers suitable for characterisation of Indian PCN resistant genotypes.

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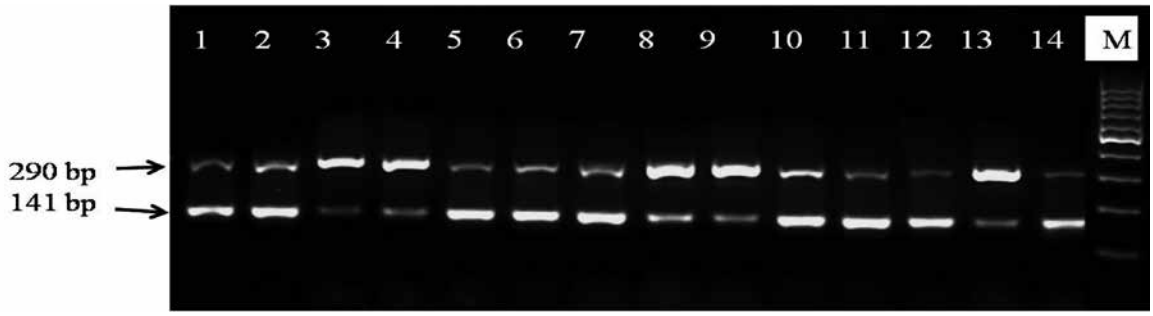


**Fig. 1.** DNA profile of different potato genotypes M : 100 bp DNA ladder, 1-14 positive samples of TG 689.

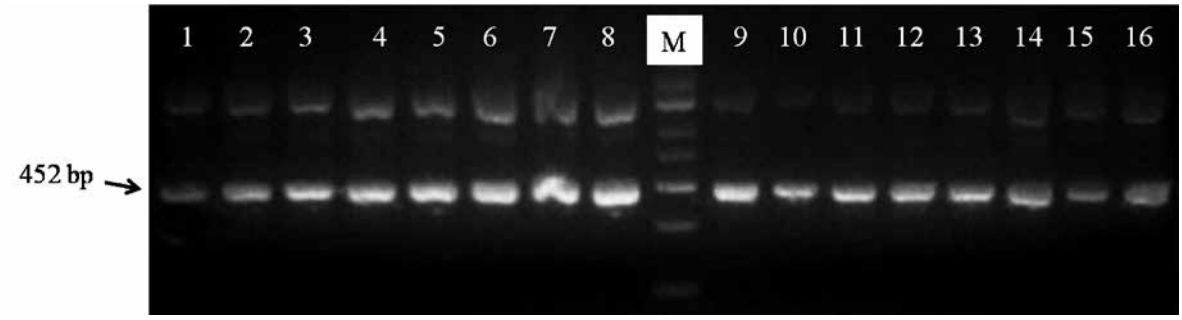


**Fig. 2.** DNA profile of different potato genotypes M : Express DNA ladder, 1-16 positive samples of 57R.

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***Short communication***

# Effect of different coloured plastic mulches on growth, yield and quality of drip fertigated bell pepper (*Capsicum annum* var. *grossum*)

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## ABSTRACT

**Among the different coloured mulches applied in bell pepper maximum rise in soil temperature was recorded under black/black plastic mulch in April (3.6ºC). It was followed by silver/black mulch with soil temperature rise of 3.3ºC as compared to no mulch application in respective month. The maximum increase in fruit yield (54.9%) was observed in bell pepper grown on silver/ black polyethylene mulch as compared to no mulch plots. It was closely followed by black/ black mulch. The maximum reduction in weed dry matter and maximum ascorbic acid content in fruit was recorded in black/ black coloured plastic mulches.**

**Key words:** Bell pepper, coloured plastic mulch, quality, yield.

Among the vegetable crops bell pepper (*Capsicum annum* var. *grossum*) is an important crop. The fruit is highly appreciated for its flavour and high content of provitamin A and ascorbic acid (Rubatzky and Yamaguchi, 7). Pepper is a warm-season crop sensitive to low temperatures and frosts. It can tolerate temperatures above 30ºC and night temperature 21-24ºC. The optimum night temperature for quality fruit production is 16-18ºC. In the era of climate change, mulching should be used for soil moisture conservation, temperature moderation, soil health maintenance, weed management and finally increased productivity (Parmanik *et al.*, 6). The benefits from the use of plastic mulches include earlier and higher yields, reduced weed populations, reduced soil evaporation, reduced fertilizer leaching, greater water use efficiency, reduced soil compaction, control of certain pests, and a cleaner harvested product (Lamont, 5). Awasthi *et al.* (2) reported that use of black as well white polyethylene mulch recording in brinjal better soil moisture content 30 cm below the mulch as compared to control plots. Therefore, an experiment was planned to study the effect of different coloured plastic mulches under drip fertigation on the performance of bell pepper.

A field experiment was conducted during *rabi* season of 2013-14 and 2014-15 at the research farm Department of Soil and Water Engineering, PAU, Ludhiana. The soil of the experimental site was loamy sand in texture with soil pH (8.2) and electrical conductivity (0.20 dS $m^{-1}$), low in organic carbon (0.30%) & available N and very high in available P (30.2 kg $ha^{-1}$) and K (350.0 kg $ha^{-1}$). Farm yard manure @ 50 t $ha^{-1}$ was applied 15 days prior to pre-sowing irrigation during the first year. The treatments comprised of four different coloured plastic mulches, *viz*. Yellow/ black, Black/ black, Silver/black and White/ black and No mulch (control). The mulch used was 25 µ in thickness, double layered and its colour facing the sky was yellow, black, silver and white in different mulches, while the surface of mulch facing the ground was black in all the mulches. The experiment was laid out in randomized block design with three replications.

The seedlings of bell pepper hybrid Indra were transplanted on 19.11.2013 and 19.11.2014, respectively. The two rows of seedlings were transplanted on a single bed at row to row spacing of 45 cm with plant to plant spacing 30 cm. Soil temperature was measured using bimetallic dial type thermometers installed at 15 cm depth in the soil. The crop was covered with low tunnels in the mid December to protect from frost and low tunnels were removed after second week of February 2014 and 2015. One drip lateral (16 mm dia) with inline emitters placed at 30 cm distance, with discharge rate of 2.2 lph (pressure =1.5 kg/ $cm^2$) was placed between the two rows on a bed. Soil temperature was recorded daily while data of growth parameters was recorded at periodic interval. The drip irrigation was applied on alternate day at 80% of ETcrop. The 100 kg N, 50 kg $P_2O_5$ and 24 kg $K_2O$ $ha^{-1}$ of N P K fertilizers were applied through fertigation in twenty equal splits. The fertilizers used were urea, phosphoric acid and sulphate of potash. The fertigation was initiated 15 days after transplanting of the seedlings. The leaf area index was measured using LP 80 Ceptometer. During each crop season fruit was picked four pickings. The quality parameters,

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*viz*. dry matter %, capsaicin content and ascorbic acid were determined using standard procedure. The soil moisture was determined gravimetrically by taking soil samples from 0-15 and 15-30 cm depth and 15 cm away from the drip line on both sides. The soil samples were collected in moisture boxes and fresh weights of soil with moisture box were recorded. The moisture content was calculated in percent. The data on weed count and weed dry matter was assessed from 0.5 m × 0.5 m area in each plot at 45 and 135 days after transplanting respectively prior to weeding. To control the weeds two manual hoeings were done after 45 and 135 days after transplanting.

The soil temperature recorded at 9.30 hours showed that up to month of March maximum soil temperature was observed under black on black mulch during both the years. Thereafter, in the months of April and May maximum value of soil temperature was recorded under silver on black mulch. It may be because of the reason that thermal conductivity of soil is high relative to that of the air, much of the energy absorbed by black mulch can be transferred to the soil by conduction as reported by Lamont (5). Besides silver mulch absorbs less short wave and long wave radiation but would emits less long wave radiation, potentially making it a better insulator, trapping more soil heat as observed by Ham *et al.* (4). However, soil temperature data recorded at 14.30 h indicated that maximum rise in soil temperature was recorded under black/ black plastic mulch in the month of April 2014 (3.8ºC) and April 2015 (3.3ºC) depending on the weather conditions. It was very closely followed by silver/ black mulch with soil temperature rise of 3.4 and 3.2ºC as compared to no mulch application in respective months during 2014 and 2015, respectively. Similar findings have been reported by Dı´az-Pe´rez (3) in bell pepper.

The pooled analysis of data showed that plant height was significantly influenced with the application of different coloured mulches except white/ black mulch which recorded plant height at par with no mulch. The leaf area index was significantly influenced by application of different coloured plastic mulches. At 150 days after transplanting (DAT) the maximum leaf area index of bell pepper plants was observed in black/black plastic mulch which was statistically at par with silver/black coloured mulch but significantly superior than no mulch, yellow/ black and white/ black mulches (Table 1).

All the mulch treatments recorded significantly higher fruit length, width and girth as compared to no mulch treatment (Table 1). The maximum fruit length was recorded in silver/ black coloured mulch. All the mulch treatments recorded significantly higher single fruit weight as compared to no mulch (control). Mulching of the soil enhanced plant biomass production as evident from plant height and leaf area index values. Slightly lower yield under black/black mulch was probably because of soil temperature rise beyond optimum limits towards end of the growing season which reduced the yield slightly as compared to silver/ black mulch. Similar findings have been reported by Dı´az-Pe´rez (3).

