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Research Review Article

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Utilization of male sterility for hybrid seed production in vegetables

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ABSTRACT

Male sterility in vegetables is commercially utilized to develop their hybrids. Substantial progress has been made in understanding the mechanism of male sterility. Different types of male sterility, and three-line and two-line hybrid breeding approaches involving Cytoplasmic male stesility (CMS) and environmental sensitive genic male sterile (GMS) lines, respectively. Special emphasis is laid on crop-wise review of reports on identification and utilization of male sterility in Solanacious fruit vegetables, cucurbits, okra, cole crops, root and bulb crops. Genetic male sterility has been successfully exploited to develop chilli hybrids, CH1 and CH 3, and two muskmelon hybrids, Punjab Hybrid and Punjab Anmol. Cabbage hybrids, H-64 and KCH-4, and Pusa Vasudha of carrot have been developed by utilizing CMS. Cytoplasmic genetic male sterility has been successfully utilized to develop chilli hybrids, Kashi Surkh, Kashi Early, Arka Meghana, Arka Harita and Arka Sweta, and onion hybrids, Arka Lalima and Arka Kirtiman.

Key Words: Male sterility, Hybrid seed, Vegetables, Cytoplosmic male sterility, Genetic male sterility

India is the second largest producer of vegetables in the world. The total area under vegetable cultivation is 8.49 million ha with a total production of 146.55 million tonnes (NHB, 2012). The prospect of increasing vegetable production by increasing land under vegetable cultivation is very limited in India. Hence it is imperative to increase the productivity of vegetables in order to meet the future demand. In this context, hybrid vegetable technology is better option to increase the productivity. Vegetable hybrids under optimum crop production and protection management produce economically more yield than improved open-pollinated varieties. Further, uniform size, earliness, better adaptability to adverse environments, better transportability, better keeping quality and resistance to stress are certain additional advantages of growing hybrid vegetables. In the past decade, vegetable hybrid technology has emerged as one of the most potential technologies in Indian agricultural production system. In coming decade also, it has to go a long way to meet the future challenges.

Presently, only about 15% area is under hybrids of vegetables, of which, 36 and 30% area are covered under tomato and cabbage hybrids respectively. In most of the vegetable crops, hand-emasculation and pollination technique are used to produce hybrid seeds. This is the most cumbersome and expensive method and may be economically feasible only in certain vegetables such as tomato, brinjal, pumpkin etc. The commonly employed mechanisms for overcoming emasculation and hand-pollination and facilitating natural pollination are use of self-incompatibility, male sterility, gynoecious lines and use of monoecious lines. As the importance of heterosis breeding has increased, male sterility provides an asset, particularly in crops like onion and carrot which produce many but small-sized flowers, making hand-emasculation tedious. It is of special interest for plant breeders to produce more efficient and economic hybrid seed.

Private sector is also involved in the development of male sterile based hybrids of the important temperate and tropical vegetables. In contrast, public sector in India, though hybrids have bred in several vegetables, genetic emasculation through male sterility has not been efficiently utilized (Kalloo *et al*. 1998). Consequently, cost of hybrid seeds is comparatively higher, which is one of the major constraints in achieving more rapid adoption of vegetable hybrid technology. Nevertheless, crops like muskmelon and chilli present very successful examples of utilization of male sterility system in India (Kalloo *et al*. 1998).

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CLASSIFICATION OF MALE STERILITY

There are three basic types of male sterility: (1) genetic male sterility, (2) cytoplasmic male sterility and (3) cytoplasmic-genetic/genic male sterility. There are two other types, *viz*. chemical-induced male sterility, and transgenic male sterility. Almost all crop plants posses male sterility if investigated properly.

Genetic Male Sterility (GMS)

The pollen sterility caused by nuclear genes is termed as genic or genetic male sterility. This type of sterility has been reported in several crop plants like barley, wheat, maize, cucurbits, cole crops, tomato, pepper and brinjal.

Features of genetic male sterility are : The GMS is controlled by a single recessive gene (*ms*) except in cabbage and broccoli, in which dominant genes are also involved in male sterility. In majority of cases, sterility is caused by single gene. However in a few cases, two or more genes control male sterility. This system consists of two types of lines, *viz*. *A* line and *B* line. *A* line refers to genetic male sterile line (*mm*) which is used as female parent in hybrid seed production. The *B* line is heterozygous fertile (*Mm*) line which is similar to *A* line except for sterility. The *B* line is used to maintain sterile line (*A*). The genetic male sterile line is maintained by crossing recessive male sterile plants with heterozygous male fertile plants. Such cross yield 50% sterile plants and 50% fertile plants. The male sterile plants are used as female parents in the development of hybrids. The fertile plants are rogued out. Crosses between recessive male sterile plants and heterozygous male fertile plants are effected every year for maintaining the male sterility.

