

Current Horticulture

(A Journal Dedicated for the Advancement of Horticultural Science)

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(A Journal Dedicated for the Advancement of Horticultural Science)

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MESSAGE



The nation is agog with discussion on various facets of Food Security Bill. Nutritional security in reality is the core of food security. This vital edifice of human nutrition is best accomplished by our contribution in research and development, leading to higher production and productivity in horticulture. As compared to field crops, horticulture is accelerating astonishingly at a rapid pace. In 2012-13, more than 250 million tonnes of fruits, vegetables etc. were produced, but we need to grow at a rate of more than 6%, if we have to meet the minimum nutritional requirement of our citizens by 2020. Paradoxically, the area under cultivable land, water and labour is limited and shrinking. Producing more fruits and vegetables for the increasing population with reduced factors of production, especially in horticulture, is a challenge in itself. *Research is the father of progress and necessity is the mother of invention.*

A scientific commitment towards crop improvement and production through scientific application of basic and strategic research such as hybridization, marker-assisted selection, double haploidy, RNAi, exploitation of male sterility etc. can revolutionize crop productivity. However, parallel improvement in input-use efficiency, especially water-and nutrient-use efficiency, a better understanding on the role of micronutrient in enhancing quality of nutrition coupled with scientific understanding of ecological parameters leading to pest and diseases management will enhance production and productivity. None of the above is possible unless we assure disease-free seed and quality planting material and technology at farmers' doorstep. Fortunately, India is blessed with one of the largest human resources for basic, strategic and applied research. But this knowledge has to be captured, documented, streamlined and managed. I am confident that publication of the *Current Horticulture* would fulfil the current gap in scientific knowledge of basic and strategic research.

My best wishes.


(N.K. Krishna Kumar)

Evaluation of SSRs (microsatellites) for detecting genetic variability in oil palm (*Elaeis guineensis*) clone

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ABSTRACT

An experiment was conducted for detecting genetic variability in oil palm (*Elaeis guineensis* Jacq.) clone, Tornado, using SSRs or microsatellites, during 2007-09, at the Directorate of Oil Palm Research, Pedavegi, Andhra Pradesh. Tissue cultured clones of oil palm were imported from ASD Costa Rica for the first time to India and were planted at farmers' fields during 2006 by a private firm. Morphological variations were studied when the plantations were three years old. Twenty-four plants of the clone, Tornado, were selected at random from a batch of 200 plants. The DNA was extracted from the spear leaves and 10ng of DNA from each sample was amplified using SSR primers. The 20 SSR primers used were designed from the reported oil palm microsatellite sites. The amplified products were separated on a 15% acrylamide gel. Seven SSR primers showed monomorphic bands. The number of alleles per locus ranged from one allele for SSR 204 to four alleles at SSR 230. In order to illustrate genetic diversity among oil palm accessions, a dendrogram was obtained from the similarity matrix by minimum evolution method. The dendrogram clustered the accessions according to their similarities calculated as proportion of shared alleles. All the palms fell into three distinct clusters, which indicates that these accessions might have at least three different origins. Based on these results six SSR primers, viz. SSR 48, SSR 119, SSR 138, SSR 176, SSR 215 and SSR 226 were selected as potential primers which can detect variation in these clones.

KEY WORDS: Accessions, Clones, Dendrogram, Genetic variation, Microsatellites, Oil palm, Simple sequence repeats (SSRs)

Detection and analysis of genetic variation can help us understand the molecular basis of various biological phenomena in plants. Since the entire plant kingdom cannot be covered under sequencing projects, molecular markers and their correlation to phenotypes provide us with requisite landmarks for elucidation of genetic variation. Molecular markers offer numerous advantages over conventional phenotype-based alternatives since they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. Microsatellites or simple sequence repeats (SSRs) are preferred for high throughput mapping, genetic analyses and marker-assisted plant improvement programmes (McCouch *et al.*, 2009). In fact, it has been demonstrated that among the four

marker-systems tested, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR), SSRs had the highest polymorphic index content (ability to distinguish genotypes) in evaluation of soybean (Powell *et al.*, 1995) and maize germplasm (Smith *et al.*, 1997).

In oil palm, the use of both RAPD and SSR markers has been reported by various researchers for genetic diversity and clonal fidelity testing from other countries like Malaysia and France. However in India, it is for the first time we report the use of microsatellite primers in oil palm. Tissue cultured clones of oil palm were planted at farmers' fields during 2006. These clones were imported from ASD Costa Rica and planted at farmers' fields in West Godavari district of Andhra Pradesh. Therefore, present study was undertaken to find out the genetic variation in field planted tissue cultured oil palm (*Elaeis guineensis* Jacq.) clone, Tornado, using microsatellite primers designed and developed at the institute.

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MATERIALS AND METHODS

Twenty-four plants of the clone, Tornado, were selected at random in the field. Leaf samples (0.5-1 g) were collected from these plants at random and brought to the laboratory. The DNA was extracted by the modified method developed (Jayanthi *et al.*, 2004) without CTAB and subsequently it was treated with RNase and purified with phenol-chloroform method. Billotte *et al.* (2006) was the first to report oil palm microsatellites. They have deposited several microsatellite sequences with the NCBI. Oil palm microsatellite primers were designed using the *oligos* V62 software using the available microsatellite sequences in the NCBI site (<http://www.ncbi.nlm.nih.gov/>). Twenty primers were designed and custom made and purchased from Imperial Biomedics, India. The PCR with microsatellite primers was carried out using the optimized protocol developed by Jayanthi *et al.* (2009) in a 12.5 µl volume containing 10 ng of template DNA, 0.05 µM of each primer, 25 µM of dNTP's (Bangalore Genei) and 0.25 unit of Taq Polymerase (Bangalore Genei) with a 10X buffer containing 15 mM MgCl₂. The PCR programme was as follows: denaturation at 95°C for 5 min., followed by 40 cycles of 1 min. at 94°C, 52°C for 1 min. and 72°C for 1 min. and a final elongation step at 72°C for 10 min.

Annealing temperature of 52°C was set as the annealing temperature of most of the primers was around 55-56°C. The PCR products was mixed thoroughly and the 5-10 ml of amplified product was loaded in 15% acrylamide gel. The gels were stained with ethidium bromide and the amplification pattern was documented with the help of a gel documentation system. The bands were scored. Identity 4 was used to calculate various genetic parameters for each accession and each SSR loci (Sefc *et al.*, 2000). A genetic distance matrix based on the proportion of shared alleles was constructed by the programme, MICROSAT (Maletic *et al.*, 1999). Mega5 was used to calculate minimum evolution phylogenetic tree (Tamura *et al.*, 2011)

RESULTS AND DISCUSSION

Of the SSRs primers, 7 showed monomorphic bands. The number of alleles per locus ranged from one alleles at 204 to four alleles at locus 230 (Table 1). The representative pattern of amplification obtained with SSR primers 226 and 48 is given in Fig. 1. Except for 8, 204, 10, 168, 215, 105, 138, 140 221, 225 and 226 all the other loci were polymorphic. Most of the alleles of the markers used resulted in very high probabilities for identical accessions. A second estimate of the information content of markers is the expected

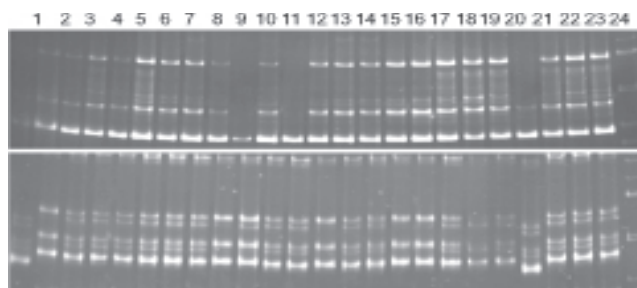


Fig. 1 Amplification obtained with SSR primers 226 and 48 with 24 palms of Tornado clones. The lanes one to twenty-four are the individual palms and the last lane in marker

percentage of heterozygous individuals. The marker evaluation using the values for expected heterozygosity is in good agreement with results obtained by PI values (Table 1). At 9 of the 20 loci, the observed level of heterozygosity was higher than the expected values (Table 1). An excess of heterozygous individuals might have been caused by selection for yield and quality. In order to illustrate genetic diversity among oil palm accessions, a dendrogram was obtained from the similarity matrix by minimum evolution method. The dendrogram clustered the accessions according to their similarities calculated as proportion of shared alleles (Fig. 2).

All the palms fell into three distinct clusters, which indicate that these accessions might have at least three different origins. These accessions might have been derived from more than one parent genotype and there was certain degree of cross-pollination between different accessions. The accessions in Cluster 1 showed higher degree of similarity with each other than the accessions from other clusters. This may indicate that members of cluster 1 might have been derived from independent explants of the same genotype. The genetic differences within this cluster might be attributed to somaclonal variation. The accessions in Clusters 2 and 3 might be from different genotypes and they might have undergone selection for yield and quality. Based on these results, six SSR primers, viz. SSR 48, SSR 119, SSR 138, SSR 176, SSR 215 and SSR 226 were selected as potential primers which can detect variation in these clones.

In oil palm, SSR markers have been reported to be reliable markers for studying genetic variability. Application of the SSR markers as DNA probes for use as quality control tool in oil palm tissue culture has been reported (Singh *et al.*, 1997). They reported that the SSR primers used were able to distinguish between the true ramets and the "rogues" when their SSR profiles were compared with the ortet. They reported that as

Table 1. Genetic parameters of SSR markers used with oil palm clones

Primer No.	Left primer sequence	Right primer sequence	Number of alleles	Probability of identity	Expected heterozygosity	Observed heterozygosity
SSR 8	GGAAAGAGGAGATGGGAGAGTGG	CTTTCCCTCTTTTGTCTGG	1	1	0	0
SSR 10	GTCAAAGTTCAGTTAAGACCACC	CACCTCTCACTATAGCTCG	1	1	0	0
SSR 41	CCAAGCACAGCAATGCC	GGTATAAGAGCCCAAAAGCG	3	0.8	0.07	0.08
SSR 48	TGAAAACAGCCTAGTGGACCC	ATACACAGCAGGATTACTGACC	3	0.375	0.5	1
SSR 105	AGC CCA TCA GAA CAT GGA C	CTA AAT TCT GAG CCC ATG CC	2	1	0	0
SSR 119	GTGAGGTGT GAT GCT GAA GGC	GGA TCG GTT ATA GCT TCC TCC	2	0.37	0.49	0.54
SSR 132	CTGGCGTACTTCCGGTACGG	TCCATGGCTCCATGCAAGGC	2	1	0	0
SSR 138	ACAGTCAAAGCCGAAGGTCC	TTGCATGTTGCTGCTAGCTCC	3	0.097	0.218	0.25
SSR 140	GATTTAGCAGGGACTTCCG	CATGAAGGACCCTTCCCTCG	2	0.8	0.08	0.08
SSR 163	GGAGAAGCGTGGCTTGAG	GCCCAACAAAAGAAAAGTAGTCC	2	0.92	0.04	0.04
SSR 168	ACCAAGCCAATACGCTG	GCATTACTTGGTTATACTTGTCC	1	1	0	0
SSR 171	GAGTTATGGAGA TGTAGGAAG G	CTG GAT CCA ATT CAC AAA CC	2	0.72	0.15	0.16
SSR 176	GATGCCCATCGATGACAG	AATGGAAAATATGCCACCC	2	0.44	0.39	0.54
SSR 185	TGTGCAAGTACATGCGTGCG	AGACCTGATCTGAACCTCGACCC	2	0.72	0.15	0.16
SSR 204	GGA CCA AAA CTA GGT AAT GGC	TTA TGA GCA GGA TGG CGA G	1	1	0	0
SSR 215	ACTCAGGGG CAA GGT AGG	CGA ACT CCC TTC AAATGTCAG C	3	0.07	0.15	0.16
SSR 221	TGT CTA CAACAA GCCATG CAC C	ATC CAG GAA ATC CAC CTG GG	3	1	0	0
SSR 225	CCA TGT GCA ATG CTT GTG TGG	ACA CCC AGC TTG GCC TA	3	0.7	0.1	0.12
SSR 226	AAGGAGCTTCGCCCTCGAGC	CCGGAGCAGAGCGGTAAGGG	3	0.59	0.24	0.04
SSR 230	TGT GTA ACG GCA AAT CAC CG	ACC TCT GTT CAC CAG GCT	4	0.92	0.04	0.04
			2.25	0.7261	0.1309	0.1605

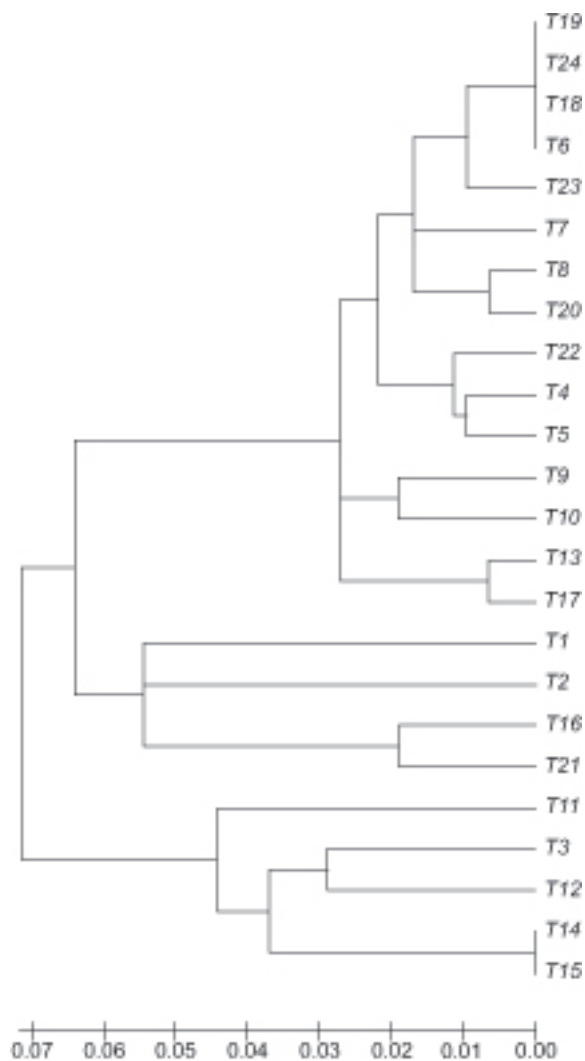


Fig. 2 Dendrogram of oil palm accessions derived from the analysis of 20 SSR loci

such, the most likely cause of the ramet having a different fingerprint profile is that it was not derived from the ortet concerned. Furthermore, they also mention that the most likely cause of the fingerprint differences was due to culture mix-up in the laboratory. Thus, it was concluded that the differences in the SSR profile are because it was not derived from the same ortet. These clones might have been derived from more than one parent genotype and also there is a probable chance of culture mix-up. The microsatellite primers can detect variation and the variation pattern obtained is repeatable unlike RAPD. This study also proved that SSR primers developed by us are reliable markers for studying variation in clonal populations.

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Standardization of preservation techniques of natural leaves for dry flower arrangements

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ABSTRACT

The experiment was conducted to standardize the preservation technology of dried leaf skeleton which can add more value to the emerging dry flower industry. Though dry flowers have been used in art and decoration for many years with a huge market potential throughout the world, the availability of plant material in abundance is one of the driving forces to nurture the hobby of preparing, crafting and aesthetically decorating dried plant material and flowers. Unlike fresh flowers, that easily lose their freshness and market value, dried flowers persist for a longer period of sale if properly preserved. Preserving dried leaf skeletons can add more value to dry flower industry. One of the important features of dried skeleton leaves is their versatility, that they can be arranged into different crafts according to ones preferred style, designs and uses. We have come up with a step-wise and optimized preparative procedure for the development of a variety of decorative dry flower arrangements having a huge potential for value-addition at commercial scale.

KEY WORDS: Bleaching, Crafting, Dry flowers, Fermentation, Leaf skeletons

Flowers have been dried since ancient time to preserve their real shape, beauty, colour and textures which make them more beautiful and attractive. The skill of making dried flower arrangements was a flourishing craft as early as the seventeenth century in England and America. But now it has passed from the hobby stage to big business in many countries. Dried flowers are commonly used as decorative items, especially for floral and wreath arrangements. The arrangements with dried flower can last for several years if flowers are well preserved. The potential for designing beautiful and artistic dried flower arrangement is endless. Dry flowers can be seen everywhere today, in supermarket, in pots, at weddings, in restaurants and even churches. Dry flower production in India is a small component of floriculture industry, but the demands of Indian dried flowers has sharply increased in a short span. Dried flowers can be used several times to meet the decorative demands throughout the year. The global dry flower industry has grown rapidly with over 60% share of profits belonging to the floriculture industry (Ranjan and Misra, 2002). The industries projected annual turnover of 2003

was more than 150 crores as reported by (Singh, 2003). India's share in the export of these items is below 1.5% in Europe and is lesser than 1.5% of the world requirement. Netherland ranks first in the export of dried flowers, followed by Columbia, Mexico, India and Israel.

The USA is a largest consumer of dried and artificial flowers (US \$2.4 million annually), followed by Germany and UK (Bhattacharjee and De, 2003). Potpourris are the major segment of dry flower industry, valued at ₹ 55 crores in India (Murugan *et al.*, 2007) alone. Leaf skeletonizing nowadays has become a popular way to make jewelry, in which leaves are skeletonized, and then somehow either plated with gold or silver. Skeleton leaf preparation in India is comparatively a new technique for producing a variety of flowers and other products, which are being used for natural, aesthetic and for interior decoration purposes (Saima *et al.*, 2012). Therefore, various techniques such as fermentation, bleaching and further processing of skeleton leaves for their potential uses in decorative arrangements were standardized.

MATERIALS AND METHODS

The plant materials selected for the study were collected at National Chemical Laboratory Garden, at

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Homi Baba Pashan Road, Pune. The procedure of leaves venation skeletons using different concentration of the Baker's yeast using *Ficus religiosa* leaves and also determined the optimal time required for the leaves to skeletonize. We used the same technique for other species of plant leaves, viz. leaves of *Bauhinia purpurea*, *Tectona grandis*, *Ficus benjamina* etc.

Skeletonization is a process that occurs spontaneously in nature but it takes a long duration to expose the inner mantle of leaves. There are wide gaps in between veins (Fig. 1a) which is not the case in chemically treated one (by fermentation method) (Fig. 1b). Thus, naturally processed skeleton leaves are not good option for preparation of major dry flower arrangements, although the condition of naturally processed skeleton leaves can be improved by the techniques of bleaching and softening (Fig. 1c) but still it cannot be used further because of the loose and deteriorated network of veins.

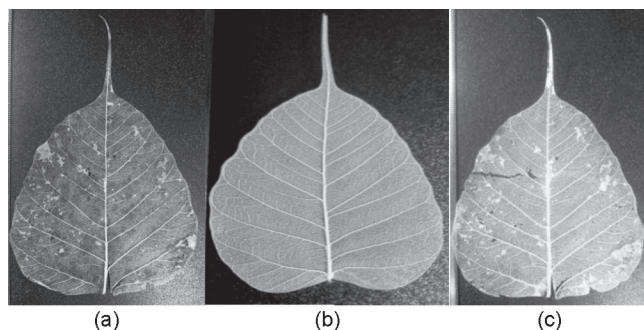


Fig. 1 Skelton formation under different processes

Fermentation

Baker's yeast was used in the fermentation of leaves for the formation of vein skeletons. The aqueous yeast solution at different concentrations was prepared by dissolving the measured quantity of Baker's yeast in distilled water. The yeast solution was used to ferment the fresh, matured and disinfected leaves of *Ficus religiosa* (Moraceae family) which were previously rinsed with water with soft rubbing to remove any dirt or dust. Various concentrations of aqueous yeast solutions prepared were 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5% (Table 1). After completion of fermentation, the leaves were taken out from the solution and kept in a vessel of clean water to remove the tissue between the veins carefully, by brushing from the middle rib to the leaf edge and also using more water to wet the leaf between brushings, if needed. The best way of separating the two parts was by carefully rubbing the leaf between the thumb and finger. After removal of soft matter, the resultant leaves were then laid perfectly flat on a piece of rough filter papers for drying.

Table 1. Effect of various concentrations of Baker's yeast on skelton formation

Bakers' yeast (%)	Response
0.0	-
0.5	+
1.0	++
1.5	++
2.0	++++
2.5	+++

The data represent the mean value of five replicates
 -No response; no change was observed in blank after 15 days
 + Low; ++ poor; +++ high; ++++ high but slight deterioration of net

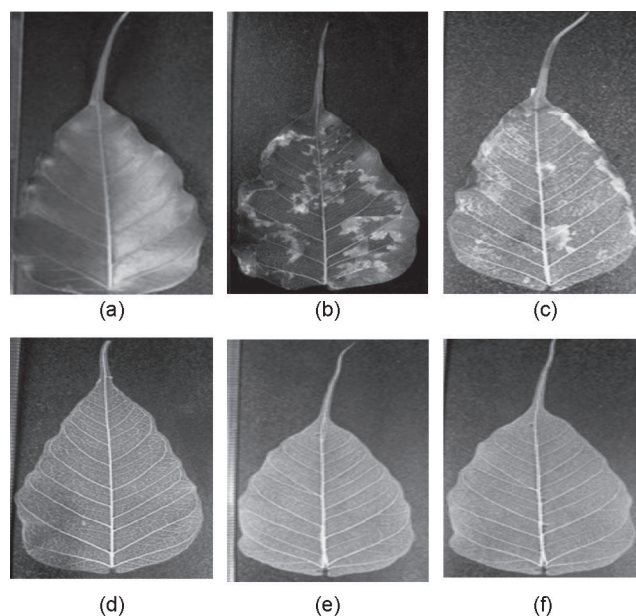


Fig. 2 Effect of Baker's yeast concentrations on skelton formation

No results were obtained in 0.0%, the control solution as shown in Fig. 2a. Formulations having 0.5, and 1.0% yeast concentrations were not found to be suitable for net formation (Figs 2b and 2c). Formulation with 1.5% concentration showed better results (Fig. 2d) and good results were obtained in 2.0% concentration (Fig. 2e). The lower and upper epidermis and other green cells of leaves were found to be floating freely at 2.0% concentration. Even at 2.5% concentration complete skeleton formation occurred but small holes were observed in venation skeletons at this concentration (Fig. 2f). All the experiments were repeated three times.

Bleaching

Sodium hypochlorite (also known as chlorine bleach) was prepared at different concentrations, viz. 0.0, 5.0, 10, 15, 20 and 25% (Table 2). The prepared leaf skeletons were immersed in these concentrations and

Table 2. Effect of sodium hypochlorite on skelton formation

Sodium hypochlorite (%)	Results
0.0	–
5.0	+
10	++
15	++
20	++++
25	+++

The data represented the mean value of five replicates
 -No response;+ low;++ good;+++ good with slight degradation of network of veins; and++++ excellent

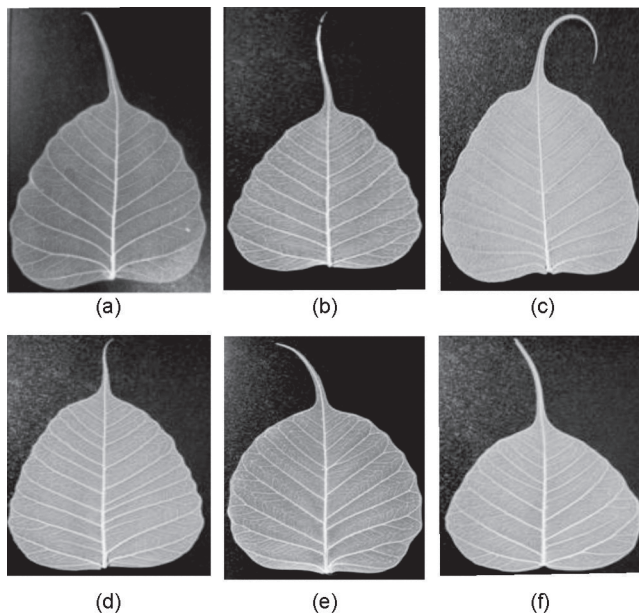


Fig. 3 Effect of sodium hypochlorite on bleaching

were kept till 1 hour. There was no result in the control (Fig. 3a). The 5.0% formulation showed very poor results (Fig. 3b). Better results were found in formulation of 10 and 15% (Figs 3c and 3d respectively), and good results were obtained in 20% (Fig. 3e) formulation, although 25% (Fig. 3f) formulation showed good transparency with slight thinning of veins.