The quality parameters showed that maximum dry matter %, ascorbic acid content (165.0 mg/100 g) in the fruit was recorded under black/ black mulch, followed by silver/ black mulch. The lowest dry matter and ascorbic acid content was recorded in no mulch (control). However, the maximum capsaicin content was recorded in the fruits from plants grown on silver/ black plastic mulch (0.34%), which was closely followed by black/ black, yellow/black and white/ black mulch. The lowest capsaicin content was observed in no mulch plots (0.23%). Awasthi *et al.* (2) also observed higher ascorbic acid in brinjal fruits under plastic mulches as compared to control plot.

The soil moisture content in 0-15 cm soil profile under different mulches was not significantly influenced under different mulches. The weed growth was influenced by use of different coloured plastic mulches (Table 2). It was observed that in plots with plastic mulch only *Cyperus rotundus* emerged

**Table 1.** Yield attributes and fruit yield of bell pepper as influenced by different coloured plastic mulches (pooled data).

| Treatment | Plant height at maturity (cm) | Leaf area index 150 DAT* | Fruit length (cm) | Fruit width (cm) | Fruit girth (cm) | Fruit weight (g) | Fruit yield (q/ha) |
|---|---|---|---|---|---|---|---|
| Yellow/ black mulch | 42.3 | 1.85 | 7.79 | 6.79 | 19.8 | 70.9 | 245.6 |
| Black/ black mulch | 43.9 | 2.30 | 8.38 | 7.55 | 20.4 | 79.6 | 280.3 |
| Silver/ black mulch | 44.7 | 2.10 | 8.77 | 7.87 | 20.8 | 83.2 | 291.6 |
| White/ black mulch | 39.2 | 1.88 | 7.98 | 7.55 | 18.8 | 73.0 | 226.7 |
| No mulch | 37.4 | 1.56 | 5.98 | 5.43 | 18.3 | 58.9 | 188.3 |
| CD (P = 0.05) | 4.09 | 0.22 | 1.23 | 1.08 | 1.28 | 9.45 | 21.4 |

DAT = Days after transplanting

**Table 2.** Effect of coloured plastic mulches on fruit quality of bell pepper, weed growth and soil moisture (pooled data).

| Treatment | Quality parameters | | | Weed growth | | Soil moisture (%) | |
|---|---|---|---|---|---|---|---|
| | Dry matter (%) | Vit C (mg/ 100 g) | Capsaicin content (%) | Weed count /m$^2$ (No) 45 DAT | Weed dry matter (g /m$^2$) 135 DAT | 0-15 cm | 15-30 cm |
| Yellow/ black mulch | 4.38 | 133.9 | 0.31 | 14 | 31.2 | 14.2 | 14.9 |
| Black/ black mulch | 5.17 | 165 | 0.32 | 11 | 16.6 | 13.5 | 12.9 |
| Silver/ black mulch | 4.65 | 160.6 | 0.34 | 12 | 18.3 | 14.4 | 13.8 |
| White/ black mulch | 4.07 | 130.8 | 0.27 | 16 | 35.0 | 16.9 | 14.0 |
| No mulch | 3.77 | 115.1 | 0.23 | 280 | 76.2 | 16.5 | 16.4 |
| CD (P = 0.05) | -- | -- | -- | -- | -- | NS | NS |

by penetrating through plastic sheet and no other weed was observed during the first count done 45 days after transplanting of the seedlings. However, at later stages (135 DAT) of crop growth some of the above mentioned weeds emerged near bell pepper plants where holes were made for transplanting of the seedlings. The weed dry matter recorded at 135 days after transplanting showed that maximum reduction in weed dry matter (78.2%) was recorded in black/ black mulch, which was closely followed by silver/ black mulch (76.0%). Similar findings have been reported by Aniekwe *et al.* (1) where 100% control of weeds has been observed under black plastic mulch in cassava in Nigeria.

The cost of mulching for one ha area assuming that 2/3$^{rd}$ area of the field has to be covered with mulch if the beds were made 60 cm wide and 30 cm space was left between the two beds. The quantity of mulch required for one ha area (10,000 × 2/3) will be approximately 6700 m$^2$, the cost of plastic mulch was Rs. 7/ m$^2$. Therefore, cost of mulching for one hectare will be (6700 ×7) = Rs. 46,900. If the produce is sold at Rs. 10/ kg, the benefit in terms of increased yield under best treatment silver/ black mulch will be = 103.3 q × 1000= Rs 1,03,300/-. Therefore, net benefit of mulching as compared to control will be Rs. 56,400/- (Rs. 1,03,300 - 46,900). From the above study it can be concluded that for growing bell pepper crop under open field conditions silver/ black and black/ black coloured mulches can be used to get higher yield. The use of silver/ black mulch recorded 54.9% increase in fruit yield.

## ACKNOWLEDGEMENT

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***Short communication***

# Stability analysis of yield and its component traits in coriander germplasm

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## ABSTRACT

**Stability analysis were done in 42 coriander genotypes including three check varieties during *rabi* 2010-11 and 2014-15 at TNAU, Coimbatore with *per se* performance. The analysis of pooled data indicated highly significant differences among the genotypes and environments for all the traits. The variance due to genotype and environments were highly significant for all the traits. Highly significant pooled deviation for umbels per plant and seed yield per hectare and highly significant G × E (linear) interaction for pods per plant and seed yield per plant indicated the preponderance of non-linear components of G × E interaction. The genotype CS 245 had shown consistent performance and stability in wider environments for seed yield per plant, whereas CS 14 has shown consistent performance in poor environment for seed yield per plant.**

**Key words:** *Coriandrum sativum*, seed yield, stability, sustainability index.

Coriander (*Coriandrum sativum* L.) commonly known as '*dhania*' belongs to family Apiaceae. Coriander seeds are also used as tonic, carminative, diuretic, stomachic and as an aphrodisiac. Although coriander has got diverse uses the knowledge on the extent and magnitude of genetic variability of agronomic and quality traits is limited. Thus, there is a great scope for crop improvement in coriander for increasing yield and quality potential in order to increase the yield, production, productivity and quality components of this important seed spice. Adequate information is not available with respect to adaptability of coriander genotypes to seasonal and environmental variations. Due to its multipurpose use, cultivation is increasing in the non-traditional areas of the country. The farmers of different states grow the landraces available with them. The majority of coriander varieties were developed from available germplasm and the performance of coriander germplasm at different years is of great importance in respect of screening them for their stability. The G × E interaction shows the differential response of genotypes to different environmental conditions and their consistency in performance over the years. An ideal variety should have a high mean yield combined with a low degree of fluctuations when grown over diverse environments (Arshad *et al*., 1).

The experimental materials comprising 42 coriander genotypes were grown during five consecutive years (from 2010-11 to 2014-15) at Tamil Nadu Agricultural University, Coimbatore. The experiment was conducted under irrigated conditions in randomized block design with three replications. The size of plot was 2 m × 2 m and seeds were sown at 20 cm × 10 cm. The observations were recorded on number of umbels per plant, number of umbellate per plant and seed yield per plant (g). The data were analyzed statistically for stability parameters based on model (Eberhart and Russel, 2). The sustainability indices (SI) were estimated as per the following formula used by earlier workers (Gangwar and Anand, 4). The sustainability index were divided into five groups, *viz*., very low (upto 20%), low (21-40%), moderate (41-60%), high (61-80%) and very high (above 80%).

The stability analysis of variance mean data (Table 1) revealed highly significant differences among the genotypes as well as environments for all the traits. Genotype × Environment (G × E) interaction was studied for seed yield per plant and its component characters, *i.e.* number of umbels per plant and number of umbellates per plant. Highly significant mean squares due to environment (linear) for all the traits indicated considerable differences among the environments and their predominant effects on the traits. This was due to variation in climatic conditions during years. Highly significant pooled deviations for number of umbels per plant, number of umbellates per plant and seed yield per plant indicated non-linear response of the genotypes due to environmental changes and greater role of unpredictable components of G × E interaction towards differences in stability of the genotypes. It is reported that both predictable and

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**Table 1.** Pooled analysis of variance of traits for stability in coriander.

| Source of variation | df | Mean square | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant height | No. of primary branches per plant | No. of secondary branches per plant | No. of umbels per plant | No. of umbellates per umbel | Seed yield per plant |
| Genotype (G) | 41 | 48.03** | 0.96** | 9.65** | 43.63** | 0.97* | 1.44* |
| Environment (E) | 4 | 596.64** | 24.90** | 579.88** | 1654.72** | 123.67** | 51.27** |
| G × E | 164 | 37.32* | 0.67 | 6.23 | 35.47* | 0.52 | 0.48* |
| Env. (linear) | 1 | 2385.92** | 99.60** | 2319.57** | 6618.82** | 494.69** | 205.09** |
| G × E (linear) | 41 | 71.15** | 0.71 | 7.05* | 45.27* | 0.75 | 0.41* |
| Pooled deviation | 123 | 25.43** | 0.64** | 5.81** | 31.43* | 0.44 | 0.49* |
| Pooled error | 410 | 4.03 | 4.03 | 0.26 | 0.91 | 5.48 | 1.38 |

unpredictable components contributed significantly towards the differences in stability of fenugreek genotypes (Mathur and Lal, 5; Gangopadhyay *et al*., 3). However, prediction for unpredictable traits can be made by considering the stability parameters of individual genotypes (Singh *et al*., 6).

The linear regression analysis facilitates identification of genotypes having wider adaptability over a range of environment. The stability analysis was done following the model of Eberhart and Russel (2), which suggested two stability parameters (i) linear regression, and (ii) deviation from such regression. According to them a stable variety will have high mean performance, regression coefficient ($b_i$) near unity, and deviation from regression ($s^2d_i$) close to zero. Therefore, all the three parameters, *i.e*., mean, linear regression and non-linear responses seems to be equally important. For the trait umbels per plant, the single genotype CS 221 (25.8, 1.17 and 0.63) recorded high mean value, significant regression coefficient along with non-significant deviation from regression indicating their stability and suitability to favourable environments (Table 2). For the same trait the genotype CS 212 (28.3, 0.93 and 6.83) recorded high population mean, non significant regression co-efficient and deviation from regression and were found stable and suitable for wider environments. Four genotypes, namely, CS 59, CS 240, CS 244 and CS 271 recorded more number of umbellate per umbel than population mean (5.5), significant regression co-efficient and non-significant deviation from regression and were found stable and suitable for favourable environments. High mean value over the population mean (0.66), significant regression co-efficient and non-significant deviation from regression was recorded in the genotype CS 114 for seed yield per plant indicating their stability and suitability to favourable environments (Table 2).

It was reported that the generalization regarding stability of a variety for all the descriptors is rather difficult (Singh and Singh, 7). In the present investigations also, genotypes did not show uniform stability and linear response pattern for all the traits. However, the overall stability may be considered on the basis of compensation pattern of different traits. For seed yield per hectare the sustainability index (SI) for all the genotypes ranged from 71.84% (CS 14) to 94.62% (CS 245). The check CS 105 recorded the highest SI (79.34%) among all the checks (Table 2), indicating low fluctuations in its performance over the locations as compared to checks. Among the seven genotypes identified for wider adaptability, four genotypes, namely, CS 245, CS 46, CS 187 and CS 228 showed high SI, thus indicating that the genotypes would give better performance consistently over the diverse environments. CS 245, which showed suitability for favorable environment also showed high SI indicating consistent performance over years in favourable environment. In case of number of umbels per plant, the genotypes qualified for wider adaptation namely, CS 134, CS 185, CS 46, CS 245 and CS 29 for favourable environment showed very high SI (Table 2). On the basis of above findings, it can be concluded that CS 245 has shown promising and consistent performance in wider environments for seed yield per plant and number of umbels per plant where as the genotype CS 14 has shown promising and consistent performance in poor environment for seed yield per plant.

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**Table 2.** Stability parameters for number of umbels per plant, number of umbellates per umbel and seed yield per plant in coriander.

| Genotype | No. of umbels per plant | | | | No. of umbellates per umbel | | | | Seed yield per plant | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | $b_i$ | $S^2d_i$ | SI | Mean | $b_i$ | $S^2d_i$ | SI | Mean | $b_i$ | $S^2d_i$ | SI |
| CS 11 (C) | 29.0 | 1.77 | 20.37 | 82.93 | 4.5 | 0.50* | 0.04* | 61.52 | 2.61 | 1.39 | 0.46 | 77.43 |
| CS 14 | 27.7 | 1.73 | 51.31 | 78.79 | 5.0 | 0.54 | 1.45 | 69.09 | 2.43 | 1.06 | 1.55 | 71.72 |
| CS 29 | 29.4 | 1.85 | 38.03 | 84.20 | 4.8 | 0.53 | 0.47 | 66.06 | 2.48 | 1.44 | 0.72 | 73.31 |
| CS 37 | 26.1 | 1.71 | 2.71* | 73.69 | 5.5 | 1.15* | -0.04* | 76.67 | 2.50 | 1.48 | 0.09* | 73.94 |
| CS 46 | 29.9 | 0.85 | 9.88 | 85.80 | 5.4 | 0.93 | 0.35 | 75.15 | 2.99 | 1.47 | 0.13 | 89.50 |
| CS 57 | 26.8 | 0.88 | 27.79 | 75.92 | 5.7 | 1.22 | 1.01 | 79.70 | 2.80 | 1.01 | 0.91 | 83.47 |
| CS 58 | 24.6 | 1.11 | 117.52 | 68.92 | 5.5 | 1.29 | 0.36 | 76.67 | 2.89 | 1.45* | 0.02* | 86.32 |
| CS 59 | 26.5 | 0.69 | 21.38 | 74.97 | 6.0 | 1.30* | 0.06 | 84.24 | 2.57 | 0.86 | 0.43 | 76.17 |
| CS 91 | 27.3 | 1.30 | 11.34 | 77.52 | 5.7 | 1.10 | 0.09 | 79.70 | 2.59 | 1.22 | 0.55 | 76.80 |
| CS 94 | 25.9 | 1.76 | 56.79 | 73.06 | 5.6 | 0.88 | 0.11 | 78.18 | 2.44 | 1.24 | 1.26 | 72.04 |
| CS 101 | 22.2 | 1.21 | 13.30 | 61.27 | 5.5 | 1.07* | -0.02 | 76.67 | 2.64 | 1.25 | 0.39 | 78.39 |
| CS 105 (C) | 25.7 | 0.85 | 26.88 | 72.42 | 5.9 | 1.02 | 0.26 | 82.73 | 2.67 | 1.28 | 1.87 | 79.34 |
| CS 114 | 22.5 | 0.64 | 45.13 | 62.23 | 5.6 | 1.07 | 1.19 | 78.18 | 2.67 | 1.23* | 0.01 | 79.34 |
| CS 116 | 31.4 | 2.17 | 74.79 | 90.57 | 5.8 | 1.18 | 0.25 | 81.21 | 2.63 | 1.03 | 0.22 | 78.07 |
| CS 119 (C) | 24.4 | 1.14 | 11.05 | 68.28 | 5.0 | 0.89 | 0.13 | 69.09 | 2.52 | 1.20* | 0.02 | 74.58 |
| CS 121 | 26.4 | 1.58 | 3.64* | 74.65 | 4.9 | 1.10 | 0.19 | 67.58 | 2.52 | 0.48 | 1.11 | 74.58 |
| CS 134 | 31.3 | 0.65* | 0.56* | 90.25 | 5.4 | 0.65 | 0.62 | 75.15 | 2.59 | 1.40 | 0.41 | 76.80 |
| CS 144 | 22.8 | 0.48 | 37.61 | 63.18 | 6.6 | 0.78 | 1.53 | 93.33 | 2.46 | 0.98 | 1.34 | 72.67 |
| CS 167 | 23.4 | 0.87 | 31.55 | 65.10 | 5.0 | 0.93 | 0.19 | 69.09 | 2.53 | 1.09 | 0.08 | 74.90 |
| CS 170 | 21.5 | 1.14 | 82.93 | 59.04 | 5.4 | 1.37 | 1.58 | 75.15 | 2.48 | 0.61 | 0.25 | 73.31 |
| CS 180 | 18.6 | 0.80 | 36.53 | 49.81 | 5.2 | 1.21 | 0.31 | 72.12 | 2.61 | 0.60 | 1.05 | 77.43 |
| CS 185 | 30.4 | 0.61 | 12.96 | 87.39 | 5.3 | 0.97* | -0.04 | 73.64 | 2.73 | 1.03 | 0.42 | 81.24 |
| CS 186 | 24.3 | 1.39 | 53.28 | 67.96 | 5.8 | 1.20 | 0.11 | 81.21 | 2.62 | 0.53 | 0.73 | 77.75 |
| CS 187 | 23.7 | 1.26 | 14.02 | 66.05 | 5.1 | 1.02 | 0.12 | 70.61 | 2.92 | 0.72 | 0.56 | 87.28 |
| CS 210 | 25.3 | 0.59 | 32.42 | 71.15 | 5.6 | 0.76 | 0.29 | 78.18 | 2.70 | 1.06 | 0.06 | 80.29 |
| CS 211 | 27.7 | 1.21 | 17.30 | 78.79 | 5.4 | 1.21 | 1.17 | 75.15 | 2.90 | 0.74 | 0.58 | 86.64 |
| CS 212 | 28.3 | 0.93 | 6.83 | 80.70 | 4.8 | 0.62 | 0.85 | 66.06 | 2.47 | 0.93 | 0.46 | 72.99 |
| CS 215 | 26.9 | 1.11 | 32.28 | 76.24 | 5.6 | 1.32 | 0.94 | 78.18 | 2.52 | 0.93 | 0.20 | 74.58 |
| CS 221 | 25.8 | 1.17* | 0.63 | 72.74 | 5.3 | 1.12 | 0.94 | 73.64 | 2.52 | 0.29 | 0.57 | 74.58 |
| CS 225 | 24.5 | 0.81 | 19.36 | 68.60 | 5.5 | 0.90* | -0.04* | 76.67 | 2.80 | 1.27 | 1.07 | 83.47 |
| CS 227 | 29.0 | 0.71 | 23.58 | 82.93 | 6.6 | 1.43* | 0.03* | 93.33 | 2.53 | 1.01 | 0.45 | 74.90 |
| CS 228 | 24.6 | 0.27 | 7.62 | 68.92 | 6.1 | 1.27 | 0.20 | 85.76 | 2.92 | 0.87 | 0.33 | 87.28 |
| CS 229 | 24.6 | -0.12 | 9.77* | 68.92 | 5.5 | 0.82* | -0.04* | 76.67 | 2.48 | 0.92 | 0.08 | 73.31 |
| CS 237 | 22.5 | 0.20 | 16.39 | 62.23 | 5.2 | 1.11* | -0.04 | 72.12 | 2.60 | 0.70 | 0.15 | 77.12 |
| CS 238 | 25.3 | 0.49 | 39.16 | 71.15 | 5.5 | 0.91 | 0.56 | 76.67 | 2.48 | 0.89 | 0.25 | 73.31 |
| CS 240 | 23.5 | 0.49* | 1.01* | 65.41 | 5.7 | 1.05* | 0.01 | 79.70 | 2.55 | 0.85 | 0.63 | 75.53 |
| CS 241 | 23.9 | 0.71 | 64.64 | 66.69 | 5.8 | 1.27 | 0.53 | 81.21 | 2.54 | 0.66* | 0.00** | 75.21 |
| CS 242 | 29.5 | 1.85 | 21.64 | 84.52 | 5.0 | 0.98* | -0.05 | 69.09 | 2.63 | 1.05 | 0.06 | 78.07 |
| CS 244 | 23.0 | 0.82 | 30.39 | 63.82 | 5.5 | 1.18* | 0.08 | 76.67 | 2.89 | 0.76 | 0.21 | 86.32 |
| CS 245 | 28.7 | 1.77 | 131.42 | 81.97 | 5.4 | 0.89 | 0.15 | 75.15 | 3.15 | 1.26 | 0.13 | 94.58 |
| CS 266 | 25.0 | 0.52 | 10.25 | 70.19 | 4.7 | 0.39 | 0.15* | 64.55 | 2.57 | 0.90 | 0.05 | 76.17 |
| CS 271 | 20.1 | 0.06 | 16.11 | 54.59 | 5.5 | 0.86* | 0.07 | 76.67 | 2.52 | 0.88 | 0.04 | 74.58 |
| Population mean | 25.71 | 1.00 | | | 5.45 | 1.00 | | | 2.63 | 1.00 | | |
| SE (mean) | 2.84 | 0.45 | | | 0.33 | 0.20 | | | 0.35 | 0.32 | | |