Effect of Time

The 20% bleaching solution was used to find out the optimum time to effectively bleach the selected plant material. Different time intervals recorded were,

Table 3. Effect of time on bleaching

Time (min.)	Result
30	–
60	++
120	+++
180	++

The data represented the mean value of five replicates
 -Low;+ low;++ good; and+++ excellent

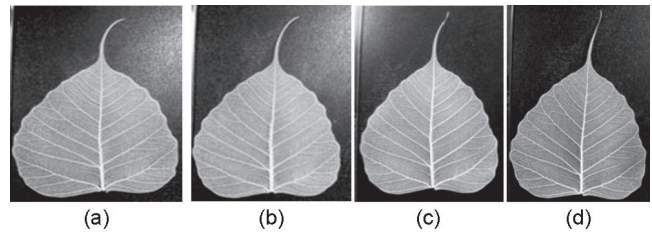


Fig. 4 Effect of duration on bleaching

30 min, 60 min, 120 min, and 180 min (Table 2). The duration of 30 min was not suitable to bleach the prepared skeletons (Fig. 4a) and good results were found at the duration of 60 min (Fig. 4b). It was noticed that maximum bleaching occurred at duration of 120 min (Fig. 4c) with deteriorated veins (Fig. 4d).

Further, softening with glycerin and colouring with different dyes and paints of bleached skeleton leaves was carried out. Softening was carried out by using glycerin, so that with softening the elasticity of selected material was increased. The material becomes more durable, soft and can be easily folded and twisted. Various greeting cards and flower and leaf arrangements were prepared by using dyed processed skeleton leaves.

RESULTS AND DISCUSSION

Fermentation

The results obtained in a number of successful reports on the preparation of venation skeletons of leaves indicate that skeletonization of leaves can be done by various ways. The selected leaves are boiled in 1 quart water and two tablespoonfull of lye to obtain the network of veins. Murugan *et al.* (2007). Skeltonization of leaves was obtained by boiling one tea spoonful of baking soda or lye per quart of water (Micheal *et al.*, 2002). The use of microorganisms to separate plant vascular skeletons is not new. Loomis and Shull (1937) suggested the immersion of leaves in an algae tank until the mesophyll was eaten away by microorganisms, leaving an intact vascular skeleton. Sharman (1942) found fermented corn stems and roots in a corn grain or pea seed infusion in pre-paring specimens for classroom work. The recovery of rubber was done from cryptostegia to isolate and measure the full extent of leaf veins as reported by Whittenberger *et al.* (1985). The method, an anaerobic fermentation process, has been used also for the isolation of protoplasts from vegetable leaves (White *et al.*, 1948).

In the experiment, an anaerobic fermentation of *Ficus religiosa* leaves was conducted by using Baker’s yeast. In this procedure various concentrations of aqueous yeast solutions were prepared and among all the concentrations prepared, the 2.0% formulation was found to be optimum concentration for the formation of network of veins.

Bleaching

Many types of foliages, pods and dry flowers can be lightened by bleaching. Bleached ornamental plants provide a striking contrast when arranged with dried flowers. The colour of prepared leaf skeletons can be lightened by immersing the material in one quart water and two tablespoon of household bleach (Murugan *et al.*, 2007). After bleaching, processed material becomes white transparent, which allows the use of dyes for colouring oxidative (e.g. hypochlorite and peroxide) and reductive bleaches (e.g. sulfite) can be used for bleaching of plant materials. The sodium hypochlorite tends to break down the coloured compounds, whereas the peroxide tends to modify them into colourless compounds (Dubois *et al.*, 1988). Thus, standardization of bleaching agents was studied under different concentrations. Among different formulations, 20% concentration was found to be the best to bleach the leaf skeletons. In this particular formulation, the complete removal of discolouration was observed.

Effect of Time

The time required by the material to stay in the solution before it is bleached vary from few minutes to an hour. The colour of skeletonized leaves can be lightened by immersing the material in household bleach solution for two hours (Murugan *et al.*, 2007).

The optimum concentration of 20% was used to study the effect of time on bleaching of venation leaf skeletons. Various time intervals recorded were 30, 60, 120 and 180 min. The best results of bleaching were obtained at the recorded duration of 120 min.

The study of preparation of venation leaf skeletons was carried out by using Baker's yeast (*Saccharomyces cerevisiae*), to standardize the optimum concentration required for the formation of venation skeletons, various formulations of aqueous yeast solution was prepared. Among all concentrations, 2.0% was found to be the optimum concentration for the formation of network of veins. Further, bleaching of processed skeleton leaves was done by using sodium hypochlorite (as a bleaching agent) which was prepared at different concentrations. The concentration of 20% was found to be suitable concentration. Further, softening followed by colouring using various dyes and paints were also done to craft the variety of beautiful flowers, and leaf arrangements. The dry flower craft technique helps upgrade the

creative facility of human mind and converts the cheapest plant raw material into wealth. The demands of dry flowers can be increased by establishing new techniques like processed skeleton leaf preparations, customer awareness by way of exhibitions, workshops, seminars etc. It would also help in employment generation.

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Tifdwarf green terminators: off-types

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ABSTRACT

The golf ball had been rolling on the Tifdwarf greens since April 1965 when it was officially released by the U S Department of Agriculture and the Georgia Coastal Plain Experiment Station. Tifdwarf grass was born as an 'off-type' from Tifgreen (T-328) which itself is a 27 chromosome hybrid turf grass released in 1956. Tifdwarf was first discovered by an Agronomist James B. (Monty) Moncrief as a small circular patch from a golf green of Tifgreen which was under evaluation before its release. Since then Tifdwarf has been used extensively as warm season putting green grass around the world. However in older greens of Tifdwarf, issues of mutations and offtypes have always been associated with it. Also today new ultradwarf Bermuda grass varieties are available which claim to offer faster putting greens. This paper analysis the tendency of Tifdwarf grass mutation and its effects on the golf putting greens.

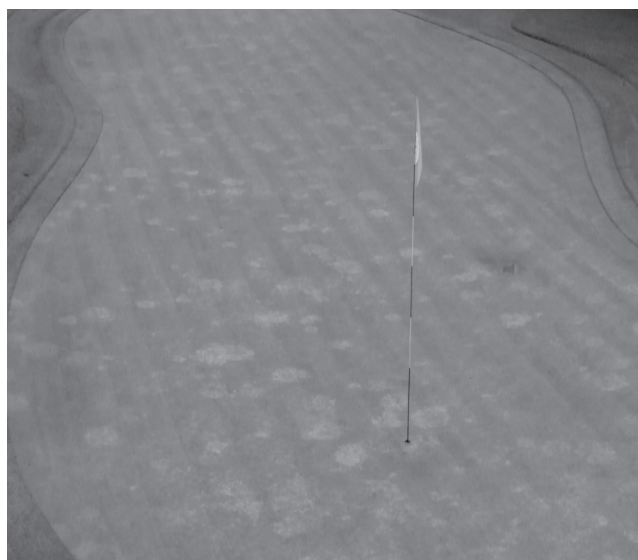
KEY WORDS: Mutation, Mutants, Off-types, Tifdwarf green terminator, Tifgrass, Turf grass

The very process of origin of 'tifdwarf' that is mutation, is now threatening its own dominance as a golf green grass. The identity crisis of Tifdwarf is an outcome of the tendency of Tifdwarf to mutate and produce 'off-types'. The natural propensity of Tifdwarf to produce off-types had been doubted and discussed since very beginning and now when it is accepted in general that sooner or later tifdwarf putting surfaces get crippled with off-types, the one big question that is looming large is whether tifdwarf will succeed in sustaining its dominance and remain the choicest putting green grass of warm climate courses? Or its off-types will turn into the terminators of its own identity in the times to come that too when the new 3G grasses have come to challenge its dominance!

Off-types and Their Origin

If you have planted an 'X' species of a turf grass hoping to get a homogenous turf cover of desired characteristics like texture, colour, appearance and growth habit, but in the base turf you figure out a grass plant or a patch of turf growing, which is different, then this 'different' plant is called 'off-type'. It is generally agreed that primarily off-types intrude your greens as: (i) contaminant in initial planting stock of sprigs or seeds that is used for laying turf; (ii) a drifting vegetative propagule or a stray seed of different grass settling eventually on your established putting greens and (iii) genetic mutations.

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Off-type patches on a tifdwarf putting green. These patches may appear light green or dark green with reference to the base turf grass depending on the season. Sometimes they merge so well that it is difficult to identify any patch at all in a putting green.

Inside Story: Within the Cells

The integrity of a species of plant or an animal is maintained by a set of 'chromosomes' found in the body cells which carries a specific genetic code also called 'genotype' of the species. This genotype is unique for each species and is passed from parents to the offsprings of a species. The expression of genotype in physical form is called 'phenotype' which translates



Within green off-types may also spread as a result of routine hole cutting practice and infestation spreads on the whole green with the passage of time. Even cultural operations like scarifying and core aeration aggravates the infestation of off-types.

into the physical attributes of the individual like in case of a grass plant the texture of leaves, inter-nodal distance, colour, responses to environment etc. So, phenotypes of all plants of a species of a grass remain the same as they carry similar genotype.

Mutations

The evolution of new species occur from the existing species and of the several mechanisms that nature has at its disposal to create new species, one important tool is 'mutation'. Mutation is phenomenon of change in the genotype of an offspring of a species. Such offsprings which have different genotypes and consequently different phenotypes than the parent plants are called mutants.

As turfgrass cells divide, spreading of genetic information to new cells increases the likelihood of species survival. For the continuity of life, every species has a natural tendency to multiply and is programmed to perpetuate. Cell division is the fundamental process that makes multiplication happen in all plants including turfgrasses.

The dividing cells in growing turf grass plants have the susceptibility to mutate maximum because during cell division the chromosome strands unwind to replicate. In such a state external agents capable of causing change in chromosome (genes) like high energy radiations, chemicals and even heat or physical factors also called mutagens easily affect genes resulting in mutations. Putting green turf grasses are always under intensive maintenance regime. So, they remain exposed

to such stresses or mutagens always and consequently tend to mutate more.

Mutant vs Off-type

All mutants in a base turf are off-types but all off-types in a base turf may not be mutants. Mutant is a plant which necessarily differs in genotype with respect to its parents while off-types are simply different plants with respect to the base turf. In effect an off-type may be considered as a weed which by definition is an undesired plant growing out of place.

The mutants or off-types in a turf grass have new characters and attributes than the parent grass type which may be desirable or undesirable from the perspective of putting in golf. If new characteristics are desirable and superior than off-type may be selected, tested for stability and then may be used as a new turf grass variety as had happened in case of Tifdwarf in 60s when it originated as an off-type of Tifgreen. If off-type has undesirable characters it is discarded.

Evolutionary importance of mutations lies in the fact that mutations result in genetic variability in the population of a species and more is the variation in population, better are the chances of its survivability in the changing environment. Those who do not change may perish in the changing environment so every species tends to mutate naturally. This is the reason why we cannot have an ideal, absolutely stable grass type for putting greens for indefinite time.

It has been observed over the years in northern parts of India that the off-type patches here in the Tifdwarf putting greens becomes prominent during July-August after few first showers and are very susceptible to waterlogging probably due to which they decline after consistent heavy rains.

After rain green management becomes extremely critical in such greens to retain and reclaim the declining off-type grass patches. In case of even a slightest error, these declining weak patches quickly wither away leaving behind barren patches on the Tifdwarf putting greens.

Maintaining off-type infested Tifdwarf greens becomes extremely tricky, and demanding especially in the months of incessant downpours, cloudy weather, reduced photoperiods and intermittent days of open sunshine with higher temperatures reaching up to 35°C or more characteristic to parts of northern India during July-August. A keen eye on green is inevitable in such situations to avoid wet-wilt and salt injuries in case you have problem soils.

Off-types vs Tifdwarf

These mutants or off-types in a tifdwarf green have different characters and attributes then the parent grass

type owing to different genotype. Due to this, off type patches that appear in putting greens behave differently than the base turf of Tifdwarf.

The off-types in Tifdwarf greens may differ from Tifdwarf in several attributes like:

- The difference may be just in colour or appearance and it may not affect putting quality.
- The off-type may have more aggressive growth rate or less aggressive growth rates during the period of stress as compared to base turf.
- The texture of leaves may be coarser or finer resulting in inconsistent greens.
- The off-types may turn hydrophobic and become a maintenance issue.

These off-types definitely behave aggressively at least in some season or part of year that is why they are observed distinctly in certain months and remain masked in other months of the year.

Managing Greens with Off-Types

Managing greens with off- types is more a tedious task, requiring extra vigil, attention and efforts as compared to pure greens. Because then the superintendent is like a Jockey trying to drive a cart with a stallion and a pony hitched together with a same stick and that too without toppling.

If off-types in the putting green have not grown extensively and are identified in initial stages as few small patches then they could be removed and replaced by a hole cutter or by resodding. However, if off-types have encroached a substantial surface and manual removal is not a practical proposition then to keep the putting surface consistent an extra attention in green keeping and superior understanding of behaviour of off-types in different seasons of the year becomes a necessity. A proper record keeping of behaviour of different green sections in different seasons of the year may help in planning the management of such greens in more effective manner.

Tifdwarf Green Terminates

When putting greens are severely infested with off- types and it becomes impractical for a green keeper to maintain the green to a satisfactory consistency. When majority of golfers putt with a doubt in the mind and when they complain greens are not true and what they say is not a lie! And when golfers and green keeper together start believing that the patches are a problem! Then it is time to replant the greens with a pure putting green grass.

In the Board Rooms of the high end international Golf Clubs where the membership demands highest

standards of putting green surfaces, the arguments for changing or not changing the off-type infested green grass often keep tossing on the table. However when such a club with good financial health decides to improve the health of its greens by replanting pure grass then the next step is to choose the right method to do so.

There may be several ‘quick-fix’ methods available to replant your greens but all such methods have their associated drawbacks as well. The ‘no-till or no-till planting’ is one such easy method that could save you a lot of money but the purity may be lost soon because in this method the probability of a left out viable contaminant of previous grass does exist to some extent which may again express itself as an off-type in the long run.

Tifdwarf should be Replanted

Tifdwarf undoubtedly gave us perfect putting surfaces for long but its tendency to mutate is now well known and should be considered before making any decision. Utmost care should be taken in sourcing the true and pure Tifdwarf for planting if it is chosen again. In any case by planting Tifdwarf on greens in present time we definitely replant risk of off-types in the long run if not in the immediate years.

SUMMARY

There is a whole new brigade of third Generation Ultra dwarf grasses like Champion, Miniverde, Novotek, Tifeagle, MS Supreme, Flora dwarf etc. which have outperformed their predecessors in delivering the speeds for the delight of golfers. These grasses are promising options that may be tested for their performance in different areas. If found successful these grasses have the potential to take the putting pleasure to a different high. However, stable performance of these ultra dwarfs in the present time should not be interpreted as an absolute insulation of these grasses from mutation in future because one species of grass may be more stable and preferred today but the probability for a change always exists!

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Effect on morphological changes in vasaka (*Adhatoda vasica*) and raktapunarnava (*Boerhavia diffusa*) under stressed and unstressed conditions

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ABSTRACT

The experiment was conducted to study the differences in morphological characters of vasaka (*Adhatoda vasica* Nees) and raktapunarnava (*Boerhavia diffusa* Linn.) growing in various unstressed (non-polluted) and stressed (polluted) habitats. Since environmental conditions play a vital role in the quality and quantity of active constituents in medicinal plants, their macro and micro-morphological characters also are affected. Both the plants, viz. vasaka and raktapunarnava showed significant variation under both unstressed and stressed habitats. The leaves of vasaka under unstressed habitat showed double leaf area, longer petiole size, more venation and with rough texture than stressed habitat. The nodal and internodal sizes were 3 times more under unstressed habitat than stressed habitat. The plants of raktapunarnava also showed variation in their macro-morphological characters under both habitats. The nodal and internodal size, petiole size, size of branches, and shoots were more under unstressed habitats than stressed habitat, but roots were more in length in stressed habitat. The size of lamina was 3 times more in unstressed than stressed habitat. The texture of leaves under unstressed habitat was smooth but it was coarse in stressed habitat. There were significant changes in micro-morphological characters in both the plants under both habitats. In vasaka, frequency of stomata, density of trichome and vein terminations were more in stressed habitat. In unstressed habitat, stomatal index, and vein islets number were more. In raktapunarnava, stomatal frequency, and density of trichomes were more in leaves in stressed habitats. While vein islet number and stomatal index were more in unstressed habitat.

KEY WORDS: Morphological characters, Stressed, Unstressed, Vasaka, Raktapunarnava

To meet the burgeoning demand of quality raw material to industries based on medicinal plants for making drugs and medicines, there is a need to maintain the natural heritage. Since our natural resource base of medicinal plants is being depleting day-by-day, adulteration or illegal export of several rare species of medicinal plants has been increased alarmingly. Ghaziabad is the second largest industrial area in Uttar Pradesh, environmental stressed conditions affect the flora, particularly of the medicinal plants and their therapeutic properties growing in and around the city. Keeping in view, an experiment was conducted to find out the differences in morphological characters of medicinal plants growing in various unstressed (non-polluted) and stressed (polluted) habitats. The medicinal plants considered for the study were vasaka (*Adhatoda vasica* Nees) and raktapunarnava (*Boerhavia diffusa* Linn.).

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MATERIALS AND METHODS

The present study was conducted on the basis of field survey and experimental work done in the laboratory. The whole experimental work was divided into four phases. In first phase, field survey, selection of both unstressed and stressed habitats (sites), ecological studies of habitats and compilation of pharmacopoeial and ethnobotanical studies, and collection of plant material from different habitats were done. In second phase, macro-morphological and micro-morphological characters were studied and analysed. The physico-chemical and phyto-chemical observations and data were analysed under the third phase. In the morphological studies, substitute plants of both the plants were compared.

In the field survey, selection of habitats (sites), and collection of plant material were done. The morphological (macromorphological and micromorphological) characters were studied. The collection of plant material was collected from polluted (stressed) and non-polluted

(unstressed habitats) sites. Different types of pollution, i.e. vehicular, industrial and urban wastes affecting sites were identified. The types of air pollution and their levels in different sites in the study area were also marked (Dutt, 2009). The data were obtained from different authentic sources like Central Pollution Control Board (CPCB), New Delhi and various Non-Governmental Organizations (NGOs). The stressed and unstressed sites were selected. The stressed site (habitat) means the area, which is affected by any type of pollution, viz. vehicular, industrial etc., however unstressed sites/non-polluted sites means low or free from any type of pollution. During the field survey in the studied area, four each major highly polluted (stressed habitats) and apparently non-polluted (unstressed habitats) sites were selected and marked for collection of samples of studied plants. The sites (habitats) are given in Table 1.

Table 1. Different sites (habitats) (stressed and unstressed) selected for study

Site (habitat)	Types of pollution
Bulandshahar Road Industrial Area, Lal Kuan, Ghaziabad	Industrial effluents
Sahibabad-Mohan Nagar Industrial Area, Ghaziabad	Industrial effluents
Hapur More, Ghaziabad	Automobiles effluents
Modinagar Industrial Area, Modinagar	Industrial effluents
*Raishpur Agricultural Area	Control site

*The site for study area

The plant material of both the plants was collected from all the studied habitats (sites). Ten samples of each species from polluted and non-polluted areas were collected and kept separately for examination. The leaves were taken from matured and flowered plants, so that they remained healthy for a longer time, containing optimum products of plant metabolism suited to exert the desirable therapeutic action. The leaves collected from polluted areas must have recorded the pollution effects of the environment. The samples of leaves after collecting the materials were kept in separate polythene bags with labelling slips (i.e. type and name of species, place of collection, date of collection etc.). The collected leaves/parts were brought to laboratory for further studies (Table 2).

MORPHOLOGICAL STUDIES

The external features of leaves like colour of both surfaces, apex, base, margins, nature of petioles, breadth of lamina and length were studied. The leaf area was calculated by the graph paper method, while leaf size

Table 2. The plants and their parts used for study

Studied plant	Parts	Study
<i>Adhatoda vesica</i>	Leaves/stems	Morphological
	Leaves	Phyto-chemical
<i>Boerhavia diffusa</i>	Leaves/stems	Morphological
		Phyto-chemical

was taken by the scale. Other studies were taken on texture and biometrical data based. The data were collected from plants of studied sites. The plant parts were examined like the surface layer, presence or absence of stomata, and their structure, presence of trichomes and their nature, venation patterns etc. In order to determine the quantitative microscopical characters, viz. vein islet number, vein termination number, stomatal index, frequency of stomata/mm², etc. were studied.

Vein Islet Numbers

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by a stomosis of veins and veinlets, such small portions or areas are termed a vein islets. The number of vein islets/mm² area is termed the vein islet number. This value has been shown to be constant for any given species and for fully-grown leaves, to be unaffected by the age of plants or the size of leaves. The determination was carried out as for palisade ratio and stained with safranin solution and mounted in Canada balsam. Place the stage micrometer on microscope stage and examine with 4X objective and a 6X eyepiece. Drew a line representing 2 mm on a sheet of paper by means of a microscopically drawing apparatus and conduct a square on the line representing areas of 4 mm². Then drew the veins and vein islets included within count the number of vein islets with the squire including those overlapping on two adjacent sides and excluding those intersected by other two sides. For each sample of leaf made three determinations and calculated the average number of vein islets mm² and about 10 determinations were calculated to get the average number used as 10X objective and a 6 eye piece.

Stomata

The previously prepared materials (leaf piece 5 mm × 5mm) transferred to a slide and prepared the mount of lower epidermis uppermost in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with 40x objective and a 6x eye piece, and marked on the drawing paper a cross(x) for each stomata and calculated the average number of stomata mm² for each surface of leaves.

Stomatal Index

The stomatal index is the percentage of the number of stomata formed by total number of epidermal cells, including the stomata, each stoma being counted as one cell. The slides were prepared as in stomatal index. The lower epidermis, uppermost, in chloral hydrate solution and put a small drop of glycerol ethanol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40 × objective and a 6x eye piece to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross for each epidermal cell and a circle (0) for each stoma. The results were calculated as follows:

$$\text{Stomatal index} = \frac{X \times 100}{E+S}$$

where, S = the number of stomata in a given area of leaf and E = number of epidermal cells (including trichomes) in the same area of leaf.

Trichomes

Trichomes are variable outgrowths of epidermal cells, which are useful in the identification of plant material. The trichome may be differentiated into a base embedded in a cell and a projecting body. The trichomes are classified into (non-glandular) and glandular hairs. Both types of trichomes were unicellular as well as multi-cellular. In our study, vasaka and raktapunarnava were studied in details and showed significant differences.

RESULTS AND DISCUSSION

The demand of the crude drugs in the pharmaceuticals and traditional herbalists is fulfilled by indiscriminate/unjustified process. The quality of herbal plants is being ignored by practioners. The practice of using medicinal plants is not under the protocol of GMPs, which includes adequate habitat, harvesting techniques, processing and manufacturing of drugs/medicines (Sharma, 2002). Since medicinal plants can change their morphological characters as well as their active constituents. In the present study we concentrated on the plant habitats along with pollution effect on their morphological variations (Murry, 1985). Due to scarcity of raw plant material of a crude drug, the demand is met by alternate/adulterative plant material which may be mixed entirely different from original plant material. Therefore, in the present study, scientific evaluation was done to identify the appropriate plant material for assured procurement (Naik, 2000).

Selection of Sites

The plants of vasaka and raktapunarnava were collected from different sites. They showed variation in

their morphological characters. The plants of vasaka grew luxuriantly in the control site, where the pH value is 7.0. The soil is aerated-brown with a humidity level of 80%. The surrounding environmental condition have 158 µg/m³ NO₂, 20.0 µg/m³ SO₂, 200 µg/m³ SPM and 105 µg/m³ RSPM. Under stressed condition, the plants change their morphological characters.

The plants of raktapunarnava were inhabitant on sandy soil having about 7.0 pH, 80% humidity and pollution-free environmental conditions under unstressed conditions. The plant material collected from stressed (Table 3) condition has alarming environmental factors, which are Bulandshahar Road Industrial Area, Lalkuan; Sahibabad-Mohan Nagar Area; Hapur More, Ghaziabad and Modinagar Industrial area, Modinagar. Of the studied sites, Raishpur Agricultural Area was found more appropriate resource site for procurement of the plant material.

Table 3. Suitable habitats for studied plants

Plant	Soil pH	Soil humidity (%)±10%	Soil type	Harvesting season
Studied plants				
<i>Adhatoda vasica</i>	7.0	80	Sandy brown	March-April and November-December
<i>Boerhavia diffusa</i>	7.0	80	Sandy black	March-April and August-September

Morphological Study

The size of petiole, leaf-area, and size of lamina were more in unstressed habitat than those of collected from stressed habitat. The venation in leaves were seen 7-19 in pairs under unstressed habitat, while it was 4-8 in pairs in stressed habitat. The texture was also different in plant material collected from both the habitats. The margins of leaves were entire in unstressed plants and slightly serrate and deformed in stressed plants (Table 4).

Under this step, stomata, stomatal frequency, stomatal index and vein number/side were studied comparatively. The stomata were anomocytic type. Their size and forms were different under stressed and unstressed habitats. The frequency of stomata was more in stressed habitat than that of unstressed one. The stomatal index was more in unstressed habitat. Similarly, vein islet number was also more (11-15/mm²). The number of trichomes was more in plants collected from stressed habitat than unstressed habitat. The density of trichomes was also more under stressed habitat. Other parameters also showed variability

Table 4. Macro-morphological characters of *Adhatoda vasica* under both habitats

Trait	Macro-morphological characters under	
	Unstressed	Stressed
Petiole size	2.5-3.5 cm long	1.0-1.5 cm long
Veination	7-19 on each side	4-8 on each side
Apex	Acute	Acute
Margin of lamina	Entire	Less straight, slightly serrate and deformed
Leaf shape	Large and lanceolate	Sharp and lanceolate
Leaf area	50–72 cm ²	25-45 cm ²
Leaf colour	A daxial: deep green and brightly shining Abaxial: pale-green	Blakish-green Pale- green
Thickness of lamina	Slightly thick	Slightly thicker
Texture	More rough on lower side than upper one	Coarse
Size of lamina	Length: 16-23 cm Breadth: 5-8 cm	7-30 cm 2-5 cm
Node and internodal size	15-25cm	5-10cm

(Table 5). *Kashyap*, (2002) showed the impact of air pollution on leaf structure and morphology. *Johri and Snehlata* (1999) studied the leaf surface and showed the impact on morphology. *Dhar* (2002) reported the trichome morphology and density of certain medicinal plants under stressed condition, which were more in

Table 5. Macro-morphological characters of *Boerhavia diffusa* Linn. under both habitats

Trait	Morphological characters under	
	Unstressed	Stressed
Petiole size	1.5-2.5 cm	0.3-1.0cm
Marginal veins	5-9 in each side	3.0-6.0 in each side
Leaf shape	Ovate and slightly lanceolate	Simple and ovate
Leaf area	6.5-18.5 cm ²	5-8 cm ²
Colour of leaf	UE: deep green; LE: pink	Dull green slightly whitish with redish lower side
Texture	Smooth	Coarse
Size of lamina	Length: 3-8 cm Width: 2.5-4.5 cm	1.0-3.0cm 0.8-5.0cm
Root length	30-45 cm	35-50cm
Shoot length	100-220 cm	32-75cm
Biomass	800-1,200 g	400-700g
Internodal size	4.5-9.5 cm	0.5-3.5cm
Stem diameter	0.5-2.5 cm	0.5-2.0cm

number. *Saha et al.* (2000) also conducted the same results which correlate the present study.

Macro-Morphological Study

The *raktapunarnava* is a creeping weed growing in wastelands. It is also found in agricultural lands as well as in roadsides. The plant material obtained from unstressed habitat were healthy and normal, but it was substandard in qualitative and quantitative characters in the material collected from stressed habitat (*Dhar*, 2002 and *Dutt*, 2009). The nodal and internodal size, petiole size, size of branches, and root and shoot length were entirely different in both unstressed and stressed habitats. The petiole size was 1.5 – 2.5 cm in unstressed habitat, while it was 0.3 – 1.0 cm in stressed habitat. The leaf area was more (6.5 – 18.5 cm²) in unstressed than stressed (5 – 8cm²) habitat. The size of lamina was three times more in unstressed habitat than that of stressed habitat. The texture of leaves from unstressed habitat was smooth but it was coarse in stressed leaves. The leaf margins were irregular in stressed habitat. The biomass of unstressed habitat was just double to that of stressed habitat. These studies are similar to those of *Sharma* (2002), *Salgare* (1998) and *Singh* (1984).

Micro-Morphological Studies

There were many differences in micro-morphological characters in between unstressed and stressed habitats. The stomatal frequency was lesser than stressed habitat. The stomatal index was more (19-23) in unstressed than stressed (11-16) habitat. In stressed habitat, the density of trichomes was more on both surfaces of leaves. The vein islet number were three times more in unstressed habitat (*Kashyap et al.*, 2004)., On the basis of morphological phyto-chemical studies, we can certify the particular plant material used in pharmaceutical industry. With the help of present studies, one can recommend the appropriate plant material.

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Effect of foliar application of micronutrients on flowering and fruiting in tomato (*Lycopersicon esculentum*)

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ABSTRACT

An experimental trial was conducted during spring-summer season of 2007, 2008 and 2009 at farmers' fields to study the response of spray of nutrients on tomato (*Lycopersicon esculentum* Mill.) yield and yield-related characters. Five farmers from each KVK- adopted villages were selected and experiment was laid out in one acre area of each farmer. The soil and irrigation water quality were almost similar for all selected farmers' fields. The nutrient treatments included spray of boric acid (B) @ 0.3%, calcium chloride (Ca) @ 0.2% and ferrous ammonium sulphate given twice after transplanting, i.e. at pre-blooming stage and 15 days after first spraying, respectively. The N, P and K were applied at the rate of 150, 75 and 60 kg/ha, respectively. The recommended cultivation practices were followed uniformly for all the fields, irrespective of the treatments. The combination of boron (B)+calcium(Ca)+ferrous ammonium sulphate (@ 0.3, 0.2 and 0.3% spray) recorded maximum net return of ₹ 1,02,464/ha with a 1:2.57 cost:benefit ratio. It gave an additional income of ₹ 20,934/ha (excluding nutrient application charges) over farmers' practices. Application of boron(B)+calcium(Ca) gave a net return of ₹ 98,564 with a 1: 2.56 cost:benefit ratio with an additional income of ₹ 17,034. The minimum cost:benefit ratio of 1:2.48 was obtained by farmers' practices with a net return of ₹ 81,530/ha. The increased returns as a result of increased quality in fruit yield of tomato with nutrient applications gave significant effect on tomato grown by most of the farmers.

KEY WORDS: Growth, Foliar application, Micronutrients, Tomato, Yield, Flowering, Fruiting

Tomato (*Lycopersicon esculentum* Mill.) is an important Solanaceous vegetable in Indian diet by virtue of its nutrients, delicious taste and various modes of consumption and uses. The productivity of tomato is being adversely affected in different areas due to deficiencies of micronutrients (Bose and Tripathi, 1996), which has been increased markedly due to intensive cropping, loss of top soil by erosion, loss of micronutrients by leaching, liming of soil and decreased availability and use of farmyard manure (Fageria *et al.*, 2002). Micronutrients are usually required in minute quantities (Benepal, 1967). They improve general condition of plants and are known to act as catalysts in promoting organic reactions taking place in plant. Tomato is most popular and remunerative vegetable crop extensively grown all over the country. Its cultivation in Delhi region is restricted to the area having good quality irrigation water, as large portion of the area is affected with ground water salinity. Tomato,

being one of the highest yielding crops, removes substantial amount of nutrient from the soil, hence requires balanced quantity of nutrients to be replenished for its sustainable productivity.

The farmers in the region apply N, P and K through inorganic fertilizers with negligible use of organic manures that leads to the deficiency of several other nutrients. The spring-summer is important season of tomato production in the Delhi region besides being grown during autumn-winter. In spring-summer crop, low flowering, poor fruit setting and fruit damage due to fruit cracking and rotting are long-standing problems of tomato growers. These contribute a considerable reduction in quality fruit yield and overall economic returns. The problem is aggravated due to existing poor soil and water quality coupled with high temperature, especially during flowering and fruiting that may hinder the proper availability of nutrients to plants even though available in the soil. Hence, an experiment was conducted to find out the effect of foliar application of micronutrients on yield of tomato.

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MATERIALS AND METHODS

A series of adaptive experimental trials were conducted at farmers' fields for three consecutive years (2007, 2008 and 2009) on the same sites to study the response of nutrients spray on tomato yield and other related attributes. Five farmers from KVK, Ujwa, Delhi adopted villages were selected and experiment was laid on one acre area of each. The soil and irrigation water quality were almost similar for all selected farmers' fields. The nutrient treatments included spray of the treatments comprising boric acid (B) @ 0.3%, calcium chloride (Ca) @ 0.2% and ferrous ammonium sulphate given twice, i.e. at pre-blooming stage and 15 days after first spray. The farmers' practices, i.e. no application of any nutrient as the control at the same variety (Krishna) at 3 different locations (replication) of the same village was treated as the control. The N, P and K were applied at a rate of 150, 75 and 60 kg /ha, respectively and the recommended cultivation practices were followed uniformly for all the fields. The pH of the solution was adjusted to neutral before application. The pooled data of 3 years on growth parameters and yield characters are presented in Table 1.

RESULTS AND DISCUSSION

The treatment combination of boron (B)+ calcium (Ca)+ Ferrous ammonium sulphate (@0.3, 0.2 and 0.3% spray) was found superior to both T₁ and T₂ treatments in respect of number of flowers than the control, number of fruits set, fruit damage due to fruit cracking and blossom end rot than the control. There was lower fruit damage and finally the yield was 22.65% more than the control. Whereas, T₂ found to be more in yield than the control.

Boron is associated with the development of cell wall and cell differentiation, and hence, helps in root elongation and shoot growth of plants. Combined efficacy of macro and micronutrients as foliar application on growth and yield of tomato by Ejaz *et al.* (2011). The increase in yield of tomato was due to increase in number of branches, flower bunch, fruits/bunch and number of fruits/plant as well as total dry matter production. The leaf area and leaf dry weight of plant increased significantly by foliar application of micronutrients (Sahu and C.R. Rajkumaray Bhol, (2012). Blossom-end rot and fruit cracking of tomato be corrected by calcium and boron spray as reported by Frank, Liebischs *et al.* (2009) and Jin-Sheng and Snapp (2004). All the micronutrient treatments except manganese and iron were found significantly effective in increasing fruits/plant and fruit weight. Improvement in growth characters as a result of application of micronutrients might be due to enhanced

Table 1. Effect of foliar application of micronutrients on yield-contributing characters and yield of tomato

Nutrient treatment	Plant height (cm)			No. of flowers/plant			No. of fruits/plant			Fruit yield (q/ha)			Average fruit yield (q/ha)
	2006-07	2007-08	2008-09	2006-07	2007-08	2008-09	2006-07	2007-08	2008-09	2006-07	2007-08	2008-09	
Boron+calcium	65.67	62.55	68.5	47	41	35	33	27	31	269.5	264.5	271.33	268.44
Boron+calcium+ferrous ammonium sulphate	77.33	81.44	69.7	56	45	41	39	33	37	282.33	276.33	278.66	279.10
Control	61.33	65.00	64.5	38	34	33	27	21	28	225.00	214.00	243.66	227.55
Economic impact of nutrient treatment													
	Gross returns (₹)			Cost of cultivation (₹)			Net returns (₹)			Benefit: cost ratio			
	1, 36, 530			55, 000			81, 530			1:2.482			
	1, 61, 064			62, 500			98, 564			1:2.577			
	1, 67, 464			65, 000			1, 02, 464			1:2.576			

photosynthetic and other metabolic activity which leads to an increase in various plant metabolites responsible for cell division and elongation as opined by Hatwar *et al.* (2003). The photosynthesis enhanced in presence of zinc and boron was also reported by Rawat and Mathpal (1984). Mallick and Muthukrishnan (1979) explained that presence of zinc activates the synthesis of tryptophan, the precursor of IAA and it is responsible to stimulate plant growth. Iron plays an important role in promoting growth characters, being a component of ferredoxin, an electron transport protein and is associated with chloroplast. It helps in photosynthesis and might have helped in better vegetative growth (Hazra *et al.*, 1987). Average fruit weight (g), yield/plant (kg), yield/ha were significantly influenced by different treatments with the foliar application of micronutrients as also observed by Singh and Verma (1991).

The response of these nutrients on quality and fruit yield of tomato was most probably due to enhanced photosynthesis and increased production and accumulation of carbohydrates and favourable effect on vegetative growth and reproductive parameters like controlling fruit damage, increasing more flowering and fruiting and leading to increase in number of fruits/plant besides, better fruit size and appearance.

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Evaluation of IPM module for insect pest management in potato (*Solanum tuberosum*): opportunities and challenges in India

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ABSTRACT

An experimental trial was laid out on potato (*Solanum tuberosum* Linn.) in potato-growing areas prone to attack by insect pests including vectors, since these are one of the important limiting factors in raising its productivity and production in India. Monitoring of insect pest especially of vectors, conservation of indigenous natural enemies, need-based application of chemicals and manipulation of cultural practices are key components of the IPM in potato as an alternate to ecologically disruptive chemical control measures. Adoption of IPM is more relevant in potato because it is often consumed directly as a vegetable. There is still a gap in available technology and the technology being adopted by farmers. This gap can be minimized by convincing the farmers about benefits of IPM. Therefore, information on IPM of important insect pests including vectors of potato and challenges in adoption of IPM schedule are important. The importance of aphids and whitefly is mainly due to their role in the spread of viruses. Insect pests of potato have been classified into four major groups on the basis of their mode of feeding and habitat: soil insects, sap feeders, defoliators and storage pests. Various strategies including eco-friendly management of major insect pests of potato is the need of the day for all potato-growing areas.

KEY WORDS: Insecticide, Insect pest, Integrated pest management, Potato

Potato (*Solanum tuberosum* Linn.) is attacked by several insect pests in field and store. Rajendran and Chandla (1986), Mishra and Agarwal (1988), Bhatnagar (2007 and 2008a) have given a comprehensive list of insect pests damaging potato directly or indirectly in different agroclimatic conditions of India. The number of insect pest species causing serious damage to potato have been few in 70s to 10-15 during the last 10 years. Of various insect pests, aphids, whitefly, mites, ants, thrips, jassids, cutworms, white grubs, defoliating caterpillars and potato tuber moth are serious ones and responsible for substantial economic losses, while the rest are sporadic and of minor importance (Saxena and Mishra, 1980). Climatic changes, killing of non-target organisms and destruction of natural enemies have brought much change in the potato pest complex situation in India (Dharpure, 2002).

Since last one decade with the change in climatic condition the new and existing insect pests, especially vector prevalence has caused heavy losses to the potato

productivity and increased the cost of plant protection. Most of the potato growers (small and marginal farmers) are dependent on traders who provide them low grade inputs keeping good margins. Their profits that hinder the transfer of recommended technology. Moreover, growers rarely follow recommend eco-friendly pest management practices. All these factors results in higher incidence of pests. Due to the lack of resistant varieties, poor adoption of improved cultural practices, commercial utilization of bioagents and poor availability of biopesticides, integrated pest management strategies are dominated by excessive use of pesticides. It is therefore, necessary to develop and follow a rational approach with greater reliance on IPM to promote sustainability and reduce use of chemicals.

IPM FOR INSECT PESTS

Cutworms were highly destructive and polyphagous pest and widely distributed in India. Five species of cutworm, viz. *Agrotis ipsilion* (Hfn.), *A. interacta* (Wlk.), *A. flammatra* Schiff., *A. spinnifera* Hb. and *A. segetum* Schiff. damage potato crop in India (Rataul and Misra, 1979). *Agrotis ipsilion* (Hfn.) is common in plains, while *A. segetum* was prevalent in hills. They are nocturnal in

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habit and cut stalks at their base or above the ground level. The infested fields sometimes look as if it has been grazed; in the grown-up crops they usually damage tender shoots and branches. After tuberization their damage is confined to the tubers, reducing market value, damaging 12-40%. Cutworms were active from October to April in plains and summer in hilly region. They have been reported from almost all potato-growing areas of North India. The female moth deposited their eggs in clusters under side of leaves or on wet soil. Beside potato, larvae feed on maize, mustard, cabbage, peas, grams and many weed plants. There were six larval instars, with larval period of 22-30 days. Pupation takes place in soil or between the folds of dried leaves. Two generations of this pest have been reported on potato crop. In general, its life-cycle was completed in 45-75 days (Saxena, 1977).

Deep ploughing of fields in summer and flooding of fields; removal of alternate hosts, weeds and crop residues, and use of pheromone 10-12 traps/ha; conservation of natural enemies like *Brosicus punctatus* Klug and *Liogryllus bimaculatus* and use bio-insecticides like *Bacillus thuringiensis* or *Beauveria bassina* @ 3 g/litre; intercropping of potato with garlic and neem-based formulation (Azadirachtin 300 ppm) @ 6 ml/litre of water on early stage larvae of cutworm, and drenching of potato ridges with Chlorpyrifos 20 EC @ 2.5 litres/ha, are advised.

White Grubs

White grubs have become more important during the last few decades especially in hilly area. They caused damage to a wide range of wild plants and cultivated crops. The damage caused by white grubs varied from 35 to 85%. The species infesting potato were *Brahmina coriacea*, *B. flavoserica*, *B. crinicornis*, *Holotrichia* sp., *H. longipennis*, *Anomala lineatopennis*, *A. dimidiata* and *Lepidiota stigmata*. The grubs caused heavy damage by feeding on newly-planted mother tubers and on roots of potato seedlings. After tuberization, they made large circular holes in tubers, rendering them unfit for market. The adult beetles come out from soil at dusk soon after the first rain in May or June, mate and feed on leaves of trees, female lays their yellow eggs in soil, grubs come out and feed on potato plants.

Deep ploughing and application of *Bacillus* sp. formulation (*B. cereus*, local isolates) @ 1×10^{10} CFU/m² or *Beauveria bassina* @ 5g/m² mixed in well rotten FYM in furrows near the seed tubers; drenching ridges with neem or jatropha seed kernels extracts in cattle urine @ 0.05% and 1.0% near the base of potato plants and Chlorpyrifos 20 EC @ 2.5 ml/litre of water as per the appearance of the pest, need to be done.

Termites (White Ant)

Termites, widely distributed in North-Eastern India, were polyphagous in nature. Two species of termites, viz. *Odontotermes obesus* Ramb. and *Eremotermes* sp. caused 4.7-6.5% damage to potato crop. Mild attack caused few symptoms but yellowing, wilting and some time death of potato plants may result from a severe infestation of termites in potato fields. Small circular holes fill with soil appeared in potato at tuberization stage. Termites first appeared in March when the day temperature was generally above 30°C, subsequently activity increased with the rise in temperature and by mid-April the damage had been increased in potato fields.

Early application of well-rotten FYM/compost and deep summer/winter ploughing; searching termite near the fields/bunds and kill the queen and seed treatment with Chlorpyrifos 20EC/ Imidachloprid 17.5SL are advised.

Red Ants

Red ants, *Dorylus orientalis* Westwood, was a serious problem in potatoes in some parts of Uttar Pradesh, Bihar, West Bengal, Assam and other parts of North-East region of India. The tubers showed a large number of small holes on surface with 70-90% tuber damage under favourable climatic conditions.

Collection and destruction of male ants through pheromone traps and use *Artisemiya* leaves cut @ 5 tonnes/ha near the ridges at the time of planting. Use Chlorpyrifos 20EC @ 2.5 litres/ha as and when ants appeared in potato before tuberization.

Sucking Pests (Sap Feeders)

The major sap feeders included aphids, whitefly, thrips, leafhoppers and mites. Among this group, aphids were of the greatest economic importance as a vector of virus diseases in potato. Whitefly has assumed significant status as a vector in North Central India but high populations of these sap feeders can also cause direct damage to crop. Thrips, mites and leafhoppers are sporadic and restricted to certain potato-growing areas. These sap feeders not only caused direct damage to potato crop but also transmit viruses, resulting degeneration of potato seed.

Aphids

Aphids were most important insect pest/vector in production of healthy seed tubers. Of the dozen species, *Myzus persicae* (Sulzer) and *Aphis gossypii* have been reported widely on potato crop in India (Chandla *et al.*, 2004). As a result of feeding the leaves curl downward, turn yellow, become wrinkled and ultimately die.

Aphids transmitted potato virus in two ways, i.e. non-persistent and persistent manner. Potato leaf roll virus (PLRV) and potato virus (PVY) caused 20-50% and 40-85% yield losses, respectively (Venkatasalam *et al.*, 2011). In north-western and central Indo-gangatic plains, population of aphids remained low from October to December. Aphids crossed the critical limit (20 aphids/100 compound leaves) in these regions only after last week of December in North-Western plains and second week of January in North-Eastern plains. The maximum population is generally observed in February and March. The seed potatoes can be grown successfully by adjusting the planting time and cutting of haulms as per the activity of aphids. Reproduction of aphids was usually parthenogenetic and viviparous. The *M. persicae* feeds on several ornamental plants, weeds and vegetables crops.

Adjusting planting dates as 15 October for North-Western plains, 25 October for Central plains and 5 November for North-Eastern plains and eliminate alternate host plants or infected diseased plants and maintain an isolation of 50 m for seed crop were advised. Use balanced fertilizer and good water management. Haulm killing and harvesting completed as per the activity of aphids or before population attaining 20 aphids/100 compound leaves; monitoring of aphid population through aphid count on 100 compound leaves or use of water and yellow sticky traps; and foliar application of Dimethoate, or Imidacloprid are recommended for controlling aphids on seed crop. Conservation of natural enemies like *Coccinella septempunctata* L., *Chrysopa* and application of *Pongamia glabra*, *Ageratum conyzoides*, *Vitex negundo* and *Acorus calamus* @ 2% is required for organic potato production.

Whitefly

The *Bemisia tabaci* (Genndius) has gradually emerged as an important competent in potato seed production programme in India. This cosmopolitan species damaged by lowering the vitality of plants through the loss of cell sap combined with injection of toxic saliva which is also linked to virus transmission (Chandel *et al.*, 2010). Whitefly is a tiny, soft, white winged insect, found mostly on lower surface of potato leaves and sucking sap from succulent leaves. Whitefly was a newly emerging vector and transmitted Gemini virus in potato, resulting apical leaf curl disease in North-Central India. Whitefly can complete a generation in 20-30 days under favourable conditions (Saini, 1998). Whitefly has a very wide host range. There may be more than 500 host worldwide (Dhawan *et al.*, 2007). Whitefly may transmit disease from one crop to another

or from weeds to crops, vegetation management is also important (Capinera, 2004).

Planting only virus-free tubers minimizes the number of infected plants and eliminate alternate host plants or infected diseased plants. Use balanced fertilizer and good water management in fields. Delay in planting of potato seed crop after 20 October was very useful, monitoring whitefly population on yellow traps.

Only foliar application of Dimethoate, or Imidacloprid were recommended for controlling whitefly on seed crop. The most effective insecticide was Imidacloprid and recommended for seed treatment (@2.5-3 ml/kg of seed) and two foliar applications at emergence with repeated application at 15 days.

Thrips

Thrip was a very small insect of 1mm with fringed wings, adhered on the apical portion of the foliage. *Thrips palmi* Karny was one of the predominant species responsible for transmission of disease in early potato in Central India. It will acquire tospovirus only at nymph stage and transmit throughout their life. The wide range of disease symptoms include leaf droop and hanging, blackening and cracking of stem and concentric ring spots in affected stems. Potato yield losses due to the disease vary greatly from place to place and year to year and may range from 15 to 30% (Khurana *et al.*, 2001).

Mites

Mites caused severe damage (20-60%) to early potato crop in Indo-genetic plains, part of Himachal Pradesh, Maharashtra, Karnataka, Gujarat, Madhya Pradesh and Western Uttar Pradesh. They sucked the sap from leaves and in severe damage, resulting in wither and waxy lower surface. Mite was very minute insect and difficult to see through necked eyes.