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***Short communication***

# Studies on stability and quality of *jamun*-mango blended ready-to-serve beverage

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## ABSTRACT

***Jamun* fruits are sweet and sour which are rich in nutrients but not acceptable due to high acidity or poor taste and flavour. However, its juice can be blended with other fruits to improve the acceptability and make use of available nutrients. Hence, an experiment was carried out to study blended *jamun*-mango juice for the preparation of *jamun*-mango blended ready-to-serve (RTS) beverages with 15% juice, 15°Brix total soluble solids and 0.2% acidity. *Jamun* and mango juices were blended in the ratio of 100% *jamun* juice as control and in the ratio of 90:10, 80:20, 70:30 and 60:40. During six months of storage, total soluble solids increased, whereas acidity decreased slightly. Anthocyanin content decreased throughout the storage period. The results of ready-to-serve beverage reveled that maximum iron (0.16 mg/100 g) was found in $T_1$ treatment (100% *jamun* juice). After six months of storage, sensory evaluation revealed that the treatment $T_3$ (80:20, *jamun*: mango) recorded the highest score for flavour (7.24), body (8.70) and for overall acceptability (7.66). The same combination retained the maximum anthocyanin content of 31.16 mg/100 g after 6 months of storage and was judged as the best.**

**Key words:** *Jamun*, mango, ready-to-serve beverage, storage.

India produced 88,977 metric tonnes of fruits and 1,62,897 metric tonnes of vegetables (Anon, 1). In fruit production, it ranks next to China. Unfortunately, a big chunk (20-30%) of this hard earned valuable produce goes waste due to inadequate post harvest infrastructure and poor utilization (1.8%) by processing industries (Verma and Joshi, 13). The demand for fruit beverages is increasing in India as well as in other countries due to increasing trend towards fast food. Fruit beverages have higher nutritional and medicinal values compared to synthetic beverages and these can be improved further by blending juice of two or more juices having nutritive and therapeutic values. Tiwari (12) standardized papaya and guava pulp for making ready-to-serve beverage. Similarly, Sharma *et al.* (10) also worked on the blending of juices of different mango varieties. Blending of fruit juices helps in improving nutrient elements, reducing cost of production by using cheaper fruits in the blends and also leads to new product development. Moreover, fruits which are rich in nutrients but not acceptable due to high acidity or poor taste and flavour can be blended with other fruits to improve their acceptability and make use of available nutrients. Some consumer avoid taking *jamun* fruit because of astringent taste but prefer it if suitable fruit products are prepared from this fruit. Hence, the study was conducted with the objective to develop value-added products from *jamun*-mango blends and to study storability of the finished product.

Mature fruits of *jamun* (*Syzigium cumini* Linn.) were obtained from the avenue trees of the R.S. Pura orchard and mango cv. Dashehari fruits were purchased from Fruit Trans-Shipment Centre (FTC) Narwal, Jammu. Both *jamun* and mango fruits were transported to the pilot plant of the Division of Post-Harvest Technology, SKUAST, Udheywalla, Jammu or further processing. The defective and injured fruits of *jamun* and mango were sorted out and healthy ones were retained for juice extraction after washing with water. The juice so obtained was passed through stainless steel strainer, homogenized followed by heating at 85°C for 30 sec. and filling in pre-sterilized glass bottles. Bottles were crown corked and pasteurized for 20 min. in boiling water, cooled, labeled and stored at ambient temperature for further use. The juice so obtained was also analyzed for physico-chemical and organoleptic characters. The *jamun* and mango juices were blended with each other in different ratio such as 100:00; 90:10; 80:20; 70:30 and 60:40, respectively for developing ready-to-serve beverage. The prepared blends were heated at 85 ± 5°C for 2 min., hot packed in pre-sterilized glass bottles followed by 20 min. processing in boiling water, cooling and labeling. RTS beverages were prepared using 15 per cent of juice from the blended ratios,

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*viz*., $T_1$ (15% juice of 100:00 *jamun*-mango blend), $T_2$ (15% juice of 90:10 *jamun*-mango blend), $T_3$ (15% juice of 80:20 *jamun*-mango blend), $T_4$ (15% juice of 70:30 *jamun*-mango blend) and $T_5$ (15% juice of 60:40 *jamun*-mango blend). Total soluble solids (15°Brix) and acidity (0.3%) of the beverage was adjusted using sugar and citric acid. The beverage was heat processed at 85°C for 30 sec. filled in pre-sterilized glass bottles of 200 ml capacity, crown corked and pasteurized for 30 min. in boiling water followed by immediate cooling, labeling and storing. The beverage was preserved with 200 ppm sodium benzoate. The ready-to-serve beverages prepared were stored at room temperature (30-42°C) for periodical physico-chemical analysis and organoleptic evaluation after 0, 2, 4 and 6 months (Ranganna, 9). Sensory appeal was determined by appearance, texture and flavour (Bourne, 3). Anthocyanins content were determine as per the method suggested by Swain and Hillis (11) and iron estimation was done as per the standard procedures described by AOAC (2). The samples were evaluated on the basis of colour, body, flavour and overall acceptability by semi-trained taste panels of 6-7 judges using 9 point hedonic scale. A score of 5.5 and above was considered acceptable (Bourne, 3). The experiment was carried out in completely randomized design and completely randomized design with factorial concept for interpretation of results through analysis of variance (Gomez and Gomez, 5).

All the treatments showed a significant increase in total soluble solids of *jamun*-mango blended ready to serve beverage during storage (Table 1). The increase in total soluble solid during storage

**Table 1.** Changes in chemical composition of *jamun*-mango blended ready-to-serve (RTS) beverage during storage.

| Treatment | Storage period (months) | | | | | $CD_{0.05}$ |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | Mean | |
| Total soluble solids (°Brix) | | | | | | |
| $T_1$ | 15.0 | 16.34 | 17.05 | 17.71 | 16.52 | Treatment (T) = 0.04 |
| $T_2$ | 15.0 | 16.45 | 17.14 | 17.86 | 16.62 | Storage (S) = 0.03 |
| $T_3$ | 15.0 | 16.52 | 17.35 | 17.43 | 16.58 | T × S = 0.07 |
| $T_4$ | 15.0 | 16.66 | 17.46 | 17.95 | 16.77 | |
| $T_5$ | 15.0 | 16.81 | 17.62 | 18.02 | 16.87 | |
| Mean | 15.0 | 16.56 | 17.33 | 17.79 | | |
| Titratable acidity (%) | | | | | | |
| $T_1$ | 0.3 | 0.27 | 0.26 | 0.22 | 0.26 | Treatment (T) = NS |
| $T_2$ | 0.3 | 0.28 | 0.25 | 0.23 | 0.27 | Storage (S) = 0.03 |
| $T_3$ | 0.3 | 0.27 | 0.26 | 0.23 | 0.27 | T × S = NS |
| $T_4$ | 0.3 | 0.26 | 0.24 | 0.20 | 0.25 | |
| $T_5$ | 0.3 | 0.28 | 0.26 | 0.23 | 0.27 | |
| Mean | 0.3 | 0.27 | 0.25 | 0.22 | | |
| Anthocyanins (mg/100 g) | | | | | | |
| $T_1$ | 34.7 | 31.16 | 28.10 | 26.60 | 30.14 | Treatment (T) = 1.52 |
| $T_2$ | 34.2 | 33.30 | 28.50 | 25.60 | 30.39 | Storage (S) = 1.36 |
| $T_3$ | 33.7 | 33.20 | 32.40 | 31.16 | 32.62 | T × S = NS |
| $T_4$ | 31.3 | 28.50 | 26.60 | 24.60 | 27.75 | |
| $T_5$ | 28.8 | 26.50 | 24.70 | 21.60 | 25.40 | |
| Mean | 32.5 | 30.53 | 28.06 | 25.91 | | |
| Iron (mg/100 g) | | | | | | |
| $T_1$ | 0.18 | 0.16 | 0.15 | 0.14 | 0.16 | Treatment (T) = 0.02 |
| $T_2$ | 0.16 | 0.15 | 0.14 | 0.13 | 0.15 | Storage (S) = 0.02 |
| $T_3$ | 0.15 | 0.14 | 0.14 | 0.130 | 0.14 | T × S = NS |
| $T_4$ | 0.14 | 0.12 | 0.11 | 0.10 | 0.12 | |
| $T_5$ | 0.13 | 0.11 | 0.10 | 0.09 | 0.11 | |
| Mean | 0.15 | 0.14 | 0.13 | 0.12 | | |

$T_1$ (100:0), $T_2$ (90:10), $T_3$ (80:20), $T_4$ (70:30), $T_5$ (60:40)

was also reported by Pandey and Singh (8) in guava ready-to-serve drink. The possible reason for the increase in total soluble solids might be due to partial hydrolysis of complex carbohydrates. After six months of storage, the maximum per cent of titratable acidity was recorded as 0.23 per cent in $T_2$ (90:10), $T_3$ (80:20) and in $T_5$ (60:40) and minimum of 0.22 per cent in $T_4$ (70:30). During storage, acidity of ready-to-serve decreased, which was in conformity with the findings of Kannan and Thirumaran (6), while working on *jamun* fruit products. Decrease in acidity during storage might be due to co-polymerization of organic acids with sugars and amino acids and loss of volatile acids during storage.

Highest anthocyanin recovery (31.16 mg/100 g) was recorded in $T_3$ (80:20) treatment with the advancement of storage period. This might be due to hydrolysis of protective 3-gycoside linkage to give unsuitable anthocyanin. These results were supported by Kannan and Thirumaran (6) in *jamun* products. The iron content of the ready-to-serve beverage decreased during storage. The treatment $T_1$ (100:00) recorded the highest iron content (0.14 mg/100 g) and the lowest iron content (0.09 mg/100 g) was in $T_5$ (60:40) and the possible reason for the decrease might be due to its heat sensitivity even at the ambient temperature, which causes the destruction of minerals during storage.

A decreasing trend in the sensory evaluation was noticed throughout the storage period in the developed samples. After six months of storage, treatment $T_3$ (80:20) received the highest score of 7.24 for flavour and 7.66 for overall acceptability (Table 2). The possible reason for decrease in flavour was due to the loss of volatile aromatic substances in storage at ambient conditions. In general, decrease in sensory score of different characteristics of *jamun*-mango blended ready-to-serve beverage, irrespective of treatments, during storage might be attributed colour changes occurring in appearance and taste of the product. Pandey and Singh (8) in guava beverage, Dwivedi *et al.* (4) in seabuckthorn beverage and Kannan and Thirumaran (6), while working on *jamun* product also reported that the organoleptic score of the products declined during storage due to formation of off-flavour, browning thus masking the original flavour/ taste. Kumar and Manimegalai (7) also reported similar findings with respect to organoleptic score in mixed fruit ready-to-serve beverages. All the samples were found to be free from microbial count upto four months of storage. However, after six months of storage only treatments $T_4$ (70:30) and $T_5$ (60:40) showed a negligible count of $1\times10^6$ (CFU/ml), which might have occurred during handling etc. and was in safe zone. Storability of ready-to-serve beverage showed that $T_3$ (80:20, *jamun*: mango) was adjudged the best by way of retaining the maximum anthocyanin content after 6 months of storage. The same treatment scored maximum points for flavour, body, colour and overall acceptability after 6 months of storage (Fig. 1).

**Table 2.** Changes in sensory quality of *jamun*-mango blended ready-to-serve (RTS) beverage during storage.

| Treatment | Storage period (months) | | | | | $CD_{0.05}$ |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | Mean | |
| Flavour | | | | | | |
| $T_1$ | 7.44 | 7.34 | 7.04 | 6.26 | 7.02 | Treatment (T) = 0.02 |
| $T_2$ | 7.44 | 7.34 | 7.24 | 6.15 | 7.04 | Storage (S) = 0.02 |
| $T_3$ | 8.76 | 7.54 | 7.34 | 7.24 | 7.72 | T × S = 0.03 |
| $T_4$ | 7.54 | 7.42 | 7.34 | 6.25 | 7.14 | |
| $T_5$ | 7.54 | 7.56 | 7.44 | 6.34 | 7.22 | |
| Mean | 7.74 | 7.44 | 7.28 | 6.45 | | |
| Overall acceptability | | | | | | |
| $T_1$ | 8.04 | 7.84 | 7.72 | 7.64 | 7.81 | Treatment (T) = 0.02 |
| $T_2$ | 7.96 | 6.75 | 7.64 | 7.56 | 7.48 | Storage (S) = 0.01 |
| $T_3$ | 8.64 | 8.34 | 7.84 | 7.66 | 8.12 | T × S = 0.03 |
| $T_4$ | 7.84 | 7.62 | 7.54 | 7.42 | 7.61 | |
| $T_5$ | 7.82 | 7.66 | 7.54 | 7.46 | 7.62 | |
| Mean | 8.06 | 7.64 | 7.66 | 7.55 | | |

$T_1$ (100:0), $T_2$ (90:10), $T_3$ (80:20), $T_4$ (70:30), $T_5$ (60:40)

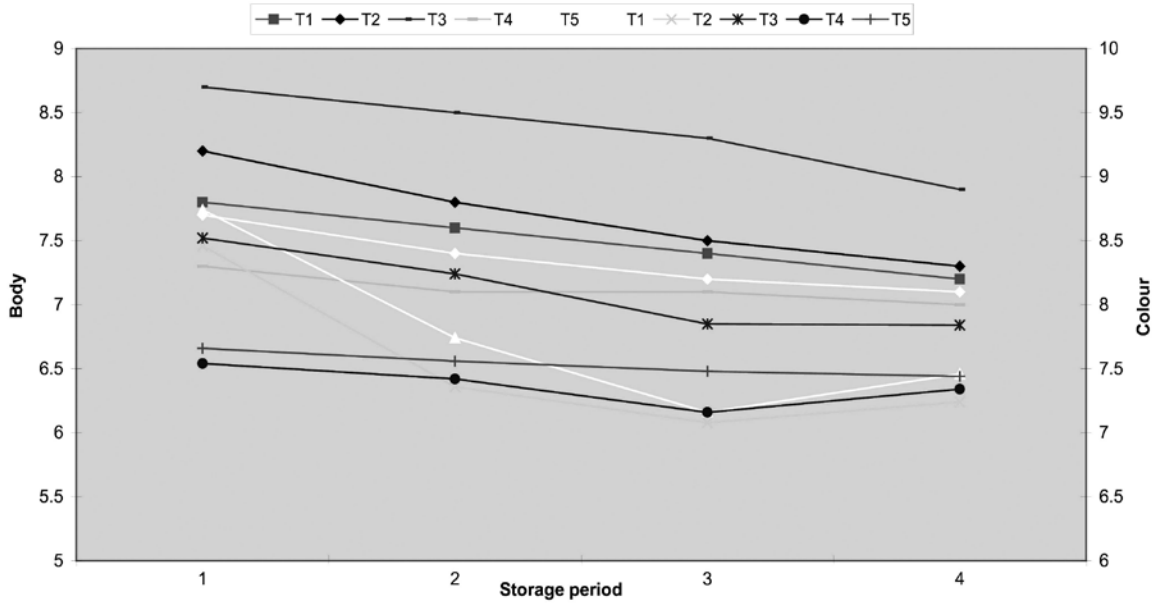


**Fig. 1.** Effect of treatments and storage period on mean score evaluation of body and colour of *jamun*-mango blended ready-to-serve (RTS) beverage.