Suitable crop rotations with non-host crops like wheat in pest-prone areas and proper isolation to potato crop with susceptible hosts like chilli and brinjal. Spraying of Dicofol 18.5EC or Abamectin benzoate 1.8 EC or Quinalphos 25 EC @ 2.0 litre/ha is advised.

Leafhoppers

The *Amrasca biguttula biguttula* Isida, *Amrasca devastans* Dist., *Empoasca devastans* and *E. fabae* Harris caused direct as well as indirect damage to potato crop. Both the nymphs and adults sucked the sap from tender parts and in severe condition plants showed hopper burn, which was very common in early planted potato crop in northern plains. The nymphs were light yellow to pale yellow in colour with well developed head capsule.

Table 1. Major insect pests of potato found in India

Common name	Scientific name	Order	Family	Plant part damaged	Description/identification
Green peach aphids	<i>Myzus persicae</i> (Sulzer)	Hemiptera	Aphididae	Leaves curl down and yellowing, major vector of potato viruses	Green or pinkish with well developed frontal tubercles, cornicles long, cylindrical and swollen in middle, in alatae abdomen with dwarf patch in centre
White fly	<i>Bemisia tabaci</i> (Gennadius)	Hemiptera	Aleurodidae	Leaves turned sticky due to secretion of honey dew and dries up	Nymphus fattened scale like and pale green and adults milky white and active
Thrips	<i>Thrips palmi</i> (Karny)	Thysanoptera	Thripidae	Leaf droop and hanging, blackening and cracking of stem	Small insect > 0.8 cm in size, prothorax without sutures wing surface pubescent
Leafhoppers	<i>Amrasca biguttata biguttata</i> (Ishida) <i>Empoasca</i> sp.	Hemiptera	Cicallidae	Suck the sap from tender parts, severe damage resulting hopper burn	Body colour ranges from white to pale yellow, wedge shaped body with red transverse marking on back and abdomen
Mite	<i>Polyphagotarsonemus latus</i> Banks	Acarina	Acaridae	Suck the sap from leaves, severe damage resulting wither and waxy lower surface	Small insect, difficult to see with necked eyes
Red ant	<i>Dorylus orientalis</i> Westwood	Hymenoptera	Formicidae	Dark brown in colour, 1-1.5 cm in length	Feed on tubers by nibbing and small but deep circular holes
Cut worm	<i>Agrotis ipsilon</i> Hufn. <i>A. segetum</i> (Schiff.)	Lepidoptera	Noctuidae	Cut the stem above the ground, deep and irregular tunnels in tubers	Dark brown/grey, hairless larvae, damage during night near the base, <i>A. ipsilon</i> is common in plains
White grubs	<i>Biahmia caricea</i> & <i>B. Longipennis</i>	Coleoptera	Scarabaeidae	Feed on rootlets, roots and tubers, makes shallow and circular holes in tubers	Light grey grubs with "c" shaped body brown head, size of grubs vary from 2-6 cm, adult black in colour
White ant (termites)	<i>Odontotermes obesus</i> (Ramb.)	Isoptera	Kalotermitidae	Damage tubers by making deep holes	Wing less soft bodied workers and division of labor in colony
Defoliating caterpillars	<i>Spodoptera litura</i> , <i>Helicoverpa armigera</i> and <i>Plusia oricalisa</i>	Lepidoptera	Noctuidae	Larvae feed on foliage and cause extensive damage under congenial conditions	Dark grey to blackish larva, Greenish or pale brown with stripes, Caterpillars move as semiloopers
Potato tuber moth	<i>Phthorimaea operculella</i> Zell.	Lepidoptera	Gelechiidae	Larvae damage foliage stems, expose tubers in field and stores	Light yellow/pinkish larvae feed on tubers while green coloured feed on foliage with dark brown head

Use of Thiomethosam 25 WG @0.3 g/litre of water when population reached 5-10 adults/plant and judicious use of nitrogenous fertilizer, as higher doses of nitrogen made the plants more succulent and prone to hopper attack.

Defoliating Caterpillars

The defoliating caterpillars, viz. *Spodoptera litura* Hb., *Helicoverpa armigera* Hb. and *Plusia oricalisa* F. were reported on potato crop from different parts of India. The larvae caused damage to feed on green foliage under congenial condition. These were migratory

population, shifted on potato crop from other major host crops like vegetables, pulses and cereals (Rataul and Misra, 1979).

Use pheromone and light trap for monitoring as well as mass collection of catches in trap and destruction of alternate hosts and conservation of natural enemies are required. Use Chlorpyrifos 20 EC spray as per economic threshold, e.g. 2 larvae/m row.

Storage Insects

Potato tuber moth (*Phthorimaea operculella*) was commonly called PTM. It was the most obnoxious pest

Table 2. Damage symptoms and recommended IPM measures for major potato pests

Insect pest	Symptoms	Recommended IPM measures
Aphids	Suck the sap from tender parts and main vector for transmission of PLRV, PVY, PVM, PVS and PVA	<ul style="list-style-type: none"> • Monitoring of aphids using yellow sticky and water traps, aphid count on 100 compound leaves • Planting, dehauling and harvesting at appropriate time • Use healthy disease-free seed material • Protect natural enemies • Use Phorate granules at planting and Imidacloprid spray before 20 aphids/100 compound leaves
White fly	Suck the sap from succulent leaves and transmission of Gemini virus, resulting in apical leaf curl disease	<ul style="list-style-type: none"> • Monitor the population build-up on potato crop • Kill weed and alternate host plants • Alternate spray of Imidacloprid and Oxydemeton methyl at 10 days interval from second week of November to third week of December
Thrips	Suck the sap from tender parts and transmission of tospovirus, resulting in stem necrosis disease	<ul style="list-style-type: none"> • Grow potato varieties K. Satlej, K. Badshah, K. Sindhuri, K. Jyoti and K. Lauvkar • Delay planting of potato by October • Imidachloroprid tuber treatment and one spray on 35 days old crop
Leafhoppers	Suck the sap from tender parts and turning of leaves upwards, hopper burn	<ul style="list-style-type: none"> • Judicious use of N • Use of Chlorpyrifos spray or Imidacloprid spray
Mite	Suck the sap, turning of leaves towards lower side and oily layer appearance	<ul style="list-style-type: none"> • Delay in planting by mid-October • Spraying of Dicofol 18.5 EC or Quinalphos 25EC @2.0 litre/ha
Red ant	Damage tuber by making small pores and weaken plants	<ul style="list-style-type: none"> • Collect male ant by using pheromone trap • Use <i>Artisemiya</i> leaves cut at planting time @ 5 tonnes/ha • Spray Chlorpyrifos
Cut worm	Cut young plants at the base and feed, deep and irregular tunnels in tubers	<ul style="list-style-type: none"> • Deep summer ploughing, removal of alternate host/weed plants • Use pheromone trap for male catch • Drenching of ridges with Chlorpyrifos
White grub	Grubs makes shallow and circular holes in tubers and feed on tubers	<ul style="list-style-type: none"> • Mass collection of adults by jarring and shaking of host tree • Use light trap for beetle collection • Use fermented FYM • Chemicals as per cutworm
Defoliating caterpillars	Directly feed on potato foliage and cause extensive damage under congenial conditions	<ul style="list-style-type: none"> • Handpicking and collection at immature stages • Spraying of Bt formulation or NPV formulation • Use light trap for adult collection • Spraying of chemical insecticide like Imidacloprid/Chlorpyrifos
Potato tuber moth	Larvae damage foliage stems, expose tubers in field and stores	<ul style="list-style-type: none"> • Removal of leftover tubers and self-sown plants • Deep planting and frequent irrigation • Use Melathion spray in stores roofs and walls • Use Lantana or eucalyptus leaves below and above potato heaps • Use CIPC treatment in store

of potato both in field and stores. The PTM larvae damaged foliage, stem, and exposed tubers in fields and stores, causing considerable losses in midhills and peninsular India. The adult of PTM was small, gray in colour having narrow wing with black and brown spots. The larvae are slender with dark head and tan bodies.

Collection of leftover tubers from potato fields after

planting and removal of self-sown potato plants. Use healthy seed, deep planting (10 cm) followed by proper earthing-up. Intercropping of potatoes with chilli, onion and pea and as far as possible, harvested potatoes should be kept in cold storage, in case of non-availability of cold stores, covering potato with 2-5 cm thick layer of chopped dried leaves of Lantana, eucalyptus and

eupatorium both below and above potato heaps reduces damage by about 90%. Use sex pheromone for mass trapping of male under storage condition. Use of CIPC (50%) @40ml applied on 1 tonne of potato with rotatory sprayer in stores. Tubers need drying before storing to avoid rottage and kept closed for 1-2 days after treatment. This chemical was recommended for sprouts suppression and reduced tuber damage by 70-80% in stores.

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Evaluation of gerbera (*Gerbera jamesonii*) cultivars under naturally-ventilated polyhouse in subtropical, sub-montane lowhills of Himachal Pradesh

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ABSTRACT

Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.) is an internationally important cut flower grown for its colourful, showy and long-lasting daisy like flowers. It is one of the nature's beautiful creations having beautiful flowers, exquisite shape, size and bewitching colours, which can be arranged artistically in different floral arrangements. A study was undertaken to evaluate four gerbera cultivars, viz. Balance (white), Dana Ellen (yellow), Rosalin (pink) and Salvadore (red) under naturally-ventilated polyhouse in subtropical, sub-montane lowhills of Himachal Pradesh. These cultivars were introduced first time at Horticultural Regional Research Station (HRRS), Dhaulakuan, during June 2012. Largest flower size (10.80 cm), maximum stalk thickness (6.53 mm), maximum number of flowers (23.33) and long vase- life (10.60 days) were observed in Balance gerbera.

KEY WORDS: Cultivars, Evaluation, Gerbera, Lowhills, Naturally-ventilated polyhouse, Subtropical

Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.) is successfully grown under different conditions in several areas of the world, meeting the requirements of various markets. This success is primarily due to the wide range in colour and shape of flowers. Gerbera is very popular and widely used as a decorative garden plant or as a cut flower. It is also commercially important and fifth cut flower in the world. There is a great demand for its magnificent flowers, particularly in European markets in the winter season and almost throughout the year in India. The growth and flowering of gerbera cultivars depend on type of season, region and growing conditions (Horn *et al.*, 1974). Nowadays, there is a trend of production of tissue-cultured plants of gerbera. New cultivars are being evaluated every year for studying their quantitative and qualitative parameters under polyhouses or greenhouses cultivation.

Good quality cut flowers of gerbera can be produced under protected cultivation. India being gifted with best climate for protected cultivation, the production of gerberas, particularly during winter months is highly profitable compared to temperate countries, where these are grown under greenhouse conditions. India being geographically located between major export markets

like Europe and East Asia, there is an increasing demand for cut flowers from our country. In Himachal Pradesh, 860 ha area is under flower cultivation. Of which, 523 ha alone is in Sirmour district. It is a 24-30 month crop and starts yielding flowers 7-8 weeks after plantation with an average yield of 240 flowers/m². A large- scale commercial cultivation of gerbera has already been done in Uttarakhand, especially in Vikasnagar, Dehra Dun and Kashipur areas which are nearer to Dhaulakuan but no such efforts have been done in state except a few progressive farmers growing in Paonta and Nalagarh. Keeping in view, present studies were undertaken under naturally-ventilated polyhouse at HRRS, Dhaulakuan, district Sirmour.

MATERIALS AND METHODS

Four cultivars of gerbera, viz. Balance(white), Dana Ellen(yellow), Rosalin (pink) and Salvadore (red) in the form of jiffy pots were procured from KF Bioplants, Pune, during second week of June 2012. These jiffy-potted plants were shifted to 6 inches plastic pots in media containing rice husk and sieved soil in a 1:1 ratio and kept under naturally- ventilated polyhouse before the onset of rains. Plants were drenched with Bavistin 1 g/litre+ Humiguard (1 ml/litre). During July first week, these plants were planted on raised beds with size 70 cm width, height 45 cm and plant- to -plant

Table 1. Performance of gerbera cultivars under naturally-ventillated polyhouse

Cultivar	Days to first flower opening	Plant spread (cm)	Flower diameter (cm)	Flower stalk length (cm)	Flower stalk thickness (mm)	No. of flowers/plant	Vase-life (days)
Salvadore (red)	64.80	39.83	9.49	44.73	5.62	14.47	9.40
Balance (white)	68.87	42.77	10.80	59.67	6.53	23.33	10.60
Dana Ellen (yellow)	73.93	46.33	9.61	63.47	6.23	21.73	10.60
Rosalin (pink)	75.93	42.70	10.51	52.80	5.25	17.13	9.67
CD (0.05)	0.84	0.98	0.16	1.25	0.09	0.63	0.42

distance of 40 cm. Plants were drenched with Bavistin 1 g/litre+ Humiguard (1 ml/litre) at fortnight intervals and NPK (19:19:19) and NPK (13:40:13) @ 1g/litre at weekly intervals. Leaf-eating caterpillars were controlled by spraying of Ultineem and ProCron (1ml/litre). The cultural practices such as fertigation and plant-protection measures were carried out uniformly for all the plants.

The evaluation was done to identify the suitable cultivars. The data were recorded on days to first flower opening, plant spread (cm), flower diameter (cm), flower stalk length (cm), flower stalk thickness, number of flowers/plant and vase-life(days). The mean data on various parameters recorded during the study was subjected to statistical analysis. During 2012-13, the trial was laid out in completely randomized block design with four treatments (cultivars) and 10 replications per cultivar (Table 1).

RESULTS AND DISCUSSION

The results revealed significant variations among all the cultivars for various parameters studied. Minimum number of days (64.80) were taken for flower opening in Salvadore, whereas maximum number (75.93) of days to flower opening were taken by Rosalin. Plant spread was maximum (46.32 cm) in Dana Ellen and minimum (39.83 cm) in Salvadore. This variation is due to varietal characters and it was reported by Dhane *et al.* (2004) and Sema Akali *et al.*, (2010). Cultivars Balance and Dana Ellen had no significant difference in plant spread. The largest flower size (10.80 cm) was noticed in Balance and smallest (9.49 cm) size in Salvadore. Cultivars Salvadore and Dana Ellen were

found to be at par with each other. Maximum stalk length (63.47 cm) was recorded in Dana Ellen, whereas minimum (44.73 cm) in Salvadore. Quality of gerbera flowers depends upon stalk length and thickness. Maximum stalk thickness (6.53mm) was observed in Balance, whereas minimum (5.25mm) in Rosalin. Number of flowers was maximum (23.33) in Balance and minimum (14.46) in Salvadore. The increase in flower yield is due to more plant spread in Balance and Dana Ellen. This would have resulted in production and accumulation of more photosynthates, leading to production of more number of flowers. The vase-life was maximum (10.60 days in Balance and Dana Ellen and minimum (9.40) in Salvadore. Cultivars Salvadore and Rosalin were found to be at par with each other and had no significant difference. Balance and Rosalin had large flowers, so these are ideal as cut flowers. Both Dana Ellen and Balance are suitable for exhibition purposes and for making bouquets.

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Effect of vegetative flushing and shoot maturity on flowering, bearing behaviour and fruit yield in litchi (*Litchi chinensis*)

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ABSTRACT

An experiment was conducted to develop an understanding of vegetative and reproductive growth patterns of litchi (*Litchi chinensis* Sonn.) Shahi trees under subtropical conditions at NRC on Litchi, Muzaffarpur, Bihar, during 2009-10 and 2010-2011. Vegetative flushes emerged during July - December were studied for developmental cycle. Growth in the form of flushes occurred in sequential pattern either naturally and/or under forced condition with varied re-growth frequency (extension growth). The flushing and shoot growth pattern were found to influence the overall floriferousness of litchi trees. There was higher percentage of panicle emergence with earlier emerged shoots, while there was a sharp decline in the intensity with the late emerged shoots. July flushes transformed to maximum (up to 90.3 and 84.0%) extent in the panicle-bearing shoots, while tagged flushes emerged during December, particularly could switch over to panicle production to the lowest extent (up to 13.3 and 10.3%), while rest did not bloom. The age of shoot and growth rate was very well monitored by the time of appearance of flushes with the interaction of climatic factors. So, monitoring the appearance of sufficient flushing during July-August, with the gradual decline in flushing capacity from September onwards, not only ensured their maximum contribution in flowering but also caused to give higher fruit setting and fruit yield. The late appearance of flushes gave rise to mixed type of inflorescences, contributing lesser towards flowering and/or sometimes remained vegetative only with reduced flowering and fruit yield.

KEY WORDS: Flowering, Fruit yield, Panicle, Shoot maturity, Vegetative flushing

Litchi (*Litchi chinensis* Sonn.) is an evergreen and important commercial fruit crop of India. It requires specific environmental and soil conditions for its successful production as adapted to subtropical climate. Flowering in litchi trees occurs once a year in the downfall of winter and happens during one growth cycle in the year round of continuous growth process. During this annual cycle, litchi trees must flower, and pollinate for fruit setting. The cyclic initiation of shoots and its extension growth, whether vegetative or reproductive has been found dependent upon the age and climatic conditions, mainly temperature (Davenport, 1986, 1990; Menzel, 1983, 1984). A healthy litchi tree may give rise to 4-5 flushing/year but induction of generative (floral), vegetative or mixed shoots from axillary or terminal is happened to be erratic and inconsistent, seems to be governed by the interaction of so many interdependent factors (shoot age, temperature and nutrient status). Under subtropical

conditions, cool temperature not only triggers bud break, also favours higher ratios of generative shoots. Above all, the erratic-bearing behaviour and poor fruit yield in litchi, has remained the major constraints for litchi growers, require immediate attention. The knowledge of flushing pattern, shoot maturity and the time of fruit bud differential under a particular set of climatic conditions for a given variety would enable the orchardists to schedule the nutrient application, irrigation and other cultural operations to have predicted and better yield and quality fruits. Hence, an experiment was conducted to understand the growth pattern which regulate bearing behaviour for predicting fruit production.

MATERIALS AND METHODS

The experiment was conducted at the National Research Centre on Litchi, Muzaffarpur, Bihar, during 2009-10 and 2010-11. The meteorological data pertaining to temperature (°C) and relative humidity (%) during the study period were recorded (Table 1). The

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Table 1. Monthly mean maximum and minimum temperature and relative humidity (%) during 2009-2011

Month	2009				2010				2011			
	Temp. (°C)		RH (%)		Temp. (°C)		RH (%)		Temp. (°C)		RH (%)	
	Max.	Min.	07.00hr	14.00hr	Max.	Min.	07.00hr	14.00hr	Max.	Min.	07.00hr	14.00hr
January	22.2	10.3	92.0	61.0	21.6	08.0	90.0	53.0	19.6	06.9	90	50
February	26.2	10.9	85.0	43.0	24.8	11.0	89.0	48.0	25.5	10.0	87	48
March	31.5	15.0	80.0	34.0	31.6	14.8	83.0	42.0	31.8	15.5	82	38
April	36.9	20.6	66.0	41.0	43.0	21.0	78.0	12.0	35.2	20.4	78	38
May	34.6	28.7	76.0	49.0	44.2	24.0	82.0	30.0	34.1	23.7	82	54
June	36.9	26.3	81.0	51.0	42.0	26.2	88.0	26.0	34.2	25.2	85	62
July	34.4	26.8	86.0	64.0	38.4	25.0	96.0	54.0	30.4	25.5	89	70
August	32.6	26.0	90.0	74.0	39.2	25.0	95.0	50.0	32.0	26.4	89	73
September	33.6	25.9	89.0	65.0	35.0	24.0	94.0	61.0	30.0	25.8	89	69
October	31.0	20.2	89.0	49.0	34.0	18.2	94.0	52.0	30.8	21.2	91	60
November	28.9	15.3	85.0	44.0	31.2	14.8	92.0	50.0	27.6	15.8	92	54
December	24.5	09.9	89.0	43.0	29.8	08.0	93.0	47.0	20.6	10.8	90	65

experiment was laid out in a randomized complete block design (RCBD) with three replications. Each replication consisted of selected three healthy trees of Shahi litchi of 7 years of age. The material consisted of tagging distinct flushes (100 each) treatment-wise at the time of emergence after harvesting of fruits from July to December in six sampling dates each in one month. The data were collected on shoot emergence and elongation, extension growth, panicle emergence, flowering and fruit-setting and fruit growth at pre-harvest level, and fruit yield and quality attributes at post-harvest level.

The phase of recurrent flushing in different months is even forced by fertilizer application, irrigation and mulching operations. All the trees having flushes and tagged shoots were studied for their vegetative and reproductive growth pattern (shoot re-growth, shoots bearing panicle, total number of flowers during flowering phases, fruit setting and finally for fruit yield and quality). The data were subjected to statistical analysis to test the significant level of the effects of different treatments and their interaction. Data were also presented in per cent values for better interpretation of the results.

RESULTS AND DISCUSSION

The occurrence of monthly temperature and relative humidity during the study period are presented in Table 1. The trend of temperature and relative humidity with variation range can very well be correlated with the vegetative growth and transformed reproductive phase of the litchi trees. There were significant differences for the period of flush emergence to growth of shoots including extension growth (Table 2). It has

been also recorded that the shoots emerged in early months (during July - August), could restart their growth as secondary and tertiary flushes but with decreased frequency, while others in later cases ceased to grow. The vegetative shoot growth occurred periodically either from terminal (apical) portion or lateral buds of stems, taking certain time to start its new growth as for flushes or for floriferous growth (for panicle emergence).

The length of flush was measured from the appeared existing ones at tagging dates, showed maximum times of extension growth in tagged samples for July (5.35 and 3.64) in both the years, followed by the sample shoots tagged during August – September, respectively, while it was recorded minimum (1.80 and 1.08) in both the years showing a definite trend. As growth of flushes proceeded, its branching was also recorded higher in case of July and August shoots with the similar trend during both the years. The tagged shoot during July recorded the maximum length of shoots (87.30cm, 79.24cm) just before the changing phase, while it was minimum length of shoots (20.40cm and 16.54cm) just before the panicle bearing stage which naturally appeared in December. Overall, tagged shoots emerged during July exhibited growth in various intensity and shoots emerged in primary flushes, recorded maximum number of extension growth coupled with branching (7.3 and 8.2) which remained almost dormant with ceased growth (1.1 and 0.6) in December flushes.

It further continued their growth in January even exposed to cool temperature. In this way, the extent of primary shoots emerged during flushes of July and August (90.3 and 84.0%, 89.6% and 75.3%) flushed in

Table 2. Growth parameters recorded during vegetative phase

Treatment	Month	Length of flush (cm)		Times of extension growth		Number of branched shoots		Number of shoots which bore panicles		Total length of shoots at the time of flowering (cm)		Total length of panicle at flowering (cm)	
		2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
T ₁	July	6.50	7.35	5.35	3.64	7.3	8.2	90.3	84.0	87.30	79.24	32.24	22.24
T ₂	August	6.66	6.96	3.84	2.28	7.2	6.2	89.6	75.3	64.10	54.32	33.32	23.32
T ₃	September	6.25	7.25	3.45	1.81	6.1	5.1	82.3	65.0	40.80	32.28	33.28	19.28
T ₄	October	6.45	7.45	3.18	1.22	4.1	3.2	47.6	48.6	38.50	30.56	29.56	13.56
T ₅	November	6.35	6.97	2.10	1.11	3.2	2.2	44.3	19.6	29.60	28.26	29.68	14.28
T ₆	December	6.25	7.10	1.80	1.08	1.1	0.6	13.3	10.3	20.40	16.54	31.32	14.62
	CD at 5%	NS	NS	2.28	1.16	2.34	2.46	11.21	14.23	16.64	13.56	NS	1.32

as secondary and tertiary flushes during both the years giving rise to more number of extension re-growth. In July, total number of extension growth observed emerging from primary, secondary and even tertiary flushes and even more numbers from primary shoots. The very small number of primary and primary-based flushes could continue their growth in shoots emerged after November and onwards as recorded only 13.3 and 10.3 number of primary flushes forming shoots with extension growth and ultimately bearing panicles (for December).

The vegetative growth pattern was observed to be dependent upon the genetic characteristics of litchi plants in addition to climatic conditions and management practices. There were significant differences in time taken for the flush emergence and rate of growth in different months. It was observed that 100 tagged primary shoots emerged as a result of monthly (respective) flushes. There were maximum number of shoots, which appeared as flushes during July and August, transformed from vegetative bud to reproductive buds and bore panicles. Two distinctly separate events must happen for flowering or vegetative growth to occur in litchi.