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***Short communication***

# Enzymatic and physico-chemical changes in pear fruits in response to post-harvest application of oxalic acid

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## ABSTRACT

**To study the effect of oxalic acid (OA) on storage behaviour of 'Nijisseiki' pear, fruits were dipped in aqueous solutions of OA @ 2, 4 and 6 mmoll$^{-1}$ for 5 min. Treated and untreated fruits were packed in corrugated fibre board (CFB) boxes and stored at 0-1°C and 90-95% RH. Fruits were analyzed for various physico-chemical changes after 0, 30, 50 and 70 days of storage. As compared to control, OA treatments were found effective in decreasing physiological loss in weight (PLW), spoilage and maintained the fruit firmness, sensory quality, total soluble solids (TSS), titratable acidity (TA), and pectin methyl esterase (PME) activity under low temperature storage. After 70 days of storage, minimum PLW (5.95%), spoilage (3.85%), and maximum fruit firmness (6.95 lbf), TSS (12.19%), TA (0.209%), sensory quality score (5.76) and PME activity (1.29 ml of 0.02 N NaOH) was registered in OA @ 6 mmoll$^{-1}$ treated fruits.**

**Key words:** Oxalic acid, pear, physico-chemical changes, storage.

Pear is one of the important temperate fruits that can be successfully grown under sub-tropical conditions of Punjab because of its wider climatic and soil adaptability. 'Nijisseiki' is recently released variety of pear under local conditions and fruits of this variety are russet brown in colour with soft-pulp. Fruits are harvested during end of June to first week of July when weather is hot and humid, which leads to heavy post-harvest losses. Therefore, there is a need to contrive a suitable technique that could reduce the after harvest losses and maintain the quality of the fruits during storage. Many storage techniques have been developed so far to extend the post-harvest shelf-life of fruits. Recently, application of OA as post-harvest treatment in fruits has received considerable attention. It is an organic acid occurring naturally in plants and fungi and is reported to maintain the membrane integrity and delaying fruit ripening process (Tarabih, 8). Oxalic acid maintained titratable acidity, soluble solids concentration and fruit firmness by reducing activities of cell wall hydrolyzing enzymes (Kant *et al.*, 4). However, information on effect of oxalic acid on storage behaviour of sub-tropical pear fruits is lacking. Therefore, the present experiment was conducted to study the effect of oxalic acid in increasing the post-harvest life of fruits of pear cv. Nijisseiki during low temperature storage.

Physiological mature, healthy and uniform fruits of pear cv. Nijisseiki were picked from the Fruit Research Farm, PAU Ludhiana and were immediately transported to Post Harvest Laboratory. Fruits were dipped in oxalic acid solution @ 2, 4 and 6 mmoll$^{-1}$, for 5 min. Control fruits were given water dip only. Fruits were air-dried, packed in CFB boxes with 5% perforation and stored at 0-1°C and 90-95% RH. Fruits were analyzed for various physical and chemical changes at 0, 30, 50 and 70 days of storage. The PLW of stored fruits was calculated by subtracting final weight from the initial weight of the fruits and expressed in per cent. Firmness of fruits was measured with the help of a stand mounted penetrometer (Model FT-327, USA) using stainless steel probe with 8 mm plunger after removal of a piece of peel from opposite points on the fruit's equator and expressed as lbf. The fruits were evaluated for sensory quality and rated by panel of five judges on the basis of appearance; taste and flavour using 9 point Hedonic scale (1-9) as described by Amerine *et al.* (1). The spoilage was calculated as the number of spoiled fruits divided by total number of fruits multiplied by hundred and expressed in percentage (%). For the determination of TSS, the fruits were grated and extracted juice obtained was filtered through a cheese cloth. TSS was measured by using digital refractometer (Atago, PAL-1, model 3810, Japan) and expressed in (%). Titratable acidity was calculated by titrating two ml of strained juice against 0.1 N NaOH solution using phenolphthalein as an indicator and expressed in percentage (%). The pectin methyl esterase activity was determined as per method described by Mahadevan and Sridhar (5). Treatments were arranged as a factorial (factors: treatments and storage intervals) experiment in completely randomized design with three replications for each treatment and every replication comprised

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of 20 fruits. The data were analyzed for analysis of variance using statistical software SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Loss in weight of fruits increased with advancement of storage period irrespective of the treatments applied (Fig. 1a). The PLW occurred at faster rate during first 30 days of storage in all the treatments then a steady increase up to 50 days was recorded. During entire storage period, the minimum weight loss was noted in the fruits treated with oxalic acid @ 6 mmoll$^{-1}$ and the maximum was recorded in control fruits. The low weight loss in fruits treated with oxalic acid might be due to decreased ethylene production which delays the ripening process (Huang *et al*., 2). A significant decrease in fruit firmness was observed with the advancement of storage period for all the treatments (Fig. 1b). By the end of storage, maximum fruit firmness of 6.95 lbf was retained by the fruits treated with oxalic acid @ 6 mmoll$^{-1}$ and the minimum (6.0 lbf) was recorded for control fruits. Razzaq *et al*. (7) also reported the reduction in fruit softening by application of oxalic acid during ripening and storage of mango fruit. The sensory quality of fruits improved up to 30 days of storage in all the treatments followed by a decline up to end of storage. This improvement was highest in control fruits which recorded sensory quality score of 8.12 after one month storage. However, after 50 days of storage, significantly higher (7.65) sensory quality rating was recorded in OA @ 6 mmoll$^{-1}$ treated fruits as compared to control (6.0) fruits. The results are in agreement with the findings of Marboh *et al.* (6) who stated that oxalic acid treated fruits retained good sensory and quality attributes during cold storage. After one month of storage, the control fruits showed spoilage of 1.62%, while oxalic acid treated fruits did not showed any spoilage (Fig. 1d). At succeeding interval (50 days), spoilage in fruits was noticed in OA @ 0, 2, 4 mmoll$^{-1}$ treated fruits. Similarly, at the end of storage studies, significantly lower spoilage of 3.85% was recorded in OA @ 6 mmoll$^{-1}$ treated fruits, while it was maximum (6.70%) in untreated fruits. Oxalic acid treatment extends the storage time and decreases decay of mango fruits due to its fungistatic action (Zheng *et al*., 9). Total soluble solids content of oxalic acid treated fruits increased upto 50 days of storage and subsequently steadily declined (Fig. 1e). However, in control fruits a sharp increase in TSS content was observed and reached maximum (14.15%) after 30 days of storage followed by a decline, which was prominent after 50 days of storage. The decline in TSS might be due to its utilization in evapo-transpiration and other biochemical activities (Marboh *et al*., 6). Regardless of treatments applied, TA of stored pear fruits decreased with the advancement of storage period (Fig. 1f). At the end of storage the significantly higher TA of 0.209% was observed in fruits treated with oxalic acid @ 6 mmoll$^{-1}$ as compared to control fruits, which recorded minimum TA of 0.152%. The PME activity of fruits increased up to 30 days of storage and it was recorded significantly higher (1.76 ml of 0.02 N NaOH) in control fruits (Fig. 1g). After 50 days of storage, a decline in PME activity was noticed in all the treatments and reached to lowest level at the end of studies. An increase in enzyme activity with advancement of storage period might be attributed to increased availability of substrates through hydrolysis of starch into sugars and the decrease at later stage of storage could be attributed to the reduced substrate level due to decomposition. A similar decrease in PME activity at later stages of storage was also reported by Jawandha *et al.* (3) in *ber* fruits. From the present investigation, it can be inferred that oxalic acid treatment @ 6 mmol l$^{-1}$ is effective in extending the storage life of Nijisseiki pear fruits under low temperature storage.

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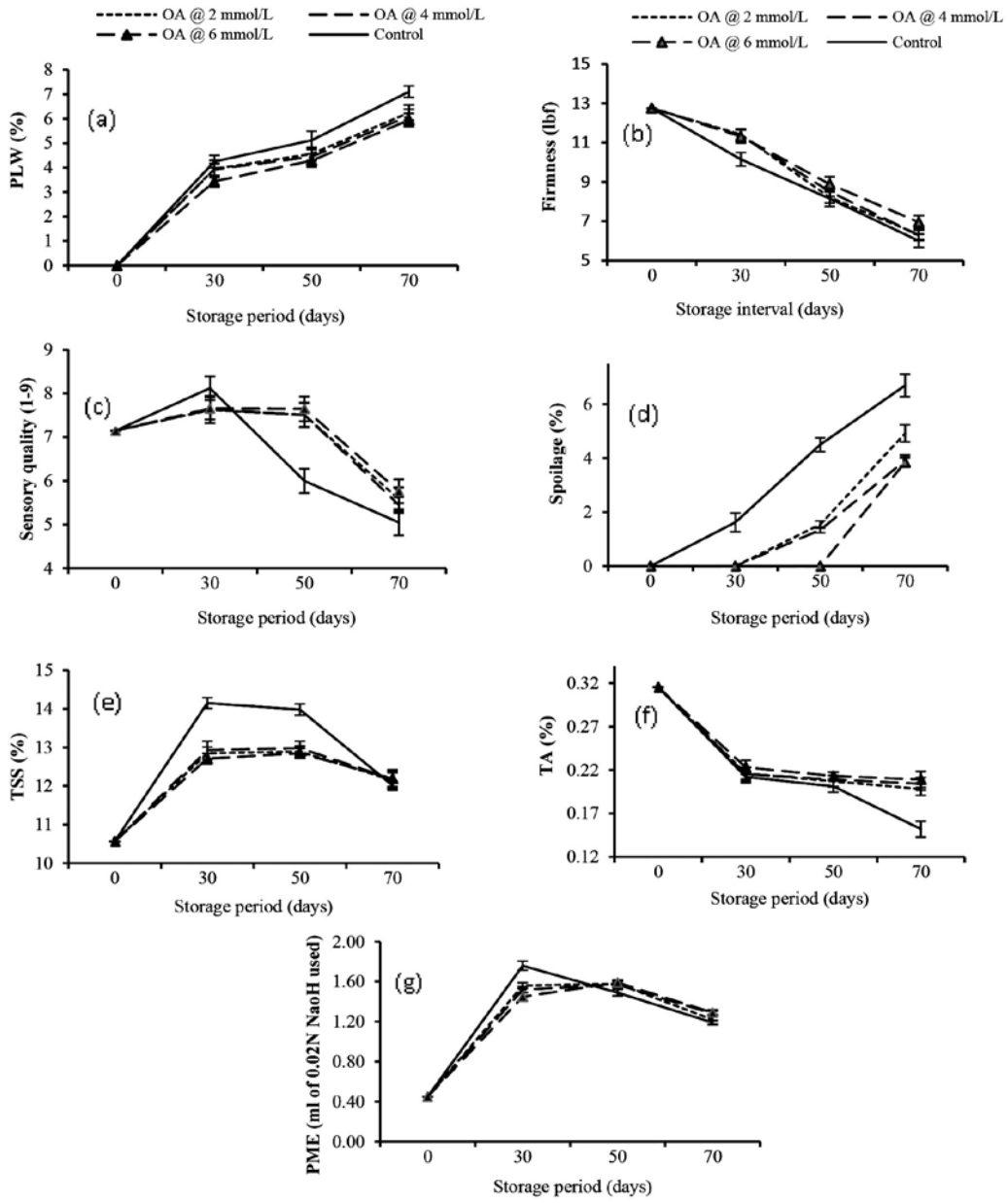


**Fig. 1.** Changes in PLW (a), firmness (b), sensory quality (c) spoilage (d), TSS (e), TA (f) and PME activity (g), in response to post-harvest oxalic acid treatments of pear fruits. Vertical bars represent ± SE of mean. LSD (P≤0.05) for PLW; Treatments (T) = 0.48, Storage periods (S) = 0.42, S × T = 0.84, for firmness; T = 0.53, S = 0.46, S × T = 0.93, for sensory quality; T = 0.46, S = 0.40, S × T = 0.80, for spoilage; T = 0.36, S = 0.31, S × T = 0.62, for TSS; T = 0.29, S = 0.25, S × T = 0.51, for TA; T = 0.012, S = 0.011, S × T = 0.022, for PME activity; T = 0.06, S = 0.05, S × T = 0.10

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***Short communication***

# *Anardana* (dehydrated wild pomegranate arils) as livelihood option for rural communities in Chenab valley of Jammu and Kashmir

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## ABSTRACT

**The study was conducted in Ramban district of Jammu and Kashmir to document present status, scope and socio-economic profile of the families involved in the collection and trade of *anardana* (*Punica granatum* L. '*Dhruni'*) towards better livelihood options for the rural communities. A random survey of Ganote, Dharam, Gool, Farmoot, Sangaldan, Gundi, Maha kund, and Chaderkot revealed that each household in these areas collects 400-500 kg of dried seed, with per household annual collection touching about 550-625 kg in Kanga and Parmote villages of Ramban. Study also revealed that the annual income from *anardana* was highest in Kanga village (Rs. 2,85,900/ ha) with 57.19% contribution to total household income, while it was lowest in Ganote Rs. 1,76,500/ ha (41.11%). However, its commercial potential is yet to be tapped. Good '*anardana*' fetches a price ranging between Rs. 300 to 400 per kg at village level, where the local commission agents working on behalf of traders at Jammu, Amritsar or Delhi procure in bulk. Some produce from nearby areas is also brought to Ramban market where it is sold in open auction. It was found that in addition to fulfilling the domestic needs, each household engaged in collection of '*anardana*' adds an average of Rs. 60,000 to their annual income.**

**Keywords:** *Anardana,* livelihood, non-timber forest products, socio-economics.

*Anardana* (*Punica granatum* L.), locally referred to as '*Dhruni*,' is an important fruit tree growing wild in hilly tracts and forests of Jammu and Kashmir state between 1000-2500 mean sea level (Saxena *et al*., 5). It also grows in upper extremities of sub-tropical forests in northern regions of India including Himachal Pradesh, Uttarakhand and Punjab. The pomegranate is a fruit-bearing deciduous shrub or small tree growing between 5 and 8 m (16-26 ft) tall. This small tree grows wild in Ramban, Rajouri, Doda and Udhampur districts where it forms extensive patches on open dry slopes. The fruits are harvested for its fleshy seeds, which are sundried to make '*anardana*', a product of commerce used in medicinal and culinary preparations. Collection of ripe fruits usually starts during August and continues upto October (Dhandar and Singh, 1). The seeds, commonly known as '*anardana*' are separated by hand and dried by spreading (Fig. 1). The conventional utilization of wild pomegranate fruit lies in the drying seeds along with pulp, which constitute the product ''*Anardana*'' (Pruthi and Saxena, 4). The dehydrated seeds are acidic (7.8-15.4%) and help in improving mouth-feel and digestion. *Anardana* is widely used as acidulent in culinary preparations and has HIGH vitamin C and minerals (Ca, Zn, Mn), and usefulness for making various digestive and other Ayurvedic medicines (Mahajan *et al*., 2).

The Jammu & Kashmir have a large variety of miscellaneous non-timber forest products (NTFP) species that are collected by its people for self-use or petty sale to generate a part of their annual income. Much of the data on the contribution of NTFPs to household economy in Jammu and Kashmir is still

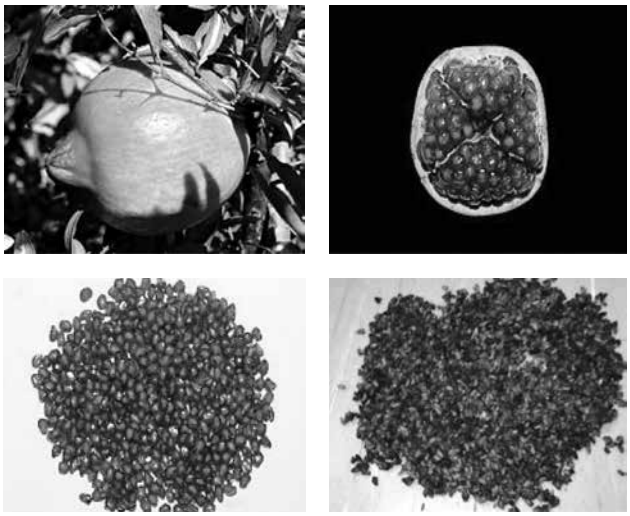

**Fig. 1.** Preparation of dehydrated aril *'anardana'* from sour pomegranate *'Dhruni'*.

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in infancy; possible reasons may be non-availability of data and lack of interest at departmental and institutional level in the subject. In addition to this, most of the plants have been an important source of products used in a variety of industries. These species need to be cultivated and conserved extensively through various government and non-government agencies through different *ex-situ* or *in-situ* programmes. This will help in boosting the local economy of the region as well as conserving these valuable medicinal resources. The present investigation was carried out with the objectives to study the status and scope of '*anandana*' towards better livelihood options for the rural communities and to study the socio-economic profile of the families involved in the collection and trade of the '*anardana*'.

Ramban district is located at 33° 14' N and 75° 17' E longitudes in the lap of Pirpanjal range of the mighty Himalaya. Ramban district is 1,156 m above msl. The boundary line of Ramban district encompasses hill station Patnitop as its southern most point, Assar on its eastern edge, Gool to the west and Banihal to the north. Terrine terrain of district Ramban is tough and hilly. Ramban shares its boundary with Reasi, Udhampur, Doda, Anantnag and Kulgam. The climate of the district varies according to altitude. The temperature rises as high to 42°C in the low-lying areas like Ramban town located in between steep mountains on the banks of Chenab River and drops to sub-zero in the high altitudes. Pirpanjal hills are considered as the gold mine of biodiversity where vegetation of several distinct zones and forest types like evergreen forests, broad leaved mix forests, scrub land and grassy pasture are predominant. Pine forests are found in the steep dry slopes and in the lower regions fruit trees like peach, lemon, olive, apricot and pomegranate are present. Middle and upper ranges have grassland type of vegetation. Majority of the population (95%) is rural and depends on agriculture and its allied sectors. The farmers have very small holdings ranging from 1-2 ha. In addition to livestock rearing, the inhabitants generally practice traditional farming and grow maize, wheat, mustered, peas and potatoes.

The present study was conducted during the year 2014-15 in the rural pockets of Ramban district of Jammu & Kashmir. For the purpose of study, 10 villages were randomly selected from the representing areas to obtain the primary data regarding the collection and trade of '*anardana*' in this region. Households (10%) living in these villages were surveyed as per procedure followed by Pfoze *et al.* (3). The information on '*anardana*' and their traditional uses was gathered through well structured questionnaires, interviews and observations with local people. The questionnaires was designed to meet the objectives of the study, tested in the field and standardized for the purpose. The secondary data was collected from research journals and various records and project reports of the forest department.

During the present study, total 200 resource persons were interviewed through questionnaires, of which 178 were male and 22 were female. All the resource persons identified were in the age group of 35-85 years out of which 85 were between age group of 30 to 50 and rests were above 50 years old (Table 1). The perusal of the secondary data collected from village amenities directory Ramban (Table 2) revealed that the maximum and minimum geographical area of 2448.8 and 235.1 ha was reported in Gool and Sangaldan villages, respectively. The total population of 20,003 was recorded from all the 10 sampled villages, out of which 10% of households living in these villages were surveyed.

The study conducted on the role of '*anardana*'in livelihood for the people of rural communities in Ramban district revealed that the annual income from '*anardana*' was highest in Kanga village Rs. 2,85,900/- ha with 57.19% contribution to total household income and found lowest in Ganote Rs. 1,76,500/ ha with 41.11% contribution to total household income (Table 3). A critical analysis of data also revealed that *Anardana* has a fair share amounting to 48.78% in the net income of every household in the study area. Although the study is restricted only to the role of '*anardana*' in the livelihood of people of Ramban, but it can be well inferred that it does has a role to play in the economy of the state (Table 3).