The resting bud must first initiate growth. Initiation is referred to here as the onset of rapid shoot development (budbreak) regardless of the type of shoot evoked. Coincident with shoot initiation, induction occurred, mainly based on conditions present at the time of initiation. Induction here refers to the temporary commitment of buds to evoke a particular shoot type, i.e. vegetative shoot (vegetative induction), generative shoot (floral induction) or mixed shoot (combined vegetative floral induction).

The data recorded on reproductive phase are presented in Table 3. The sequence of floral formation exhibited obvious phase change, leading to flowering and fruiting. The data showed that earliest flush tagged

in July and August bore maximum number of pure panicles having maximum number of flowers in different phases of appearance during both the years. The fruit setting percentage responsible for quantum of fruit yield was also recorded comparatively higher in the panicle emerged during July, August and September. The number of flowers/ panicle were also recorded higher in shoots emerged during July, August and September during both the years. The fruit yield during July (28.36 kg/plant; 39.66 kg/plant) and August (26.66 kg/plant; 29.63 kg/plant) during both the years under study showed a clearcut indication that flushes which appeared earlier gave significantly higher fruit yield with more number of fruits/ panicle. It was recorded minimum (08.33 kg/plant; 18.63 kg/plant), in plants on which emerged primary flushes were tagged in December (Table 3).

Effect of Flushing and Shoot Maturity on Vegetative Growth

The individual shoot emerged as a result of respective flushing took enough time to pass various growth phases with varied pace to be the category of stem with continued growth as type of vegetative and/ or to become generative shoots. The vegetative growth initiated in the form of flush was never continuous and consistent in the same tree or trees of the same orchard but exhibited periodical quiescence. The number and intensity of flushes varied greatly in the same tree may be attributed due to the fact that differences in temperatures and radiation received by the side and portion of the tree. During July, total number of quaternary flushes emerged from primary, secondary and tertiary flushes. The very small number of primary and primary-based flushes could continue their growth in shoots emerged after November onwards. These observations confirmed earlier findings of Khan (1960) who stated that growth flushes in litchi trees emerging

Table 3. The parameters recorded during the reproductive phase of the growth cycle on litchi

Treat ment	Month	Number of flowers/panicles		Male/hermaphrodite/ female (Nos.)		Sex ratio (M:H+F)		Fruit setting (%)		Fruit yield (kg/plant)	
		2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
T ₁	July	1490	1730	826:296:366	936:360:288	1.24:1	1.44:1	1.27 (19)	1.32 (23)	28.36	39.66
T ₂	August	1470	1680	916:373:391	903:387:387	1.19:1	1.16:1	1.29 (19)	0.95 (16)	26.66	29.63
T ₃	September	1330	1260	734:335:261	672:192:384	1.23:1	1.14:1	1.05 (14)	1.26 (16)	22.32	29.33
T ₄	October	930	980	509:209:262	560:80:320	1.08:1	1.40:1	1.18 (11)	0.91 (09)	11.66	24.36
T ₅	November	610	780	258:192:160	436:106:238	1.26:1	1.23:1	0.98 (09)	1.02 (08)	11.33	19.66
T ₆	December	230	360	118:060:052	196:56:108	1.05:1	1.20:1	1.69 (09)	1.38 (05)	08.33	18.66
CD at 5%		160.2	183.2	–	–	–	–	–	–	5.52	7.31

from July to December, consequently produced as many as five flushes during single growing season.

The present findings also revealed that pattern of re-flushing and cessation of growth during different months indicated that growth generally occurs in at least alternate month's pattern. Each flush, after it is initiated, grows for sometimes, stops and breaks out again till growth finally ceases and found greatly influenced by environmental condition. In this way, development of each new shoot is followed by a period of dormancy (Popenoe, 1920) which helps the shoots to attain proper physiological maturity for flower bud differentiation. This seems to be the reason for more re-flushing frequency, as very less number of flushes emerged in October, November and December, as secondary flushes which consequently gave rest period to tertiary flushes.

Among re-flushed flushes, mean number of flushes produced in July, August and September were found to be statistically at par with each other. Cumulatively, higher percentage of total primary flushes sprouted in subsequent months during vegetative cycle of litchi tree and its ceased after they sprouted once in November or December as primary flushes. In the same way, out of total tagged primary flushes of July, flushes sprouted in February as floriferous flushes and that of December flushes continued their growth even as vegetative or remained ceased and could not grow in any subsequent month.

These data clearly indicated an occurrence of definite pattern in vegetative growth of litchi trees grown under subtropical conditions (i.e. under a particular set of climatic conditions), showing an increase in the frequency of ceased flushes and decrease in re-growth frequency of flushes with the passage of time for consistently transforming into floriferous growth, which is in perfect agreement with findings by (Davenport, 2003). Changes in climatic conditions conducive to vegetative growth seems to bring gradual

changes in endogenous levels of certain hormones which are further responsible for stimulating vegetative growth (Menzel and Simpson, 1995; Davenport, 2000).

Effect of Flushing and Shoot Maturity on Reproductive Growth

There was considerable variation in actual time of transition for vegetative to floriferous condition in litchi, since it is dependent upon the climatic conditions, particularly the fluctuations in temperature, previous year cropping condition and stem age (Davenport, 2000; 2003). According to the weather data, it is evident that there is a sharp change in climatic conditions at the end of September and October with the advent of cold and dry weather and cool condition for two months at least for resting and transition phase. In case of flushes, appeared in September onwards remained vegetative in spite of exposure of low temperatures during the actual flowering season in the region.

Floral induction in litchi occurs in response to cool temperatures perceived by mature leaves which are necessary for floral initiation (Menzel and Simpson, 1995). Floral panicles originate from terminal or subterminal buds of the most recent vegetative flush. The mature leaves also appear necessary for floral induction. Litchi tend to produce inflorescences from terminal buds. Litchi flowering is dependent on bud release during cool florally inductive temperatures, i.e. low temperature. This is largely regulated by maturity of the flush. Vegetative growth in litchi is through recurrent flushing with interval between successive flushes dependent on the prevailing weather conditions (Olesan *et al.*, 2002). There is only a small part of this cycle when the new shoots are being receptive to floral induction and that being around the time of early flush development when expanding buds are no more than a few millimetres in length (Batten and McConchie, 1995). Therefore vegetative shoots that are not mature by late autumn (180-210 days), often do not flower,

because the cyclic nature of flush development means they will initiate new growth only after cool florally inductive winter conditions have passed.

Previous researches of temperature effect on floral bud differentiation in litchi were based on field production and comprehensive analysis of meteorological data (Menzel and Simpson, 1995). Though the results showed that low temperature in conjunction with shoot maturity (stem age) had impact on panicle emergence, many researchers specifically confirm the parameter of only low temperature requirement. Menzel and Simpson (1995) and Young (1970) believed that low temperature was favourable for flower bud differentiation, because relative cold weather was conducive in inhibiting nutritive (vegetative) growth, increasing liquid density of trees, controlling the flushing of spring shoots and the winter shoot promoting reproductive growth and finishing floral bud differentiation. If temperature was high, lack of enough cold accumulation would result in flowering reversion (Young, 1970).

Thus, continuous flushing pattern was observed in vegetative growth of trees in Shahi litchi with gradual decrease in re-flushing frequency of primary and subsequent flushes during the same year. The time of appearance of flushes and its age played their major role in production of healthy panicles with minimum share in production of vegetative growth and the shoots emerged from flushing at latter part of the growth significantly contributed towards production of vegetative growth during current year but resultant reproductive phase transformation was much higher in the early appeared flushes getting sufficient time for shoot maturity. Overall, it may be concluded that as flush grows older, its capacity to produce reproductive panicles increased and regrowth as vegetative flushes decreased. Flowering in litchi trees occurred once a year in winter and happened during one growth cycle in the year round continuous growing process.

Contribution of only some percentage of primary flushes in blooming rather than whole still needs further investigation which may involve biochemical and hormonal assays of these flushes.

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Genetic resources and crossability relationship among various species of *Abelmoschus*

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ABSTRACT

The extensive interspecific crossing was done in the genus, *Abelmoschus*, at the National Bureau of Plant Genetic Resources, New Delhi, during 2008-2011, to find out crossability relationship among various species of *Abelmoschus*. The result indicated that development of F₁ interspecific hybrids of cultivated okra with wild species was comparatively easy but F₁s were sterile. Restoration of fertility through colchicine treatment was successful in the crosses of okra with *A. tuberculatus* and *A. tetraphyllus*. Further, pollen-pistil interaction appears to play an important role in the success of interspecific hybridizations between *A. esculentus* and *A. tetraphyllus* var. *tetraphyllus*, *A. moschatus* and *A. tetraphyllus* var. *pungens*. This appeared due to the predominant presence of pre-zygotic barriers operating at all the stages of reproductive pathway during the passage of pollen tube to the ovary, resulting in limited fertilization, followed by low seed setting and embryo formation. In some cases, incompatibility resulted in seed abortion due to post-zygotic barriers. Utility of *A. caillei* as a potential bridging parent to transfer desirable genes in okra breeding programmes appears to be beneficial. Under field epiphytotic conditions, some accessions of *A. angulosus* var. *grandiflorus*, *A. crinitus*, *A. ficulneus*, *A. tetraphyllus* var. *pungens* and *A. enbeepeegearensis* did not express any YMV symptom and are hence believed to be resistant. An elaborate programme for screening against YMV encompassing all the taxa along with hybrid derivatives in hot spots is necessary for identifying desirable donors. All the wild species including subspecies and taxonomic varieties of the Indian origin are conserved in the National Gene Bank at NBPGR, New Delhi, and are expected to be useful sources for several economically important traits in breeding programmes.

KEY WORDS: Chromosomes, Crossability, Epiphytotic conditions, Genetic resources, Wild *Abelmoschus* species

The Indian subcontinent has more than 10 species of *Abelmoschus*. Of them, *A. manihot* is the only species not occurring in India. Our studies have indicated that Western Ghats and North-Eastern India besides Chotanagpur Plateau are important for wild species conservation. The genus *Abelmoschus* is a native of South and South-East Asia and Africa (Borssum Waalkes, 1966). Even though the genus was originally established and accepted by Gaertner (1791) and Moench (1794), later authors did not recognize it. However, following Hochreutiner (1924) who clearly defined the taxonomic traits of *Abelmoschus*, there is a general consensus for its

acceptance as a separate genus distinct from *Hibiscus*. The genus is characterized by asymmetrical, spathaceous, deciduous calyx which fall off as a unit as against the campanulate or cupular regular, accrescent calyx in *Hibiscus*. The genus derives its name from the Arabic word, *Kaab-el-misk* meaning musk seed (Sivarajan and Pradeep, 1996) and the type species is *A. moschatus* Medik. The *Index Kewensis* lists more than 30 species in Asia and Africa, four each in the Americas and Australia, two-third of them relegated to synonyms. The taxonomy of the genus is complex especially that of infraspecific categories of *A. manihot* and *A. moschatus*.

In the revision of the genus in 1966, Borssum Waalkes retained only 6 species; *A. manihot*, *A. moschatus*, *A. angulosus*, *A. crinitus*, *A. ficulneus* and *A. esculentus*. He simplified Hochreutiner's infraspecific sub-division of *A. manihot* and regrouped those under two subspecies; subsp. *manihot* (L.), which include all the

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cultivated forms and subsp. *tetraphyllus*, comprising all the wild forms. Within the subsp. *tetraphyllus*, he recognized two varieties: *tetraphyllus* and *pungens*, both based on morphological distinctness and geographical discontinuity. Variety *tetraphyllus* is predominantly distributed in lower elevations from sea-level to 400 m that are subjected to annual dry season, whereas var. *pungens* grows at higher elevations (400-1600 m). There are no reports of occurrence of *A. manihot* subsp. *manihot* in India either in cultivation or in the wild. *A. enbeepegearensis* was newly described from lower elevations of Western Ghats (Joseph *et al.*, 2013).

The International okra workshop under the aegis of IPGRI (IBPGR 1991) in an attempt to resolve the taxonomic issues of the genus proposed elevation of two subspecific ranks of *A. manihot* as two distinct species considering distinct chromosome number and ploidy levels as additional traits. They proposed that subsp. *tetraphyllus* should be treated as a separate species as its 2n number (2n = 130,138) is much higher than that for *A. manihot* (2n = 60,66,68). Tyagi (2002) based on chromosome counting from flower buds and root tips from 7 accessions showed that 2n number for *A. manihot* does not exceed 66. Ugale *et al.* (1976) reported 2n = 130 and Joshi and Hardas (1976) reported 2n = 138 for subsp. *tetraphyllus*. Martin (1982) also treated *A. tetraphyllus* as a separate species distinct from *A. manihot*.

The *A. moschatus* is another complex species with many infraspecific types although in India we have only two distinct entities subsp. *moschatus* and subsp. *tuberosus*. The subsp. *moschatus* is called *Kasthurivenda* (*kasthuribhindi*) and is cultivated and occurs as an escape in peninsular India. It is truly wild in secondary forests in North-East region comprising Tripura, Mizoram and Arunachal Pradesh. The subsp. *tuberosus* characterized by tuberous roots and red flowers is a slightly less robust form, occurring rarely in North-East India as an extension of its distribution in Myanmar (Bisht and Bhat 2007). Even though Bates (1968), and Hamon and van Sloten (1995) recommend for its separate species status. We support the placement adopted by Borssum Waalkes (1966), as F₁ and advanced generations of direct and reciprocal crosses of both subspecies were found to be highly fertile behaving like any natural species.

The *A. angulosus* in peninsular India is another polymorphic taxon especially with respect to indumentums, leaf size, shape and flower colour. Sivarajan and Pradeep (1996) recognized 3 taxonomic varieties in peninsular India and Sri Lanka. The var. *grandiflorus* is more common, distributed in the lower elevations in Western Ghats, up to 300 m, whereas var. *purpureus* is restricted to fringes of Shola forests in higher elevation of Western Ghats at Bababudangiri

(Karnataka), Silentvalley National park, Mathiketten Shola NP and Periyar Wild Life Sanctuary (Kerala) and the Nilgris in Tamil Nadu. The var. *angulosus* is extremely rare spotted at a few places in Munnar - Marayur and Munnar - Shanthanpara stretch and its habitat is highly threatened. Being niche specific *ex situ* seed multiplication is also faced with many problems related with its adaptability. Further, it was found to be highly susceptible to fruit-borer.

The *A. tuberculatus* is a species endemic to India (Pal *et al.*, 1952). Morphologically much allied to cultivated okra, Borssum Waalkes considered it only as a synonym of *A. esculentus* even though the protologue itself details its crossability barrier and sterility of F₁ hybrid with *A. esculentus*. The International okra workshop and many Indian workers recognize it as a valid species. Paul (1993) recognized two taxonomic varieties, *viz. deltoideifolius* and *tuberculatus*. Since all the species of okra offer good scope as genetic resource for its improvement. Therefore, a preliminary attempt was made to evaluate the crossability relationship of okra with other species of *Abelmoschus* and scope for incorporation of useful traits from wild species.

MATERIALS AND METHODS

Thirteen taxa of *Abelmoschus*, *viz. A. esculentus*, *A. angulosus* var. *grandiflorus*, *A. angulosus* var. *angulosus*, *A. angulosus* var. *purpureus*, *A. caillei*, *A. crinitus*, *A. enbeepegearensis*, *A. ficulneus*, *A. moschatus* subsp. *moschatus*, *A. moschatus* subsp. *tuberosus*, *A. tetraphyllus* var. *tetraphyllus*, *A. tetraphyllus* var. *pungens* and *A. tuberculatus* were raised in pots at NBPGR Regional Station, Thrissur, Kerala, during 2008-2011. Standardized cultural practices were followed periodically to ensure optimum growth of all the species. Time of anthesis of each taxa was standardized by recording the flower opening at 10 min interval from 5.00 to 8.00 AM for 10 days. Anther dehiscence was studied by observing the dusty nature of pollen-grains and attachment of pollen-grains on stigmatic surface. Emasculation of flowers of female parent was done on previous day evening in flowers ready to open on the next day and pollination was done the next day between 8.00 and 10.00 AM. Pollen-grains collected from male parents were dusted on stigma of emasculated flowers in sufficient quantities to ensure quantum of pollen-grains for fertilization.

Cytogenetical Studies

Seeds of various *Abelmoschus* species were germinated on moist filter papers in Petri-plates at 25°C and the root tips of about 0.5-1.0 cm length were excised, pre-treated with saturated solution of ñ-

dichlorobenzene/0.002M 8-hydroxyquinoline for 3 hr at room temperature and subsequently fixed in freshly prepared Carnoy's fluid (1:3 glacial acetic acid : 96% ethanol mixture) for 24 hr. The fixed root tips were hydrolyzed with 1N HCl for 10 min at 60°C and stained in 1% leuco-basic fuchsin. The stained root-tips were subsequently squashed in 1% aceto-carmin. For meiotic analysis, flower buds of appropriate size were fixed in freshly prepared 1:3 glacial acetic acid: 96% ethanol mixture for a minimum of 24 hr at room temperature and later stored in 70% ethanol at 10°C till use. Anthers were squashed in 1% acetocarmine solution with ferric chloride solution as mordant. A mixture of 25 pollen mother cells was considered for determination of chromosome number and meiotic analysis.

Pollen-Pistil Interaction

The pre- and post- fertilization barriers were studied in selected cross-combinations of species that are difficult to cross. Pollen-pistil interactions were observed in *Abelmoschus* crosses involving four wild *Abelmoschus* species that have greater potential as donors, namely *A. tetraphyllus* var. *pungens*, *A. moschatus*, *A. tetraphyllus* var. *tetraphyllus* and *A. caillei*, African cultivar and one popular cultivar, Pusa Sawani (*A. esculentus*). Emasculation of unopened flower buds was done by removing the petals and undehisced anthers with a sharp knife in the afternoon between 4:00 and 6:00 PM and were covered with butter paper bags to prevent drying of stigmatic surface and cross-pollination due to insect activity. Cross-pollination was made by tapping the pollen-grains directly over the stigmata of emasculated flowers in the following morning between 9:00 and 10:00 AM and the flowers were again covered with paper bags. The pistil from crossed and self-pollinated flowers were collected 1, 2, 4, 8, 12 and 24 hr after pollination (HAP) and fixed in FAA solution (5% formalin: 5% acetic acid: 96% ethanol) for 12 hr and then transferred to 70% ethanol for further processing.

Alcohol preserved pistils of *Abelmoschus* species were gently rinsed in tap water and hydrolyzed in 4N NaOH and transferred to 50 % of sodium hypochlorite solution for 20 min. Pistils were stained with 0.001% decolorized aniline blue dissolved in 0.1% K₃PO₄ solution (Kho and Baer, 1968).

Quantification of fertilization barriers was done as pollen- grains observed on stigma (Phase I), percentage of pollen-grain germination and growth of pollen tube subsequent to penetration of stigma (Phase II), style and up to the ovary (Phase III) and inside the ovary (Phase IV). Pollen germination percentage was calculated as the proportion of pollen-grains that germinated to total number of pollen-grains observed on stigma. Growth of pollen tube estimated as their per

cent occurrence in various regions of gynoecia was calculated with reference to the percentage of total pollen-grains that germinated.

RESULTS AND DISCUSSION

Floral Biology

Anthesis was initiated in all *Abelmoschus* species in the morning. Flower opening is a long process in which the tubular corolla starts spreading gradually after day break. However, ready- to- open buds could be identified in all taxa in previous day by their extended size and visible expression of yellowish red colour. Floral visitors are very few. Anther dehiscence coincides with full opening of the corolla. The time of anthesis in different taxa is presented in Table 1.

Table 1. Time of anthesis in different taxa of *Abelmoschus*

Taxa	Time of anthesis (AM)
<i>A. esculentus</i>	06.40 – 07.30
<i>A. moschatus</i>	07.15 – 07.45
<i>A. tuberculatus</i>	07.00 – 07.45
<i>A. ficulneus</i>	07.30 – 08.30
<i>A. tetraphyllus</i>	07.15 – 07.45
<i>A. crinitus</i>	08.00 – 09.00
<i>A. enbeepeegearensis</i>	09.30 – 10.00
<i>A. caillei</i>	07.00 – 07.45
<i>A. angulosus</i> var. <i>grandiflorus</i>	08.00 – 8.45
<i>A. tetraphyllus</i> var. <i>pungens</i>	07.30 – 07.45

Hybridization

The crossability relationships of Indian taxa of *Abelmoschus* with cultivated okra and between various wild species was worked out at NBPGR Regional Station, Thrissur (Table 2). Wide hybridization attempted to develop interspecific hybrids between various species of *Abelmoschus* was found to be partially successful (Fig. 1). While it was possible to raise F₁ hybrids between majority of the species through emasculation and hand pollination, the F₁ was found to be highly sterile. In cases of cross of *A. esculentus* with *A. tuberculatus*, *A. tetraphyllus*, *A. angulosus* var. *grandiflorus* and *A. caillei*, the F₁ was robust, prolific bearing but the pods developed through back cross were either with chaffy seeds or apparently filled but embryo aborted (Fig. 1). However success in restoring fertility was achieved through amphidiplodization by colchicine treatment.

Colchicine treatment of emerging seedlings at two-leaf stage with 0.1% colchicine for four days at six hour interval gave good success. The synthetic amphidiploids in these crosses behave like normal species but were intermediate between both parents for many characters. The crossed seeds as well as herbarium specimens of

Table 2. Inter and intra-specific hybrids developed at NBPGR RS, Thrissur

Female parent	Male parent	Fruit setting (%)	Status of F ₁
<i>A. esculentus</i>	<i>A. angulosus</i> var. <i>grandiflorus</i>	100.00	F ₁ shy seed setting, chaffy seeds
<i>A. angulosus</i> var. <i>grandiflorus</i>	<i>A. esculentus</i>	16.43	Seeds unfilled
<i>A. esculentus</i>	<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	92.31	F ₁ vigorous with prolific bearing. Sterile seeds with aborted embryo. Pods spiny, 12-15 cm long. Fertility restored through colchicine treatment
<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	<i>A. esculentus</i>	19.23	Vigorous with prolific bearing. Sterile seeds with aborted embryo, pods spiny, 12-15 cm long
<i>A. esculentus</i>	<i>A. moschatus</i> subsp. <i>moschatus</i>	57.14	Few filled seeds
<i>A. moschatus</i> subsp. <i>moschatus</i>	<i>A. esculentus</i>	11.54	No germination
<i>A. esculentus</i>	<i>A. moschatus</i> subsp. <i>tuberosus</i>	47.06	Fruits set but no seed setting
<i>A. moschatus</i> subsp. <i>tuberosus</i>	<i>A. esculentus</i>	0.00	—
<i>A. esculentus</i>	<i>A. caillei</i>	38.89	Vigorous F ₁ , seeds set but no germination
<i>A. caillei</i>	<i>A. esculentus</i>	25.00	Filled seeds (vigorous F ₁ s but sterile)
<i>A. esculentus</i>	<i>A. ficulneus</i>	35.48	Unfilled seeds
<i>A. ficulneus</i>	<i>A. esculentus</i>	0.00	—
<i>A. esculentus</i>	<i>A. tetraphyllus</i> var. <i>pungens</i>	100.00	Fruit set but no seed setting
<i>A. tetraphyllus</i> var. <i>pungens</i>	<i>A. esculentus</i>	0.00	—
<i>A. esculentus</i>	<i>A. tuberculatus</i>	30.00	F ₁ vigorous with prolific bearing. Sterile seeds with aborted embryo. Pods spiny, 12-15 cm long. Fertility restored through colchicine treatment
<i>A. tuberculatus</i>	<i>A. esculentus</i>	85.71	F ₁ vigorous with prolific bearing. Sterile seeds with aborted embryo, pods spiny, 12-15 cm long. Fertility restored through colchicine treatment
<i>A. esculentus</i>	<i>A. crinitus</i>	25	F ₁ performance under study
<i>A. crinitus</i>	<i>A. esculentus</i>	0	—
<i>A. esculentus</i>	<i>A. enbeepeegearense</i>	30.0	F ₁ performance under study
<i>A. enbeepeegearense</i>	<i>A. esculentus</i>	25.0	F ₁ prolific flowering, no fruit setting resembling maternal parent

F₁s of direct or reciprocal crosses are available for utilization and consultation by breeders. Interspecific cross compatibility between okra and various wild taxa, viz. *A. moschatus* (Akhond *et al.*, 2000), *A. angulosus* (Samarjeewa 2003), *A. tuberculatus* (Kuwada 1966; Pal *et al.*, 1952) and *A. manihot* (Kuwada 1961, 1974) was studied by various workers with the intention of incorporating useful traits and creating genetic diversity. However, there are no reports on any study to assess cross compatibility with other species. Wide hybridization using all the available taxa were attempted (Table 3) and following observations were made in the crosses with *A. esculentus*.