A random survey of Ganote, Dharam, Gool, Farmoot, Sangaldan, Gundi, Maha kund, Chaderkot area revealed that each household in these areas collects 400-500 kg of dried seed, with annual collection of dry seeds touching about 550-625 kg in Kanga and Parmote villages of Ramban. Collection of ripe fruits usually starts during August and continues

**Table 1.** Age group of informants.

| Age group | Male | Female |
|---|---|---|
| > 70 | 17 | - |
| 61-70 | 29 | - |
| 51-60 | 59 | 10 |
| 41-50 | 49 | 12 |
| 30-40 | 24 | - |

**Table 2.** Composition of geographical area and population size of villages in Ramban district.

| Pilot area (village) | Village-wise area (ha) | | | Population | | |
|---|---|---|---|---|---|---|
| | Geographical | Cropped | Irrigated | Male | Female | Total |
| Ganote | 1,651.5 | 200.3 | 0.8 | 1,065 | 1,032 | 2,097 |
| Kanga | 1,158.6 | 76.5 | 32.4 | 875 | 869 | 1,744 |
| Dharam | 1,582.7 | 189.8 | 10.9 | 1,431 | 1,310 | 2,741 |
| Gool | 2,448.8 | 498.9 | 29.9 | 3,495 | 3,221 | 6,716 |
| Farmoot | 723.6 | 112.9 | 13.4 | 390 | 225 | 615 |
| Sangaldan | 235.1 | 87.4 | 24.3 | 376 | 334 | 710 |
| Gundi | 2,140.4 | 189.0 | 17.8 | 1,335 | 1,215 | 2,550 |
| Maha kund | 419.7 | 194.6 | 3.6 | 948 | 826 | 1,774 |
| Chaderkot | 322.54 | 23.45 | 2.0 | 617 | 439 | 1,056 |
| Pernote | 796.8 | 143.3 | 14.9 | 1,256 | 1,172 | 2,428 |
| Total | 10,683.0 | 1572.8 | 135.1 | 10,532 | 9,471 | 20,003 |

Source: Village Amenities Directory (6)

**Table 3.** Comparison of net annual income per household from '*anardana*' with traditional crops and labour.

| Village | Net income per household (in Rs $yr^{-1}$ $ha^{-1}$) | | | | Contribution of '*anardana*' to total household income (%) |
|---|---|---|---|---|---|
| | Crop | *Anardana* | Labour | Total | |
| Ganote | 1,57,825 | 1,76,500 | 95,000 | 4,29,325 | 41.11 |
| Kanga | 1,19,000 | 2,85,900 | 95,000 | 4,99,900 | 57.19 |
| Dharam | 1,59,587 | 1,89,250 | 94,000 | 4,42,837 | 42.74 |
| Gool | 1,40,800 | 1,77,862 | 90,000 | 4,08,662 | 43.52 |
| Farmoot | 1,12,912 | 2,61,275 | 94,000 | 4,68,187 | 55.81 |
| Sangaldan | 1,45,612 | 2,61,187 | 92,000 | 4,98,799 | 52.36 |
| Gundi | 1,37,225 | 1,79,437 | 92,400 | 4,09,062 | 43.87 |
| Maha kund | 1,14,250 | 2,29,650 | 92,400 | 4,36,300 | 52.64 |
| Chaderkot | 1,27,225 | 1,99,437 | 92,400 | 4,19,062 | 47.59 |
| Parmote | 1,19,250 | 2,19,650 | 92,400 | 4,31,300 | 50.93 |
| Mean | 1,33,368 | 2,18,014 | 92,960 | 4,44,343 | 48.78 |

upto October. The fruits are usually hand plucked by bending the branches with the help of a long stick. The seeds, commonly known as '*anardana*' are separated by hand and dried by spreading. After keeping some produce for self use the remaining stock is sold (Mahajan *et al*., 2). Good '*anardana*' fetches a price ranging between Rs. 300 to 400 per kg at the village level, where the local commission agents working on behalf of traders at Jammu, Amritsar or Delhi procures it. Some produce from nearby areas is also brought to Ramban market where it is sold in open auction. It may be noted that in addition to fulfilling the *bonafide* domestic needs, each household engaged in collection of *'anardana'* adds an average of Rs. 60,000 to its annual income. The rind also has good medicinal value and is used by India's herbal industry. However, its commercial potential is yet to be tapped because mainly of the difficulty in drying the rind. Its traditional uses, however, include preparing of ink for writing on *Takhti* by school children, in dying leather, for making FYM by mixing with cattle dung.

*Anardana* has a fair share amounting to 48.78% in the net income of every household in the Chenab valley of Jammu & Kashmir. Although the study is restricted only to the role of Anardana in the livelihood of people of Ramban, but it can be well inferred that it does has a role to play in the economy of the state. The state harbours a large variety of miscellaneous NTFP species that are collected by its people for self-use or petty sale to generate a part of their annual income. *Punica granatum* of Ramban district

and its adjoining areas, which not only finds its way in international markets but is also, sold at local markets @ 300-400 Rs/kg of seeds. About 1,100 tonnes of *Anardana* is produced annually valued at Rs. 38 crores at current market price. These are just examples to highlight the importance of wild collected *Anardana* in the state and their impact on the rural economy. Survey also revealed that each household in these areas collects 400-500 kg of dried seed, with per household average annual collection of dry seeds touching about 550-625 g. It may be noted that in addition to fulfilling the *bona-fide* domestic needs, each household engaged in collection of '*anardana'* adds an average of Rs. 60,000 to its annual income.

## ACKNOWLEDGEMENTS

The authors are thankful to the farmers of the Ramban district for providing valuable information and cooperation during PRA and the field survey to carry out this work. We greatly appreciate faculty members for valuable discussions, suggestions and constructive comments.

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# Book Review

## Integrated Development of Horticulture in the North-East India


***Authored by*** **: S.P. Ghoah, S.V. Ngachan and A.K. Jha**

Published by the Director, ICAR Research Complex for NEH Region, Umroi Road, Umiam, Meghalaya 793103, 232 p.

North Eastern region in India comprising of eight states have the most diverse and unique eco-systems, where horticultural crops play a significant role in the rural economy. This book is a valuable, ready reference dealing with the technology generated by the NARS and the efforts of Developmental agencies under central and state sponsored development programmes, which have really changed the traditional horticulture into technology-led development activities. This book has seven well planned chapters, *i.e.* dealing with technological advancements, cropping systems, crop diversification, research and development efforts, innovative technologies, marketing and post-harvest management; and future opportunities. It has well specified sections like varietal wealth, indigenous crops, improved production technologies, crop protection, post-harvest management in diverse crops, namely, fruits, vegetables, root & tuber crops, spices, plantation crops, mushrooms etc. Specific issues, namely, genetic resources, indigenous crops, plant protection and nutrient management, post-harvest management and value addition, marketing, small farm holdings, organic farming, GAP, off-season cultivation, protected cultivation, etc. ensuring profitability and livelihood security. On the whole, it is a complete book dealing with different facets of Horticultural sector, and would serve as a ready reckoner for all horti-agriculturists, scientists, policy planners, extension workers, development agency staff, students as well as progressive farmers.

Sanjay K. Singh
Editor-in-Chief, Indian Journal of Horticulture

# Hort News

The Executive Council of the Horticultural Society of India feels pride in Achievements made by its esteemed members in selection to Prestigious Positions and also getting national recognitions for their contributions from the Indian Council of Agricultural Research, New Delhi.

| | |
|---|---|
|  | Dr A.K. Singh, MD National Horticulture Board, Gurugram joined as the Deputy Director General (Horticulture Sciences), Indian Council of Agricultural Research, New Delhi |
|  | Dr B.N.S. Murthy, Principal Scientist, IIHR, Bengaluru joined as Horticulture Commissioner, Ministry of Agriculture and Framers Welfare, Govt. of India |
|  | Dr Pritam Kalia, Scientist Emeritus, IARI, New Delhi received Rafi Ahmed Kidwai Award for Outstanding Research in Agricultural Sciences-2016 |
|  | Dr S.K. Singh, Head of the Division, IARI, New Delhi received Bharat Ratna Dr C. Subramaniam Award for Outstanding Teacher-2016. |

| | |
|---|---|
|  | Dr S.K. Pandey, Ex-Director, CPRI, Shimla received Hari Om Ashram Trust Award for the Biennium 2014-15 |
|  | Dr Jitendra Singh, Professor, College of Hort. & Forestry Agri., Jhalawar, Rajasthan received Dr Rajendra Prasad Puruskar for technical books in Hindi |
|  | Dr Vijay Rakesh Reddy S., Scientist, ICAR-CIAH, Bikaner, Rajasthan received Jawaharlal Nehru Award for P.G. Outstanding Doctoral Thesis Research |

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***Review/ Strategy paper*:** This article is received through invitation. It should be comprehensive, up-to-date and critical on a recent topic of importance. The maximum page limit is of **16 double-spaced typed pages** including tables and figures. It should cite latest literatures and identify some gaps for future. It should have a specific **Title** followed by the **Name(s) of the author(s)**, **Affiliation**, **Abstract, Key words,** main text with subheadings, **Acknowledgements** (wherever applicable) and **References**.

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**Abstract**: A concise summary (200 to 300 words) of the entire work done along with the highlights of the findings.

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