***A. angulosus* var. *angulosus*:** The taxa is highly niche specific and hence it failed to grow up to flowering

stage at Thrissur. Raising of cultivated okra at high altitude along with wild species is required for wide hybridization. Though the species is highly susceptible to fruit-borer, genotypes with hybrid vigour, YMV resistance, perennial nature and adaptability to high altitudes, and high rainfall and low sunshine hours may be achieved through crossing.

***A. angulosus* var. *grandiflorus*:** Vigorous F₁ could be obtained when *A. esculentus* was treated as female parent. As the wild parent is late flowering (October), staggered sowing of okra is required to obtain synchronized flowering. F₁s were vigorous but failed to set fruits during early period of growth. However, as the season advanced, prolific fruit bearing was observed but were without viable seeds. The sterility may be

Table 3. Crossability relationship at a glance among different taxa of *Abelmoschus*

Interspecific crosses attempted (species used as female in rows and as male in columns)	<i>A.angul- osus</i> var. <i>angul- osus</i>	<i>A.angul- osus</i> var. <i>grandi- florus</i>	<i>A.angul- osus</i> var. <i>purpu- reus</i>	<i>A.cailliei</i>	<i>A.crinitus</i>	<i>A.escul- entus</i>	<i>A.ficul- neus</i>	<i>A.enbeep- eegear- ense</i>	<i>A.moscat- tus</i> subsp. <i>moscha- tus</i>	<i>A.moscat- tus</i> subsp. <i>hatus</i>	<i>A.tetraph- yllus</i> var. <i>tetraph- yllus</i>	<i>A.tetraph- yllus</i> var. <i>ylus</i>	<i>A.tuber- culatus</i>
<i>A. angulosus</i> var. <i>angulosus</i>	5	-	-	-	3	5	-	3	-	-	-	3	-
<i>A. angulosus</i> var. <i>grandiflorus</i>	-	5	-	0	-	0	0	-	0	0	4	-	0
<i>A. angulosus</i> var. <i>purpureus</i>	-	-	5	-	-	-	-	-	-	-	-	-	-
<i>A. cailliei</i>	5	6	-	5	0	5	-	-	2	0	6	0	0
<i>A. crinitus</i>	3	-	-	-	5	-	-	3	3	0	-	-	-
<i>A. esculentus</i>	-	6	-	7/8	3	5	2/4	-	3	2	5/8	2	5/8
<i>A. ficulneus</i>	-	-	-	-	0	3	5	0	0	0	4	0	7
<i>A. enbeepgearens</i>	-	-	-	-	5	-	-	5	6	6	-	-	-
<i>A. moschatius</i> subsp. <i>moschatius</i>	0	1	-	4	3	4	4	2	5	7	4	0	0
<i>A. moschatius</i> subsp. <i>tuberosus</i>	-	-	-	0	3	3	-	3	7	5	0	0	4
<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	7	0	-	5	-	7/8	0	-	-	-	5	7	3
<i>A. tetraphyllus</i> var. <i>pungens</i>	0	-	-	0	0	0	-	0	0	0	-	-	5
<i>A. tuberculatus</i>	-	-	-	3	0	6	5	3	-	-	-	3	5

-Not attempted; 0, no fruit setting; 1, fruit setting; 2, fruit setting but no seed setting; 3, fruit setting and seed setting; 4, fruit setting, seed setting but no germination; 5, fruit setting, seed setting and F₁; 6, fruit setting, seed setting, F₁ and F₂/BC1; 7, fruit setting, seed setting, F₁ and F₂/BC1 and 8, colchichoploids – C1 germinated and viable seeds harvested.

overcome by colchicine treatment. Transfer of desirable traits like virus resistance, long crop duration, increased number of fruits/plant and photo periodic sensitivity are achievable.

A. angulosus var. *purpureus*: Like var. *angulosus* this taxa also failed to grow up at Thrissur conditions. Hence it is essential to attempt hybridization at high altitude areas. Traits like virus resistance, perennial nature and adaptability to high altitudes may be incorporated from this species through distant hybridization.

A. tetraphyllus var. *tetraphyllus*: Vigorous F_1 s could be obtained in direct and reciprocal crosses without any difficulty. Even swarms of natural hybrids of this species are found growing among the cultivated species. But hybrids were sterile with unfilled seeds. Pods had intermediate size between cultivated and wild parent. The F_1 sterility could be overcome through colchicines treatment. The wild characters like prickly hairs on fruit surface and thin fruit wall with less mucilage content need to be eliminated through repeated back crossing with cultivated okra. Hybrid vigour, long fruiting duration, high branching and relative tolerance to fruit-borer are the positive traits which could be transferred from the wild parent. Successful crossing of okra with *A. tetraphyllus* was also reported by Kuwada (1961).

A. tetraphyllus var. *pungens*: Even though the natural habitat of this taxa is at cooler, high altitude areas, it was possible to grow the species at Thrissur conditions with flowering and fruiting. The cultivated parent needs to be sown during September – November to ensure availability of flowers of both species simultaneously. High fruit setting was observed when *A. esculentus* was treated as female parent but the seeds were chaffy.

A. tuberculatus: This species is very close to the cultivated *A. esculentus* and hence hybrids could be easily obtained in both directions. The F_1 s showed vigorous growth and prolific bearing and fruit size was on a par with cultivated parent. The F_1 s were completely sterile but fertile lines could be raised through chromosome doubling. Successful hybridization of okra with *A. tuberculatus* was also reported by Kuwada (1961).

A. moschatus subsp. *moschatus*: Even though successful wide hybridization with *A. moschatus* was earlier reported by Joshi *et al.* (1974), F_1 s could not be obtained through conventional hybridization technique. Relatively high fruit setting was obtained when *A. esculentus* was fertilized with pollen-grains of *A. moschatus*, seed were found to be unfilled due to abortion of embryo. Hence embryo rescue is needed to obtain

F_1 s. Resistance to jassids is the desirable trait observed in *A. moschatus*.

A. moschatus subsp. *tuberosus*: Okra showed fruit setting when fertilized with the pollen-grains of *A. moschatus* subsp. *tuberosus*. But seeds were completely unfilled and failed to germinate and hence embryo rescue is needed to realize hybrids with this taxa. Dwarf and branched nature of plant and perennating ability by means of tap root tuber, field tolerance to virus and ornamental red flowers are the traits to be exploited from this species.

A. enbeepeegearensis: This newly described species is also a material to be exploited in breeding programmes for transferring virus resistance and perennial nature. Crosses were attempted in both directions and viable hybrid seeds harvested in a cross of okra with this wild species. The hybrid is yet to be studied.

A. caillei: Successful F_1 hybrids with *A. esculentus* were obtained in both direct and reciprocal directions. Even though F_1 s were vigorous with prolific-bearing, generation could not be advanced due to sterility in F_1 s. Chromosome doubling is needed to advance the hybrid. Long crop duration and thicker pods are the traits to be transferred from *A. caillei*.

A. ficulneus: Fruit setting was observed when *A. esculentus* was fertilized with the pollen-grains of this drought tolerant and fruit borer resistant species. But the seed were unfilled. Embryo rescue within one week of crossing may be helpful to obtain hybrids.

A. crinitus: Viable F_1 hybrids were raised from crosses of cultivated okra genotype with *A. crinitus* as pollen parent. The F_1 was, however, sterile. YMV resistance may be transferred from *A. crinitus* even though it was found to be susceptible to jassids.

Based on fertility of inter-specific hybrids, it can be assumed that primary gene pool of okra comprises only land races and other varieties falling under *A. esculentus*, whereas *A. tuberculatus*, *A. crinitus*, *A. tetraphyllus* and *A. angulosus* fall in secondary gene pool.

Analyses of Crossability Barriers

A. esculentus (σ) pollen-grain germination behaviour on stigma of wild *Abelmoschus* species (φ)

When *A. esculentus* was selfed, pollen germination was 87.64% which was significantly ($P < 0.05$) higher than the interspecific crosses with *A. tetraphyllus* var. *tetraphyllus* (55.01%), *A. moschatus* (36.04%), *A. tetraphyllus* var. *pungens* (36.72%) and *A. caillei* (89.7%). After pollen tube penetration into stigma, rate of pollen

tube growth was substantially reduced in style of *A. tetraphyllus* var. *tetraphyllus* (40.46%), *A. moschatus* (28.50%) and *A. tetraphyllus* var. *pungens* (31.55%) which is significantly ($P < 0.05$) lower than the control. In *A. tetraphyllus* var. *tetraphyllus* × *A. esculentus* (34.23%), *A. moschatus* × *A. esculentus* (26.52%) pollen tube required 6 hr to reach Phase II, whereas *A. tetraphyllus* var. *pungens* × *A. esculentus* took 12 hr with very low frequencies (5.05%). Even though overall pollen germination was observed to an extent of 36.72%, very few pollen tubes (2.16%) entered Phase IV and reached the ovule; but after 24 HAP fruit dropping was the main obstacle to get viable seeds in *A. tetraphyllus* var. *pungens* × *A. esculentus* (Table 4).

Structural abnormalities were also evident like heavy callose accumulation at the entry point of ovary, bifurcating of growing tip, swollen tip and twisting of pollen tube were commonly observed in *A. moschatus* pistil (Fig. 1).

Pollen-grains behaviour of wild *Abelmoschus* species

When *A. esculentus* was used as a female parent, significantly ($P < 0.05$) lower pollen germination percentage (39.54%) was observed (Table 5) with *A. moschatus* and *A. tetraphyllus* var. *pungens* (41.24%). In spite of high pollen germination (77.01%) in initial phase in *A. esculentus* × *A. tetraphyllus* var. *tetraphyllus* occurrence of branched pollen tube and reverse direction of pollen tube growth resulted in reduced (36.2%) pollen tube growth (Fig. 1). Occurrence of barriers was not so severe in *A. esculentus* × *A. caillei* as compared to other interspecific crosses.

Crossability Barriers

Species specific adhesion mechanism was observed in *Abelmoschus* species demonstrating the importance of adhesion for successful fruit and seed setting in interspecific crosses. Pollen-grains adherence was very

Table 4. Pollen behaviour of *A. esculentus* (%) on stigma of wild *Abelmoschus* species (&)

Pollen recipient (♀)	HAP (h)	Pollen observed (No.)	Pollen germination (%)	No. of pollen tubes travelling through transmitting tissue			
				Phase I	Phase II	Phase III	Phase IV
<i>A. esculentus</i>	1	250	230(92.00)	202(80.08)	-	-	-
	2	310	285(91.93)	264(85.16)	247(76.67)	196(63.22)	-
	6	230	189(82.17)	170(73.91)	140(60.86)	129(56.08)	105(45.65)
	12	290	245(84.48)	228(78.62)	201(69.31)	178(61.37)	140(48.27)
			1080	949(87.64*)	864(79.44*)	588(51.7*)	503(45.16)
<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	1	300	170(56.66)	135(45.00)	-	-	-
	2	340	187(55.00)	128(37.64)	-	-	-
	6	260	148(56.92)	100(38.46)	89(34.23)	60(23.07)	-
	12	270	139(51.48)	110(40.74)	102(37.77)	87(32.22)	68(25.18)
			1170	644(55.01*)	473(40.46*)	191(18.0*)	147(13.82)
<i>A. moschatus</i>	1	365	110(30.13)	98(26.84)	-	-	-
	2	285	105(36.84)	80(28.07)	-	-	-
	6	279	109(39.06)	89(31.89)	74(26.52)	-	-
	12	257	98(38.13)	70(27.23)	58(22.56)	49(19.06)	34(13.22)
			1186	422(36.04*)	337(28.50*)	132(12.3*)	49(4.76)
<i>A. tetraphyllus</i> var. <i>pungens</i>	1	357	123(34.45)	103(28.85)	-	-	-
	2	268	110(41.04)	97(36.19)	-	-	-
	6	270	107(39.62)	95(35.18)	-	-	-
	12	346	110(31.79)	90(26.01)	70(20.23)	65(18.78)	30(8.67)
			1241	450(36.72*)	385(31.55*)	70(5.05*)	65(4.69)
<i>A. caillei</i>	1	347	302(87.03)	290(83.57)	-	-	-
	2	327	290(88.68)	278(85.01)	250(76.45)	-	-
	6	220	197(89.54)	172(78.18)	159(72.27)	115(52.27)	90(40.90)
	12	340	318(93.54)	290(85.29)	254(74.70)	170(50.00)	140(41.17)
			1234	1107(89.70*)	1030(83.0*)	663(55.8*)	285(25.56)

Figures in parentheses denote percentage value and bold letters indicate percentage of average value calculated on total pollen-grains germinated; *, significant at $P = 0.05$; ±, standard error.

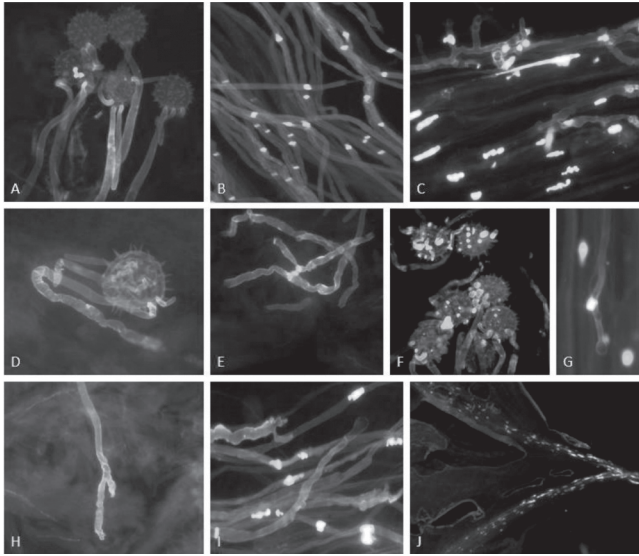


Fig. 1 Pollen germination and their behaviour as observed in interspecific crosses of *Abelmoschus*. A. Germinated pollen-grain of ACA in Phase II in AES pistil; B, normal growth of pollen tubes with uniform callose plug size; C, deformation of callose of plugs and branched pollen tube in AES \times AMT; D, germinated pollen-grain of AMT that grows in reversed direction; E, crooked pollen tube of AMO; F, heavy callose deposition on pollen-grain in AES \times AMO; G, balloon like tip in AES \times ACA; H, I, branched tip and swollen tip in AMP \times AES; J, callose accumulation in pollen tube at entry point of AMT ovary. [*A. esculentus* (AES), *A. caillei* (ACA), *A. manihot* subsp. *tetraphyllum* var. *pungens* (AMP), *A. manihot* subsp. *tetraphyllum* var. *tetraphyllum* (AMT), *A. moschatus* (AMO)].

low in *A. moschatus* \times *A. esculentus*, *A. tetraphyllum* var. *tetraphyllum* \times *A. esculentus* and *A. tetraphyllum* var. *pungens* \times *A. esculentus* which might be due to comparatively large size of *A. esculentus* (σ) pollen which could not adhere to smaller stigmatic papillae of female parent preventing the primary interaction as discussed earlier in *Abelmoschus* species (Abdullah *et al.*, 2000). In this study, occurrence of non-hydrated pollen-grains indicated a possibility of rejection and depletion of pollen-grains of *Abelmoschus* species by stigmatic papillae. Also, low percentage of pollen germination may result in reduction of pollen tube number growing towards the micropyle end which reduces the chances of seed setting in the studied species.

In addition, normal metabolism of pollen tube was hindered in unfavorable condition, as a result, pollen tubes were malformed, which reduced further growth to micropyle. This unfavourable condition might be responsible for unexpected fruit dropping in *A. tetraphyllum* var. *pungens* \times *A. esculentus* which leads to

low fruit setting. This analysis can be explained for the reason of low fruit setting, less number of seed setting and ultimately reducing the crossing efficiency in *Abelmoschus* crosses (Patil *et al.*, 2013).

Overall observation of pollen tube behaviour reflects that structural abnormalities may be a result of high inter-specific morphological distinctness of these species. Furthermore, study demonstrated that the diversity in callose response in relation to interspecific crosses indicates the temporal variations in process of pollen tube inhibition and its role in pollen-pistil interaction and reproductive isolation among *Abelmoschus* species crosses (Patil *et al.*, 2013).

Self-Fertility of F_1 and Back Cross

Back crossing and selfing were performed in the following crosses and its reciprocal namely, *A. esculentus* \times *A. tuberculatus*, *A. esculentus* \times *A. angulosus* var. *grandiflorus*, *A. esculentus* \times *A. caillei*, *A. esculentus* \times *A. ficulneus*, *A. tetraphyllum* \times *A. esculentus*, *A. caillei* \times *A. angulosus* var. *grandiflorus*, *A. caillei* \times *A. tetraphyllum*, *A. moschatus* subsp. *moschatus* \times *A. moschatus* subsp. *tuberosus* and *A. tetraphyllum* var. *tetraphyllum* \times *A. angulosus* var. *grandiflorus*. The F_1 s of *A. esculentus* \times *A. tuberculatus* showed hybrid vigour with a height up to 1.5m and prolific-bearing (30-40 pods on open pollination). Normal fruit setting and apparently normal seeds were observed but the seeds were found to be embryo aborted in both selfed and back-crossed pods. Premature fruit fall and prickly hairs on fruit inherited from wild parent were common features observed. The number of days of pod retention in mother plants varied from a minimum of 6 days in the cross of *A. tetraphyllum* \times *A. ficulneus* and a maximum of 20 days in the cross *A. moschatus* subsp. *tuberosus* \times *A. tuberculatus*.

Hybrids of Ornamental Value

Abelmoschus moschatus subsp. *tuberosus*, is unique in its brick red petal colour and dwarf habit. More known by its synonyms: *A. sagitifolius* and *A. moschatus* subsp. *rodophyllum*, its natural distribution ranges are grasslands and open forest areas in Myanmar, Malaysia, Thailand, Vietnam, China and Australia, extending to North-eastern India. It is an introduced ornamental in Kerala with weak bi-annual habit and small red flowers. F_1 hybrids involving this with *A. crinitus*, *A. enbeepeegearensis* and *A. moschatus* subsp. *moschatus* were found to be dominant for red flower colour and has perennating nature. F_1 hybrids of these three taxa with *A. moschatus* subsp. *tuberosus* were produced and evaluated along with their parents for ornamental traits. The hybrids involving *A. crinitus* and *A. moschatus* subsp. *moschatus* exceeded both the parents for traits

Table 5. Pollen behaviour of wild *Abelmoschus* species (%) on stigma of *A. esculentus* (&)

Pollen recipient (♀)	HAP (h)	Pollen observed (No.)	Pollen germination (%)	No. of pollen tubes travelling through transmitting tissue			
				Phase I	Phase II	Phase III	Phase IV
<i>A. esculentus</i>	1	310	287(92.58)	254(81.93)	-	-	-
	2	290	270(93.10)	264(91.03)	240(82.75)	225(77.58)	-
	6	258	237(91.86)	215(81.33)	195(75.58)	180(69.76)	150(58.13)
	12	260	226(86.92)	212(81.53)	190(73.07)	170(65.38)	137(52.69)
		1118	1020(91.1*)	945(83.95*)	625(57.85)	575(53.18)	287(27.50)
<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	1	267	190(71.16)	160(59.92)	-	-	-
	2	280	219(78.21)	190(67.85)	125(44.64)	90(32.14)	-
	6	315	250(79.36)	210(66.66)	145(46.03)	110(34.92)	-
	12	290	230(79.31)	190(65.51)	157(54.13)	120(41.37)	102(35.17)
		1152	889(77.01*)	750(64.98*)	427(36.20)	320(27.10)	102(8.79)
<i>A. moschatus</i>	1	310	126(40.64)	105(33.87)	-	-	-
	2	270	165(24.07)	102(37.77)	89(32.96)	-	-
	6	297	139(46.80)	97(32.65)	75(25.25)	50(16.83)	-
	12	300	140(46.66)	106(35.33)	92(30.66)	70(23.33)	56(18.66)
		1177	570(39.54*)	410(34.90*)	256(22.21)	120(10.04*)	56(4.66)
<i>A. tetraphyllus</i> var. <i>pungens</i>	1	280	129(46.07)	105(37.50)	-	-	-
	2	295	119(40.33)	98(33.22)	75(25.42)	-	-
	6	290	110(37.93)	90(31.03)	79(27.24)	65(22.41)	-
	12	305	124(40.65)	102(33.44)	87(28.52)	60(19.67)	45(14.75)
		1170	482(41.24*)	395(33.79*)	241(20.29)	125(10.52*)	45(3.68)
<i>A. caillei</i>	1	320	297(92.81)	280(87.50)	-	-	-
	2	290	259(89.31)	245(84.48)	215(74.13)	190(65.51)	-
	6	310	279(90.00)	230(74.19)	196(63.22)	169(54.51)	156(50.32)
	12	265	225(84.90)	202(76.22)	180(67.92)	164(61.88)	139(52.45)
		1185	1060(89.3*)	957(80.59*)	591(51.31)	523(45.47)	295(25.69)

Figures in parentheses denote percentage value and bold letters indicate percentage of average value calculated on total pollen-grains germinated; *, significant at $P = 0.05$; \pm , standard error.

such as prolificacy of flowering, vigour, extended life span, flower colour, shape, flower yield per day and adaptability. These hybrids perennate by virtue of their tuberous tap root and could be multiplied by stem cuttings. It needs very less care and can be grown easily throughout the year in tropical climate both as a plant for flower beds and as potted plants. By virtue of their adaptability to varied season, contrasting leaf, flower and anther colour, prolificacy and perennation, it is an ideal choice for ornamental gardens. Intraspecific cross of *A. moschatus* subsp. *moschatus* and *A. moschatus* subsp. *tuberosus* segregated for flower colour and shades in F_2 generation which offer further scope for selection of ornamental types but *A. crinitus* hybrids were sterile. However, *A. crinitus* hybrids were the most versatile for their multi-petaled red flowers formed by the modification of stamens. *A. enbepeegearensis* hybrids

were weak and poor in adaptability but the flowers were having bright red velvety petals. Propagation in all the three hybrids are easy through vegetative stem cuttings.

Wide range of variation in plant height, number of branches and flower colour pattern and shades was observed in 153 F_2 population of the cross *A. moschatus* subsp. *moschatus* \times *A. moschatus* subsp. *tuberosus*. Plant height varied from 40.0 to 248.0 cm and number of branches 3 to 14. Flower colour variation was characterized based on RHS colour chart. The colour of flower were deep red, moderate red, vivid red, strong pink, deep pink, strong red, deep yellowish pink, strong purplish pink, brilliant yellow, light yellowish green, vivid purplish red, light orange yellow with red stripes, vivid reddish orange, moderate pink, moderate reddish orange and strong yellowish pink.

Cytogenetical Studies

The *A. moschatus* complex, comprising two subspecies, viz. *A. moschatus* subsp. *moschatus* and *A. moschatus* subsp. *tuberosus* had somatic chromosome number of $2n = 72$, even though a few aneusomatic cells with $2n = 68$ were also recorded. Similarly, out of the three subspecies belonging to *A. angulosus* complex, *A. angulosus* var. *grandiflorus* had $2n = 72$ without the occurrence of any aneusomaty. In case of *A. esculentus*, we have confirmed the somatic chromosome number of $2n = 130$, although few aneusomatic cells of $2n = 230$ were encountered. In *A. tuberculatus*, chromosome number has been confirmed as $2n = 58$. The highest somatic chromosome number has been observed in *A. caillei* as $2n = 200$ with few deviant cells also. In *A. manihot* complex comprising subspecies *A. tetraphyllus* var. *tetraphyllus* and *A. tetraphyllus* var. *pungens*, somatic chromosome counts have been recorded as $2n = 132$ and 66 , 132 respectively.

Male meiotic studies of various species of *Abelmoschus* revealed normal chromosome associations in *A. caillei* (2.87IV+0.12III+94II+0.12I), *A. esculentus* (3.86IV+55.87II+1.53I), *A. ficulneus* (2.31IV+ 0.12III+ 29.25II+3.31I), *A. tuberculatus* (2.18IV+23.82II+1.41I), *A. moschatus* subsp. *moschatus* (0.08III+35.44II+0.96I), *A. tetraphyllus* var. *tetraphyllus* (5.64IV+ 0.29III+ 51.08II+ 5.71I) and *A. enbepeegrearensis* (0.23IV+35II+0.28I), which were on expected lines. The recombination frequencies were estimated as 117, 107.80, 54.87, 51.84, 55.56, 100.43 and 62.67 for *A. caillei*, *A. esculentus*, *A. ficulneus*, *A. tuberculatus*, *A. moschatus* subsp. *moschatus*, *A. tetraphyllus* var. *tetraphyllus* and *A. enbepeegrearensis* respectively with the terminalisation coefficient values of 0.78, 0.80, 0.71, 0.71, 0.66, 0.77 and 0.79. The anaphase I distribution has shown by and large equal distribution of bivalents in all the species presently studied.

Male meiotic studies in F_1 hybrids had shown differential behaviour in terms of chromosome associations for example 3III and 30II were encountered at M1/diplotene/ diakinesis in the cross involving *A. moschatus* subsp. *moschatus* \times *A. moschatus* subsp. *tuberosus*. The chromosome numbers in F_1 hybrids were as per expectations barring the incidence of tivalents. Similar patterns was also observed in F_1 hybrids of cross involving *A. moschatus* subsp. *tuberosus* \times *A. moschatus* subsp. *moschatus*, while the F_1 of *A. moschatus* subsp. *tuberosus* \times *A. crinitus* was characterized in exhibiting number of univalents and bivalents (Fig. 2). However majority of PMCs had 69 chromosomes.

The somatic chromosome numbers recorded for *A. tuberculatus* was in accordance with the earlier reports by Joshi and Hardas (1953); Kuwada (1966, 1974);

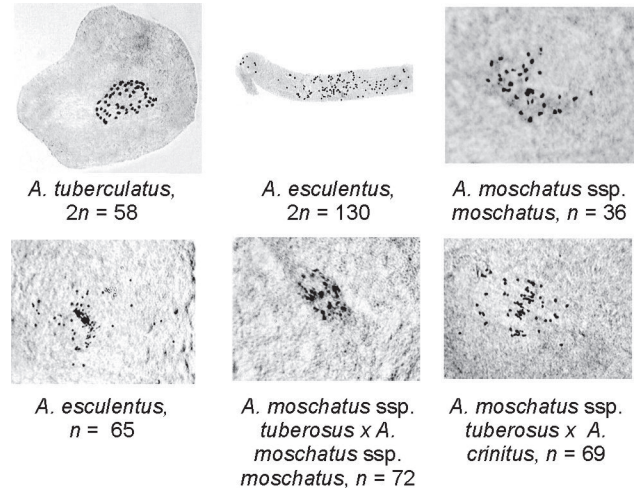


Fig. 2 Male meiosis of parent and F_1 hybrids of *Abelmoschus*

Gadwal *et al.* (1968) and Joshi *et al.* (1974). On contrary, the somatic chromosome numbers for *A. moschatus* subsp. *moschatus* and *A. moschatus* subsp. *tuberosus* were $2n = 72$. However, somatic chromosome number for *A. angulosus* var. *grandiflorus* of $2n = 66$, is being reported for the first time. The study also confirms the somatic chromosome counts for *A. esculentus* as $2n = 130$ (Merita *et al.*, 2012). The chromosome counts for *A. tetraphyllus* var. *tetraphyllus* is $2n = 132$ are at variance to the published reports of $2n = 130$ and $2n = 138$ by various workers (Ugale *et al.*, 1976; Joshi and Hardas, 1976). Likewise, for *A. tetraphyllus* var. *pungens*, the somatic chromosome number is $2n = 132$, while single accession had $2n = 66$. These reports are also at variance with the reports of $2n = 138$ by Gadwal (1968). Extremely small size and large number of chromosomes, unrevealing centromeres and stickiness among the chromosomes might be responsible for such discrepancies found in the chromosome number reports.

Genetic Erosion and Conservation

India is blessed with high representation of almost all wild taxa of *Abelmoschus*. While *ex situ* conservation in seed genebank and field gene banks are being taken up by NBPGR, *in situ* conservation is mostly passive as component vegetation of wild life sanctuaries and national parks. The general level of threat faced by majority of wild *Abelmoschus* species are low but endemic and niche specific taxa like *A. angulosus* var. *angulosus* and var. *purpureus* in Western Ghats needs *in situ* protection. In *A. crinitus* through soft wood grafting on locally adapted *A. moschatus*, vigorous growth and seed production could be achieved. The *A. tuberculatus* and *A. ficulneus*, both conspicuously absent in Western Ghats are normally not adapted to high rainfall season

Table 6. *Ex-situ* conservation of *Abelmoschus* species at NBPGR and its Regional Station

Species	No. of accessions at Thrissur	No. of accessions at NBPGR, New Delhi
<i>A. tetraphyllus</i> var. <i>tetraphyllus</i>	118	142
<i>A. tetraphyllus</i> var. <i>pungens</i>	3	22
<i>A. angulosus</i> var. <i>grandiflorus</i>	11	10
<i>A. angulosus</i> var. <i>angulosus</i>	1	-
<i>A. angulosus</i> var. <i>purpureus</i>	2	-
<i>A. crinitus</i>	1	5
<i>A. enbeepeegearensis</i>	2	1
<i>A. moschatus</i> subsp. <i>moschatus</i>	15	100
<i>A. moschatus</i> subsp. <i>tuberosus</i>	2	4
<i>A. ficulneus</i>	5	83
<i>A. tuberculatus</i>	5	126
<i>A. caillei</i>	24	2
<i>A. esculentus</i>	863	2317

and acidic soils prevailing here. However, through periodic lime application, satisfactory growth and prolific fruiting could be ensured. The *ex situ* conservation of various wild *Abelmoschus* taxa at NBPGR Regional Station, Thrissur, and in the national gene bank are given in Table 6.

Biotic and Abiotic Stress Reaction

The *A. moschatus* is reported to be tolerant to drought and low temperature (Akhond *et al.*, 2000). Further, it is said to be resistant to powdery mildew (Prabhu *et al.*, 1971) and highly resistant to jassids (Sandhu *et al.*, 1974). Compared to any other species *A. tuberculatus* is more close to cultivated okra. Bisht *et al.* (1997) based on a study of selected characters has classified the genetic diversity in 49 collections of *A. tuberculatus* available in the national genebank. Though its vitamin C content is at par with cultivated okra, its heavy bearing may be advantageous in yield improvement. Of late, red mites and African mealy bugs have become severe epidemics in okra in some parts of the country. While *A. moschatus*, *A. crinitus*, *A. tuberculatus* and *A. angulosus* were susceptible, some of the *A. tetraphyllus* collections showed field resistance to red mites. The *A. angulosus* var. *grandiflorus* and *A. crinitus* showed field resistance to YMV over the years at Thrissur.

Synthetic Species

The F₁ hybrid of CO₁ and local land races of okra with *A. tuberculatus* was found to be prolific-bearing, producing over 130 fruits/plant. However, hybrids were found to be intermediate for fruit size and studded with prickly tubercles. The heavy bearing nature, resistance to fruit-borer and fruits with seeds and mucilage offer scope for utilization in a similar way as okra but the prickly tubercles on pericarp is a dominant character. They do not soften even after cooking which is deterrent to its use in culinary preparations. Similarly, the amphidiploid derivatives of the cross between *A. esculentus* (landraces) with *A. tetraphyllus* were found to be fertile and behaving like any normal species. However, F₂ derivatives had more of wild characters, especially small, thin pods and less mucilage.

CONCLUSION

The *Abelmoschus* is a tropical genus with about 10 species with all but *A. manihot* occurring in India. Western Ghats and North-Eastern India besides Chotanagpur Plateau are important for wild species conservation. Development of F₁ hybrids with wild species following wide hybridization can be readily achieved, but F₁ is sterile in majority of combinations. Restoration of fertility through colchicine treatment was successful in the crosses of okra with *A. tuberculatus* and *A. tetraphyllus* at NBPGR. Further, pollen-pistil interaction appears to play an important role in the success of interspecific hybridization between *A. esculentus* and *A. tetraphyllus* var. *tetraphyllus*, *A. moschatus*, and *A. tetraphyllus* var. *pungens*; and this appears to be due to the predominant presence of pre-zygotic barriers operating at all the stages of reproductive pathway with limited fertilization (low seed set) and embryo formation, followed by seed abortion (post-zygotic barriers). Utility of *A. caillei* as potential bridging parent to transfer desirable genes in okra breeding programmes appears to be beneficial. Under field epiphytotic conditions, *A. angulosus* var. *grandiflorus*, *A. crinitus*, *A. ficulneus*, *A. tetraphyllus* var. *pungens*, and *A. enbeepeegearensis* did not express any YMV symptom and are hence believed to be resistant. An elaborate programme for screening against YMV encompassing all the taxa along with hybrid derivatives in hot spots is necessary for identifying desirable donors. All the valid species including subspecies and taxonomic varieties of Indian origin are conserved and are available to breeders for research purposes.

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Seasonal variation in nutrients of mango (*Mangifera indica*) cv. Alphonso and Kesar leaves

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ABSTRACT

An experiment was conducted on seasonal variation of nutrients in leaves of mango (*Mangifera indica* L.) cv. Alphonso and Kesar at ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat), during 2010-2012. There was a decrease in leaf nitrogen content in Alphonso on 1 October, while in Kesar, it was 1 November during first year (2010-11), and 1 October as well as 1 November during second year (2011-12). Later on, continuous decrease up to 1 March after flowering was observed in both the varieties during both the years. Phosphorus level of mango declined from 1 July to 1 September during 2010-2011 and 2011-2012, respectively. Highest phosphorus was recorded on 1 October which was reduced in later stage of period. This trend was found in both Alphonso and Kesar as well as during both the years. On the contrary, potassium content recorded increase from 1 July to 1 September than it decreased from 1 October to 1 June during 2011-12 in Alphonso. In Kesar, potassium content was decreased from 1 July to 1 December. However, it was observed stable up to 1 June in growth and development of mango fruits during both the years.

KEY WORDS: Mango, Alphonso, Kesar, Nutrients and Seasonal variation, Leaf

Mango (*Mangifera indica* L.), the most important fruit of the Anacardiaceae family, is known to originated in Southeast Asia mainly from Indo-Malayan region, possessing the pride position among tropical and subtropical regions and grown commercially in 111 countries. There are more than 1,000 mango cultivars found in Indian subcontinent but hardly 20 cultivars are popular and commercially grown in various parts of the country. However, orchards are poor or less productive. A number of reasons have been assigned for poor productivity of mango orchard. Irregular or biennial bearing is one of the major hurdles in the commercial spread of different varieties and it is entirely an inherent problem of flowering physiology which is exclusively different from the problems of unfruitfulness or shy-bearing. A large number of horti-techniques have been assessed and employed to improve mango production. Leaves are considered as an important storage organ for organic nutrients and carbohydrates, which exhibit the better source and sink relationship. Leaf nutrient status varies with vegetative or

reproductive stage through which a tree passes (Zidan and Maximos, 1962; Avilan, 1971). Nutritive need of mango is very important and is very critical. Plant leaf tissues contains fair amount of nutrient elements. However to estimate the contents, leaf tissue analysis is the correct diagnosis approach. Leaf analysis values have been and are being used as a powerful tool in ascertaining the nutritional status of plant which provide a sound basis in the understanding and remedy of many complex problems.

MATERIALS AND METHODS

An experiment on seasonal variation of nutrients in leaves of mango cv. Alphonso and Kesar was conducted during 2010-2011 and 2011- 2012 at Agriculture Experimental Station, Navsari Agricultural University, Paria. A group of 30 trees of each varieties, viz. Alphonso and Kesar having uniform size and canopy were selected. Leaf samples include leaf lamella and petioles were collected from the middle of shoots of 4 -6 months old selected trees of both varieties as per the method given of Bhargava and Chadha (1993). Total nitrogen of leaf sample was determined by the micro-kjeldahl method and phosphorus was determined by vanadomolybdophosphoric acid yellow colour method

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given by Jackson (1973). Potassium content in leaf samples was determined by flame photometer as per the method given by Piper (1950).

RESULTS AND DISCUSSION

Leaf nitrogen content of cv. Alphonso declined from 1 July 2010 (1.56%) up to 1 October 2010 (1.34%) and then exhibited little rise and fall up to 1 February 2011 (1.34%). On 1 March 2011, leaf nitrogen content was recorded minimum (1.15%) in 2010-2011. In 2011-2012, it showed slight fall and rise from 1 July 2011 (1.83%) up to 1 April 2012 (1.79%). Significantly minimum leaf nitrogen content was found on 1 October 2011 (1.62%) (Table 1).

In Kesar, leaf nitrogen content showed significant increase from 1 July 2010 (1.49%) to 1 January 2011 (1.61%) (Table 2). It was found significantly minimum on 1 August 2010 (1.45%), followed by 1 October 2010 (1.56%). Leaf nitrogen significantly decreased from 1 February 2011 (1.50%) to 1 March 2011 (1.48%) during 2010-2011, while during 2011-2012, it recorded increase from 1 July 2011 (1.80%) to 1 August 2011 (1.81%) and decreased from 1 September 2011 (1.78%) to 1 April 2012 (1.71%).

There was a decrease in leaf nitrogen content in Alphonso on 1 October during both the years, whereas in Kesar, it was on 1 November during 2010-11, and on 1 October and 1 November during 2011-12. Then, there was a gradual decreases up to 1 March after flowering

in both varieties during both years. Thus, decreased level of nitrogen in September and October during first year (2010-2011) and in November during second year (2011-2012) might have led to fruit bud differentiation and induced flowering. Development in source-sink relationship plays a vital role to decide for transition of vegetative to reproductive growth in fruit crops including mango. These findings confirmed to those of Avilan (1971) and Gupta and Narasimham (1980) in mango. Further, nitrogen in leaves of Alphonso and Kesar was found optimum during both the years.

Sharma *et al.* (1978) reported a range of 0.95-1.45%, while 1.88% nitrogen content was reported by Kumar and Nauriyal (1969). Later on, it decreased on 1 March. Photo assimilates translocated from leaves to developing fruits from pea to marble-sized and before maturity stage. Urban *et al.* (2004) reported similar results and are in agreement with the present study. Growing organ essentially require nitrogen to form the protein body which resulted in maximum depletion of this element from leaves (Pathak and Pandey, 1978, Anusuya *et al.*, 2011 in mango; Baloda *et al.*, 2004 in mulberry; Singh and Ali, 2007 in olive).

There were significant decreases in leaf phosphorus content from 1 July 2010 (0.16%) to 1 January 2011 (0.12%) with little increase on 1 October 2010 (0.16%). From 1 February 2011 (0.15%) leaf phosphorus content increased till 1 June 2011 (0.19%). During 2011-12, leaf phosphorus increased from 1 July, 2011 (0.18%) to 1

Table 1. Nutrients content in leaves of mango cv. Alphonso during 2010-2011 and 2011-2012

Date/monnth	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
1 July	1.56	1.83	1.70	0.16	0.18	0.17	0.85	0.83	0.84
1 August	1.52	1.79	1.66	0.13	0.16	0.15	0.84	0.86	0.85
1 September	1.38	1.72	1.55	0.13	0.19	0.16	0.82	0.86	0.84
1 October	1.34	1.62	1.48	0.16	0.20	0.18	0.82	0.85	0.84
1 November	1.36	1.72	1.54	0.14	0.18	0.16	0.82	0.83	0.83
1 December	1.41	1.76	1.59	0.13	0.17	0.15	0.81	0.83	0.82
1 January	1.37	1.78	1.78	0.12	0.15	0.13	0.79	0.82	0.80
1 February	1.34	1.74	1.54	0.15	0.15	0.15	0.80	0.80	0.80
1 March	1.15	1.75	1.45	0.16	0.13	0.15	0.82	0.78	0.80
1 April	1.65	1.79	1.72	0.17	0.13	0.15	0.83	0.75	0.79
1 May	1.98	1.82	1.90	0.19	0.15	0.17	0.83	0.75	0.79
1 June	1.85	1.80	1.83	0.19	0.16	0.18	0.82	0.72	0.77
S Em ±	(M) 0.019	0.011	0.102	0.009	0.008	0.017	0.010	0.016	0.024
	(Y) -	-	-	-	-	-	-	-	-
	(MxY) -	-	0.015	-	-	0.008	-	-	0.013
CD (5 %)	(M) 0.055	0.034	NS	0.026	0.025	NS	0.031	0.048	NS
	(Y) -	-	-	-	-	-	-	-	-
	(MxY) -	-	0.046	-	-	0.025	-	-	0.039
CV (%)	2.18	1.15	1.67	10.29	9.30	9.78	2.21	3.50	2.28

Table 2. Nutrients content in leaves of mango cv. Kesar during 2010-2011 and 2011-2012

Date/monnth	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
1 July	1.49	1.80	1.64	0.15	0.20	0.18	0.87	0.84	0.86
1 August	1.45	1.81	1.63	0.14	0.18	0.16	0.86	0.87	0.87
1 September	1.63	1.78	1.71	0.13	0.17	0.15	0.86	0.87	0.87
1 October	1.56	1.72	1.64	0.16	0.19	0.18	0.85	0.86	0.86
1 November	1.54	1.75	1.65	0.15	0.18	0.17	0.83	0.84	0.84
1 December	1.57	1.76	1.66	0.12	0.18	0.15	0.79	0.82	0.80
1 January	1.61	1.72	1.66	0.12	0.17	0.15	0.80	0.81	0.80
1 February	1.50	1.72	1.61	0.13	0.16	0.15	0.82	0.79	0.80
1 March	1.48	1.75	1.62	0.12	0.15	0.14	0.83	0.80	0.82
1 April	1.69	1.71	1.70	0.13	0.14	0.14	0.82	0.78	0.80
1 May	1.75	1.76	1.76	0.18	0.15	0.17	0.83	0.77	0.80
1 June	1.85	1.80	1.83	0.17	0.17	0.17	0.82	0.79	0.80
S Em.± (M)	0.079	0.019	0.062	0.007	0.004	0.012	0.013	0.005	0.012
(Y)	-	-	-	-	-	-	-	-	-
(MxY)	-	-	0.057	-	-	0.005	-	-	0.009
CD (5 %) (M)	0.232	0.056	NS	0.020	0.011	NS	0.039	0.014	0.040
(Y)	-	-	-	-	-	-	-	-	-
(MxY)	-	-	0.16	-	-	0.016	-	-	0.028
CV (%)	8.609	1.877	5.96	8.313	3.647	6.04	2.745	1.034	2.04

October 2011 (0.20%) and reached to maximum level. Then it started to decline from 1 November, 2011 (0.18%) to 1 April 2012 (0.13%) and recorded minimum phosphorus content in Alphonso leaves.

During 2010-2011, leaf phosphorus showed significant decrease from 1 July 2010 (0.15%) to 1 January 2011 (0.12%) in Kesar. Sudden and significant increase was recorded on 1 October 2010 (0.16%). On the other hand, leaf phosphorus content was significantly maximum on 1 July 2011 (0.20%) during 2011-2012. It significantly decreased from 1 August 2011 (0.18%) to 1 April 2012 (0.14%) and registered an increase during 1 May (0.15%) and 1 June 2012 (0.17%).

The phosphorus level of mango was decreased from 1 July to 1 September 2010-2011 and 2011-2012. October had registered the highest value for phosphorus accumulation, then again it started to decline. This trend was found in both the cultivars during both the years. Under South Gujarat agroclimatic condition, normally three vegetative flushes occur in a year, and flowering takes place from December to February depending upon the congenial condition. The phosphorus level of leaves in both cultivars was within optimum range (0.022-0.210 %) (Young and Koo, 1969). Increasing trend in phosphorus content was found in leaves on 1 October and 1 November. Higher phosphorus content was found in leaves of both varieties during October and November, which is the

probable fruit bud differentiation stage. The higher phosphorus level and comparatively low nitrogen might have induced fruit bud differentiation and flowering. This has resulted in fruits harvested in June, i.e. before July, thereby terminating the sinking of nutrient into leaves (Koo and Young, 1972).

In the present study, decreasing level of phosphorus after December and January during both the years in Alphonso as well as in Kesar can be correlated with advancing leaf age as well as vital role of phosphorus in the formation of seed in plants. The decline in phosphorus in mango leaves during fruit development could be due to its utilization in greater quantities for formation of stone as eulogized by Kumar (1970), Dhillon *et al.* (1987) in mango; Singh and Randhava (1961) in orange; Singh and Ali (2007) in olive.

Further, leaf potassium content of mango cv. Alphonso decreased from 1 July 2010 (0.85%) to 1 January 2011 (0.79%) and it significantly increased from 1 February 2011 (0.80%) till 1 May 2011 (0.82%) (Tables 1 and 2). On other hand, leaf potassium content during 2011-12 was significantly increased from 1 July 2011 (0.83%) to 1 September 2011 (0.86%). Then, it decreased from 1 October 2011 (0.85%) till 1 June 2012 (0.72%).

In Kesar, leaf potassium content recorded significant decrease from 1 July 2010 (0.87%) to 1 December 2010 (0.79%), the minimum level. Then potassium content of leaf exhibited little rise and fall up to 1 June 2011 (0.82%).

During 2011-12, leaf potassium content was significantly increased from 1 July 2011 (0.84%) to 1 September 2011 (0.87%), then it decreased from 1 October 2011 (0.86%) till 1 May 2012 (0.77%).

Rapid decrease in potassium content in leaves of both cultivars at initial stage corresponded with the period of rapid growth of shoots. Similar observation has been reported by Koo and Young (1972). Decreasing or stability in potassium content after flowering, fruit setting and fruit growth might be due to utilization of potassium by fruits in their development. The result of Pathak and Pandey (1978), Chadha *et al.* (1984), Dhillon *et al.* (1987) are in agreement with the present findings.

Pooled data of nitrogen, phosphorus and potassium content in leaves did not show significance. However, interaction of month (M) and year (Y) recorded significant difference, indicating the role of important nutrients present in leaves after application of fertilizer during monsoon. Availability of nutrients, higher absorption and photosynthesis resulted in accumulation in leaves. During the period of fruit bud differentiation, flowering and early stage of fruit development might have resulted in the depletion of nutrients in plants.

From 1 March to 1 June during both the years, increase in nitrogen, phosphorus and potassium was recorded, indicating better source-sink relationship because of availability of second half dose of nutrients generally applied during February. Increased nutrient contents in leaves translocated to the developing fruits of Alphonso and Kesar. Fluctuation in nutrient contents particularly nitrogen plays an important role in fruit setting, drop and retention up to maturity stage. These finding are in agreement with those of Zidan and Maximos (1962) in mango cv. Pyri and Alphonso, Avilan (1971); Chadha *et al.* (1984) in Dashehari and Chausa mango. Rajput *et al.* (1987) has reviewed the factors affecting mineral composition of mango leaves in relation to flowering which also supported our present findings.

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Radiation induced mutagenesis in annual Chrysanthemum (*Chrysanthemum coronarium*) cv. Local White

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ABSTRACT

An experiment was conducted to induce novelty using gamma irradiation in annual chrysanthemum (*Chrysanthemum coronarium*) cv. Local White at IARI, New Delhi, during 2007. The seedlings were irradiated at 0, 2.50, 5.00, 7.50, 10.00 and 15.00 Gray gamma rays. The favourable effect of radiation was perceptible at lower doses (5.00 Gy). Beyond the dose of 7.50 Gy gamma radiation the seedlings did not survive. From the mutated population, 14 types of novel promising mutants comprising semi-double, double and miniature forms were isolated. The flower form varied from marigold, carnation, ranunculus, chrysanthemum, gerbera and dahlia flower types in those seedlings that were exposed to 5.00 Gy gamma radiation.

KEY WORDS: Radiation induced mutagenesis, Annual Chrysanthemum, Gamma radiation, Seedlings

The commercial cultivation of annual chrysanthemum (*Chrysanthemum coronarium*) has gained momentum in recent times due to its wider acceptance in different markets. Variability available in the species is very negligible. Only white and yellow cultivars with single and semi-double forms are available but the double variants are in great demand in the market. Creation of variability therefore has enormous commercial significance. Mutation breeding played a major role in the improvement of ornamental crops. Plant mutant database of International Atomic Energy Agency lists 3,218 mutants belonging to cereals, pulses, oilseeds, fruits, vegetables, flowers besides medicinal and aromatic crops (IAEA 2013).

Of the 3,218 documented mutants, 278 belong to the genus chrysanthemum and none belong to *C. coronarium*. Man-made efforts to induce mutations using physical and chemical mutagens were successful with varied frequencies. Earlier efforts to induce mutations were aimed at using UV, X-rays besides alpha, beta and fast moving neutrons. Gamma rays remain the most potent to induce desirable variability in a number of ornamental crops (Datta 2005). Therefore, an experiment was conducted to inducing novelty in flower colour and form in annual chrysanthemum by employing gamma irradiation.

MATERIAL AND METHODS

The annual chrysanthemum cultivar Local White (single flower form) was grown in isolation to purify the cultivar for three consecutive years with selfing bags to facilitate self-pollination. The selfed seeds were sown and the off-types were rogued out. The purified seeds from cv. Local White were sown during September 2007. The seedlings of 10 cm height (20-25 days old) were irradiated in a gamma chamber with ⁶⁰Co as the radiation source at the Nuclear Research Laboratory (NRL), IARI, New Delhi. One hundred seedlings each were irradiated at 0.2.5, 5.00, 7.50, 10.00 and 15.00 Gy gamma radiation, whereas 100 untreated plants were planted as the control. The LD₅₀ was determined based on the survival percentage of irradiated seedlings. The irradiated seedlings were transplanted on ridged beds under open field conditions at a distance of 70 cm × 70 cm. Standard agro-techniques and pest control measures were followed to raise a healthy crop. Radio-sensitivity and post-radiation recovery were assessed by recording the percentage of survival and establishment of plants in the field.

Morphological observations comprising vegetative and flowering attributes were recorded when the plants were in full bloom (120 days after transplantation). Perceptible morphological variations and colour differences were recorded to select novel mutants and to compute the mutation frequency at various radiation

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Table 1. Effect of gamma irradiation on survival of annual chrysanthemum seedlings (30 days after radiation)

	Radiation dose (Gy)					
	0 (control)	2.50	5.00	7.50	10.00	15.00
Survival rate (%)	99.00 ± 2.24	95.20 ± 1.58	50.60 ± 0.86	20.00 ± 0.46	0.00	Nil
Plant height (cm)	26.40 ± 0.32	30.60 ± 0.84	20.70 ± 0.32	16.20 ± 0.32	–	–
Number of branches	9.00 ± 0.41	7.00 ± 0.12	4.00 ± 0.12	2.00 ± 0.06	–	–
Number of leaves/branch	6.00 ± 0.28	6.70 ± 0.18	4.62 ± 0.08	2.00 ± 0.14	–	–
SEM ±						

doses. The mutants were compared based on morphological traits and floral attributes.

Promising novel mutants were bagged with muslin cloth bags to encourage self-pollination to raise self-pollinated seeds to raise the M2 population. The isolated M2 population was selfed and the subsequent generations were raised.

RESULTS AND DISCUSSION

Standardization of Radiation Dose

The data on influence of radiation dose was recorded 30 days after transplantation. The survival percentage and growth decreased as the radiation dose increased (Table 1). The favourable influence of radiation was perceptible at lower doses (2.50 Gy). Among the irradiated population, survival percentage (95.20 ± 1.58), plant height (30.60 ± 0.84), number of branches (7.00 ± 0.12) and number of leaves/branch (6.70 ± 0.18) were more at 2.50 Gy dose. At this lower dose of radiation the plants were more vigorous and tall in stature. The leaves were more succulent and lush green compared to the control and other doses of irradiated plants. Only 20% of seedlings survived when the radiation dose was increased to 7.50 Gy. When the radiation dose was increased beyond 7.50 Gy gamma rays the seedlings did not survive (Table 1). LD 50 dose was found to be at 5.00 Gy. At higher dose, the plants were shorter (16.20 ± 0.32) and produced lesser branches (2.00 ± 0.06) when the plants were subjected to 7.50 Gy gamma radiation.

Mutant Selection and Mutation Frequency

The variability in vegetative and floral characters was recorded by critical visual observations. Perceptible variation in flower form and colour were not observed at lower doses of radiation (2.50 Gy). However, novel mutants were recorded when seedlings were exposed to 5.00 Gy radiation dose. The mutated population exposed to 7.50 Gy radiation dose produced abnormal flowers compared those identified at 5.00 Gy. From the

mutated population irradiated with 5.00 Gy dose 14 new promising mutants (14%) comprising altered flower form and colour were isolated. The single flower form in the parent cultivar is modified into semi-double, double, miniature forms at 5 Gy of radiation. Out of which 8 were found to be promising and possessed commercial significance. The flowers with varied flower form appeared like marigold, gerbera, carnation, ranunculus, chrysanthemum and dahlia flower types in those seedlings that were exposed to 5.00 Gy gamma radiation (Fig. 1).

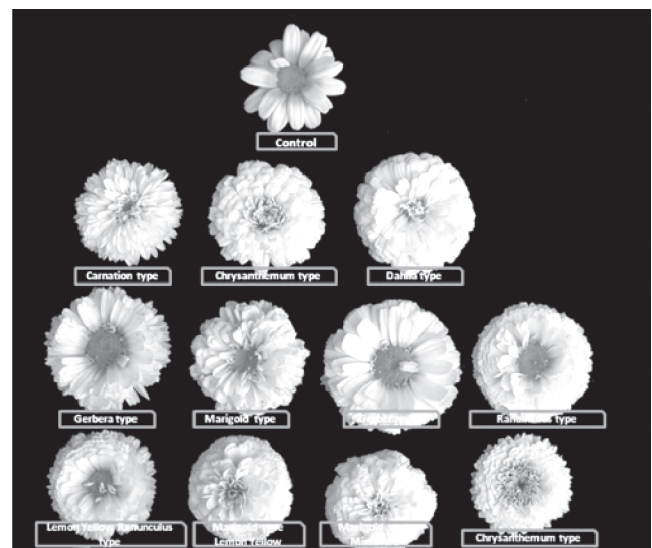


Fig. 1 The flowers in varied forms

Effect of Radiation on Vegetative Traits

Significant differences were noticed in the vegetative traits in six flower form mutants and two flower colour mutants. Highest plant height (114cm) and length of leaf lamina (10.71 cm) were recorded in those plants that produced gerbera type flowers, whereas minimum (84 cm) was recorded in those plants that produced marigold type semi-double flowers

Table 2. Flower form and colour mutants observed at various doses of gamma radiation

Dosage (Gy)	Flowers form mutants	Colour of mutants	Remarks
2.50	Nil	Nil	Enhanced vegetative growth, lush green foliage
5.00	12 Marigold type double Marigold type semi-double Marigold type miniature Chrysanthemum type double Ranunculus type gerbera type Dahlia type	2 (Lemon yellow Ranunculus type Lemon yellow marigold type)	Flower form comprise semi-double and double forms
7.50	4	4	Flower forms comprise abnormal flowers which are misshapen with distorted flower structure
10.00	Nil	Nil	–
15.00	Nil	Nil	–
0.00 (control)	Nil	Nil	Nil

(Table 2). The significantly more number of branches (41) were found in those plants that produced ranunculus type of flowers compared to the control. Plants that produced chrysanthemum type of flowers produced maximum number of leaves (35.64), leaf width (6.37 cm) and length of lower lobes (2.79 cm). The radiation dose also brought about perceptible changes in colour of flowers. Lemon yellow coloured flowers resembling ranunculus and marigold type of flowers were recorded among the novel mutants (Table 3).

Effect of Radiation on Floral Traits

The number of flowers produced per plant was highest in those plants that were not irradiated (292 flowers). Among the induced mutants, highest number of flowers/plant was recorded in those plants that produced chrysanthemum type of flowers. Flower diameter was found to be more in gerbera type of mutants. Among mutants of the peduncle length (8.92 cm), thickness (0.40 cm) and length of corolla tube (0.82 cm) were more in marigold type mutant, whereas length (3.23 cm) and width (1.87 cm) of ray florets was more in gerbera type mutant. The parent cultivar Local White produced flowers that were pure white (150 D). Minor variations in flower colour were evident in the mutants that produced flowers that were near to white (1D, 4D and 12B). Significant colour variation was noticed in two mutants that produced lemon yellow coloured (8C) flowers that resembled marigold and ranunculus types (Table 4).

Induced or spontaneous mutations have played a major role in development of present day commercially important chimeras. These include not only the

variegated leaf pattern selections, but also some colour sports such as the 'William Sim' carnation, 'Indianapolis' chrysanthemums (Stewart and Dermen 1970), many poinsettia cultivars (Stewart and Arisumi 1966), African violet (Lineberger and Druckenbrod, 1985), Ficus (Beardsell and Norden 2004). The survival percentage and growth decreased as the radiation dose increased. The favourable influence of radiation was perceptible at lower doses (2.50 Gy). Such favourable influence of radiation at lower doses (hormosis) was well documented in some of the crops (Luckey 1991). At this lower dose of radiation the plants were more vigorous and tall in stature. The leaves were more succulent and lush green compared to the control and other doses of irradiated plants. Only 20% of the seedlings survived when the radiation dose was increased to 7.50 Gy. When the radiation dose was increased beyond 7.50 Gy gamma rays, the seedlings did not survive.

Novel mutants were recorded when seedlings were exposed to 5.00 Gy radiation dose. The mutated population exposed to 7.50 Gy radiation dose produced abnormal flowers compared to those identified at 5.00 Gy. From the mutated population irradiated with 5.00 Gy dose 14 new promising mutants comprising altered flower form and colour were isolated. The initial action of most ionizing radiation is on the water which constituted about 98% of the total number of molecules in soft tissues. Ionizing radiation produced a variety of oxygen species from water) (Gould 1968, Luckey 2005). Each of these will avidly attack nearby material to make strange compounds and atomic fragments (free radicals) which can change the structure of DNA and RNA, drastically alter metabolic pathways. This could be the possible reason for the induction of novel types

Table 3. Effect of gamma irradiation (5Gy) on vegetative traits, six flower form and two flower colour of mutants

Mutant	Plant height (cm)	Leaf length of lamina (cm)	Number of branches	Number of leaves / branch	Leaf width (cm)	Leaf length of lower lobes (cm)
Flowers form mutants						
Marigold type double	91.70	10.10	36.00	32.00	5.41	2.58
Gerbera type	114.20	10.71	34.00	34.66	6.47	2.25
Marigold type semi-double	84.00	9.50	37.66	35.00	5.49	2.43
Ranunculus type	94.00	9.33	41.00	26.00	5.43	2.75
Carnation type	99.10	7.62	29.00	30.00	4.34	2.49
Chrysanthemum type	98.70	10.03	38.00	35.64	6.37	2.79
Colour of mutants						
Lemon yellow						
Ranunculus type	91.7	10.10	36	32	5.41	2.58
Lemon yellow marigold type	114.20	10.71	34	34.66	6.17	2.25
Control white	102.10	10.45	32.00	27.00	6.52	2.73
CD (5%)	2.31	1.22	3.54	3.89	0.84	0.86

Table 4. Effect of gamma irradiation (5Gy) on floral traits

Mutant	Inflor-escence width (cm)	Number of flower buds/plant	Flower diameter (cm)	Disc diameter (cm)	Peduncle thickness (cm)	Length of peduncle (cm)	Length of corolla tube (cm)	Length of outer florets (cm)	Width of outer florets (cm)	Colour (RHS colour chart)
Flowers form mutants										
Marigold type double	17.70	230	6.39	-	0.25	8.20	0.82	2.18	0.93	150 D
Gerbera type	13.49	225	7.55	-	0.32	7.97	0.46	3.23	1.87	1 D
Marigold type semi-double	16.17	130	5.65	2.29	0.40	8.92	0.48	2.67	1.25	4 D
Ranunculus type	16.07	180	7.20	1.28	0.27	5.57	0.54	2.56	1.19	4 D
Carnation type	18.90	127	5.53	-	0.33	6.43	0.63	2.23	1.12	4 D
Chrysanthemum type	12.65	284	5.16	-	0.33	5.68	0.51	2.11	1.06	12B
Colour of mutants										
Lemon yellow										
Ranunculus type	17.7	230	6.39	-	0.25	6.60	0.82	2.18	0.93	8C
Lemon yellow marigold type	13.49	225	7.25	2.29	0.32	8.20	0.46	2.23	1.17	8C
Control (Local White)	21.50	292	6.60	2.45	0.34	6.60	0.50	2.94	1.33	150 D
CD (5%)	1.24	6.25	0.92	0.16	0.12	0.96	0.21	0.64	0.84	-

in a white coloured annual chrysanthemum cultivar Local White that produced a totally different flower forms and colour when exposed to gamma radiation.

Significant differences were noticed in vegetative traits in six flower form mutants and two flower colour mutants. Highest plant height and length of leaf lamina were recorded in those plants that produced gerbera type flowers, whereas minimum was recorded in those plants that produced marigold type semi-double flowers. The significantly more number of branches

were found in those plants that produced ranunculus type of flowers compared to the control.

The number of flowers produced per plant was highest in those plants that were not irradiated. Among the induced mutants, highest number of flowers/plant was recorded in those plants that produced chrysanthemum type of flowers. Flower diameter was found to be more in gerbera type of mutant. Among mutants, peduncle length, thickness, length of corolla tube were more in marigold type

mutant, whereas length and width of ray florets were more in gerbera type mutants. The parent cultivar Local White produced flowers that are pure white (150 D). Minor variations in flower colour were evident in the mutants that produced flowers that are near to white (1D, 4D and 12B). Significant colour variation was noticed in two mutants that produced lemon yellow coloured (8C) flowers that resembled marigold and ranunculus types.

Mutation by using both physical and chemical mutagens has successfully produced quite a large number of new and promising varieties in different seeds and ornamental plants, and was considered to be a most successful tool for breeding ornamental plants (Datta 1992,1997). Mutation breeding has been more successful in ornamental plants because changes in any phenotypic characteristics like colour, shape or size of flowers and chlorophyll variegation in leaves can be easily detected (Datta 2009). In addition, heterozygous nature of many ornamentals offers high mutation frequency. The capability of Gamma-rays in inducing desirable mutations in ornamental plants is well understood from a significant number of new varieties developed via direct mutation breeding. Recent genetic engineering techniques appear to be most promising and exciting for development of desirable transgenic ornamentals, but this technology is at the early stage of development.

Every technique has its own advantages and disadvantages. After more than three decades of applied mutagenesis work, it is established beyond doubt that mutation breeding will constitute an excellent supplement to conventional methods in practice. Studies have clearly proved that mutation breeding techniques using nuclear radiation can be exploited for creation of new and novel ornamental cultivars of commercial importance, by inducing genetic variation in already adapted, modern genotypes and can also enrich the germplasm of ornamental horticulture. Although mutation breeding is a random (chance) process, reports are available for directive mutation in flower colour with some startling colours. In the present investigation a range of favourable, novel mutants were induced in garland chrysanthemum for the first time.

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BOOK-SHELF

THE SCIENCE OF HORTICULTURE. Vol. I and II. Peter, K V. (Editor), pp. I–XIII + 406 (Vol. I), ₹ 1800. ISBN 978-93-8023547-9; pp. I–XVI + 347 (Vol. II), ₹ 1600. ISBN 978-93-8023548-6.
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The book spreads over 31 chapters, 16 in Vol. 1 and 15 in Vol. 2. Each chapter is contributed by a renowned specialist in his or her own field of specialisation. And each chapter is well documented, referenced, and supported by tabular and graphic information. The reader would come across terms like “Halo Priming” in vegetable crops, a novel term (wrongly spelt as “Helo” Priming Chapter 12, Vol. 2, in Table of Contents) curiously enough, even to a practising agriculturist. I mention this just to drive home the point that one benefits a lot, both intellectually and practically, in reading both the books. The chapter on “Geographical Indications (GI) and Trade Related Aspects of Intellectual Property Rights (TRIPS)” contributed by a specialist in a law firm contains a wealth of information on intellectual property rights, World Trade Organisation (WTO), its charter etc.

Though all the chapters are well documented, some stand out in academic excellence, with a wealth of new scientific information, such as Chemistry of Fragrance (Chapter 3, Vol. 2), Methodology for Gene Sequencing (Chapter 12, Vol. 1) and Transgenic Ornamentals with Reduced Ethylene Production and Perception (Chapter 14, Vol. 1). Chapter 6 (Vol. 2) on the Principles of Preservation and Packing to improve quality and extend shelf-life of fresh horticultural produce is especially very informative in the context of India, as the country, though second only to China in vegetable production stand to lose a huge quantity of freshly harvested produce for the lack of adequate preservation and packaging infrastructure to extend the shelf -life of the produce. These are but a few examples of the depth and extent to which each chapter has been treated by the concerned specialist

Prof . K.V. Peter has done an admirable job bringing together all the information under one cover. Both these volumes will be widely read and practically utilized for piloting a real “Fruit and Vegetable Revolution” in India.

Professor K P Prabhakaran Nair
 Formerly Professor, National Science Foundation,
 The Royal Society, Belgium, and Senior Fellow,
 Alexander von Humboldt Foundation,
 The Federal Republic of Germany

Orange-fleshed cucumber for nutritional security

Cucumber (*Cucumis sativus* L.) is reported to have originated in foothills of the western Himalayas. Rich diversity is present throughout the country with more prevalence in Maharashtra, Rajasthan, Uttar Pradesh, Uttarakhand, Haryana, Bihar and North-Eastern states.

Evaluation of Indian Cucumber

The NBPGR, New Delhi, has conducted a number of explorations and collected the diversity from all over India. In a trial conducted during *kharif* 2012 at NBPGR, New Delhi, 90 accessions of Indian cucumber germplasm were characterized and evaluated for different agromorphological traits. Significant morphological variations were noted for fruit colour, size, shape and texture. Mature fruit colour ranged from light brown to dark brown, bright yellow orange to orange. Two accessions, IC420405 and IC420422, collected from Mamit district of Mizoram in 2004, showed unique fruit colour (orange) along with orange flesh which is a rare type. The morphological characteristics of matured fruits in both the accessions are presented (*see* Box). Both the accessions flowered 60 days after sowing with the onset of winter as compared to other accessions (40 days after sowing) and have soft, sparse and white spines in contrast to other accessions having hard and black spines.

Character	IC420405	IC420422
Fruit shape	Columnar	Columnar
Fruit colour	Yellow orange	Lemon yellow
Spine colour	White	White
Spine density	Sparse	Sparse
Flesh colour	Orange	Orange-yellow
Fruit texture	Smooth	Smooth
Fruit length (cm)	16-18	14-16
Fruit diameter (cm)	7-8	6-7
Seed cavity length (cm)	14-16	12-14
Seed cavity diameter (cm)	4-5	4-5
No. of seeds/fruit	400-450	150-200
Seed length (cm)	0.4-0.5	0.4-0.5

Previously orange-fleshed cucumber was known to be derived from a landrace, Xishuangbanna Gourd (*Cucumis sativus* var. *xishuangbannaensis*), from the Prefecture Xishuangbanna of the Yunnan Province in Southwest China, very close to North- Eastern part of India. The fruit shape Xishuangbanna gourd are of three types, viz. long and narrow, column-like and round but the length:diameter ratio of all three types is 3:1. However, orange-fleshed cucumber of China (Xishuangbanna gourd) is closely related to Indian population of cucumber germplasm. This could be due to migration of cucumber germplasm from North-eastern part of India, a primary centre of origin to Xishuangbanna, China, since both are geographically close to each other.

Future Prospects

The common green/white cucumber is low in nutritional quality while orange cucumbers are rich in carotenoid (~700 µg/100 g flesh weight), which makes this unique germplasm best suited for improving the nutritional quality of cucumber. Orange-fleshed Indian cucumber could be utilized in breeding programme to develop carotene-rich cucumber which in turn will play a significant role in meeting global nutritional security. There is a need to conduct more exploration trips to north-eastern regions to collect more diversity in orange cucumber. Since cucumber is cheap and easily available round- the- year, it can help in eradicating the vitamin A deficiency in nutritionally vulnerable group of our population. India being the centre of origin for cucumber is known to be the treasure house of its diversity. The common cucumber is green/white-fleshed and is low in nutritional quality. The orange-fleshed cucumber contains more than ten times carotenoid as compared to green/white cucumber and can play a vital role in eradication of malnutrition.

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Farmers' choicest mango variety—Vanlaxmi

A new promising farmers' variety of mango, known as Vanlaxmi, from Gujarat has been identified by AICRP (STF) centre AES, Paria. Its fruits are very attractive with red blush and good taste, attractive yellow crisp pulp, good shelf-life, high-yielding and free from spongy tissue. A progressive farmer Mohanlal Dayalji Patel, Village Palan, Taluka-Valsad, District Valsad, in Gujarat, has 45 plants of Vanlaxmi planted in 1996 at a distance of 5m × 5m. He is popularizing by giving grafts. He has already distributed about 2,000 grafts. This variety has a very good export potential.

Mango Vanlaxmi

The mango variety, Vanlaxmi, was originated in farm of Thakorbbhai Ambalal Patel's "Patel Farm and Nursery" located at Udwada, district Valsad, in Gujarat. The nursery is very reputed and popular among farmers. There were Vanraj and Jamadar mango trees close to each other and some branches were touching each other in his farm.

During 1981-82, flowering also came simultaneously on both the varieties. He harvested a few fruits from Vanraj from the branches touching to Jamadar and sowed the stone in his field. One stone germinated and came into fruiting after 7-8 years. He was much impressed by looking the attractive red blushed fruits, which were sweet in taste and had longer shelf-life. He also found other desirable tree and fruit characters in the variety.

During 1995, he named the variety as Vanlaxmi by taking "Van" from Vanraj and "Laxmi" from his mother name Laxmiben Patel. They started preparing grafts from mother stock and sell grafts every year from their nursery.

A Successful Journey of Mohanlal Dayalji Patel

Mohanlal Dayalji Patel is a progressive mango farmer. At present, he is totally dependent on mango farming for his annual income, which comes mainly from his 7 acres of mango farm containing 10 mango varieties (Kesar, Langra, Dashehari, Amrapali, Chausa, Vanlaxmi, Fazri, Vashi Badami, Dadamio, Totapuri). All varieties are performing very well in his farm, including North Indian mango varieties such as Langra, Dashehari, Amrapali, Chausa and Fazri.

Mohanlal Patel was among the first farmers who took it as challenge to plant a new variety Vanlaxmi on large scale. He planted 45 grafts of Vanlaxmi at a spacing of 5m × 5m during 1998. He raised plants taking all necessary care and now he is getting reward from it. He owns 12 years old 30 tree + 5 years old 15 trees of Vanlaxmi which are fruiting excellently. At present, trees of Vanlaxmi are completely healthy, growing well and have attained the average height of 3.4m. The canopy does not touch each other and about 3-5 ft space is still available between the trees.

He takes good care of his mango orchard and add about 50kg poultry manure/tree/year in addition to inorganic chemical fertilizers. He applies micronutrient mixture @ 250-300g/tree in every 2-3 years to maintain the micronutrient status in soil. He also uses azotobacter cultures in basin.

He applies two irrigations to mango trees every year, i.e. first in January to facilitate flowering and second in April to support the needs of growing fruits. He uses pesticides in mango as per the need only.

Mohanlal Patel is very wise in marketing of Vanlaxmi mangoes. He tried local market, door-to-door market, distant market and also sent some quantity of fruits abroad in search of better prices and to popularize Vanlaxmi variety. Many a times, he also gives some fruits free of cost to relatives, mango traders and persons involved with mango industry in search of better market. At present, local traders are not aware of the importance of this variety and hence, demand it at a cheaper price.

There is an urgent need to undertake extensive research on Vanlaxmi mango variety not only at regional level but also at national level. This variety has yield and quality characters suitable for becoming an ideal mango variety which is good for table purpose and excellent for domestic as well as export market. The variety proved its yield potential at farmers' fields and can play important role in future mango cultivation.

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