Certain mutants, which although produce functional pollen-grains, fail to self-fertilize, either due to non- dehiscence of pollen-grains and their special flower morphology. These mutants are often termed as functionally sterile, for example genotypes with exerted stigma in tomato (Georgiev, 1991), brinjal and several, other vegetables (Kaul, 1998).

EGMS Line

Certain GMS lines are conditional mutants, meaning thereby, in particular environment male sterile mutant plants turn into male fertile. After determination of critical environment for sterility and fertility expression, such GMS mutants are classified into Environmental Sensitive Genic Male Strile (EGMS) lines. The EGMS lines (mostly temperature sensitive) have been reported in several vegetable crops. Initially, EGMS lines were thought to be of very less practical value, as they were unstable. But now they are considered to represent most efficient system for hybrid seed production. However, from practical view point, it is necessary to identify critical temperature or photoperiod for the fertility/ sterility expression in temperature and photoperiod sensitive male sterility, respectively (Table 1).

Table 1. Environmental sterile male sterile mutants

Vegetable	Mutants	References
Brussels, sprouts	TGMS	Nieuwhof, 1968
Broccoli	TGMS	Dichson, 1970
Pepper	TGMS,	Daskalov, 1972;
	TCMS	Shifriss, 1997*
Carrot	TGMS	Kaul, 1998
Tomato	TGMS	Rick, 1948

HYBRID SEED PRODUCTION FOR GMS BASED HYBRIDS

The hybrid seed is produced in the open in an isolated field, called **'hybrid seed production block'.** The female and male lines are planted alternatively in the ratio of 2:1 (Fig. 1). The female line produces both the male fertile and male sterile plants in the ratio of 1:1. The female line is first prepared for cross. pollination for hybrid seed production by removing the male fertile plants. In general, male sterile plants are morphologically not distinguishable from sister fertile plants, except in a few cases, were male sterile flower size is smaller than that of fertile flowers, e.g. tomato and chilli.

A hybrid seed crop is inspected at different stages of plant growth and development to ensure the genetic purity. The first inspection is done before flowering. The off type plants for foliage (leaf size, leaf shape, leaf colour) and plant type characters should be removed. The diseased plants and extra early-flowering plants should also be rogued out. The second inspection is conducted at flowering stage. The plants which do not confirm to the purity requirements regarding the flower orientation, flower colour, spread of the plant and leaf characters such as size, shape, colour etc. should be removed. Third inspection is conducted at the fruiting stage. The plants showing variation in fruit shape, colour, size and position of fruits should be rogued out. Removal of off type plants at this stage helps to

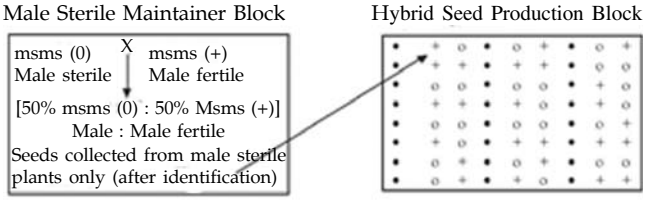


Fig. 1. General scheme of hybrid seed production utilizing GMS

avoid mechanical admixtures and further chances of outcrossing of true-to-type plants with off types.

Maintenance of female and male lines in/GMS

Genetic constitution of male sterile plant is '*msms*' and that of maintainer is *Msms* (heterozygous). The male sterile plants are maintained by crossing it to heterozygous male fertile plant because progeny of such crossing produces the population of male sterile and male fertile plants in a 1:1 ratio (Fig.1). The female parent is planted in a separate block called '**Male Sterile Maintainer Block**'. Roguing is done at three different stages as described under hybrid seed production. In this male sterile maintainer block, female parent (male sterile) bears both the male fertile and male sterile plants in the ratio of 1:1. At the time of flowering, male sterile plants are identified and tagged. The male fertile plants in this block serve as a pollinator for male sterile plants. There is no need to rogue out the male fertile plants in this block. The fruits from male sterile plants are picked when ripe, dried and their seeds are extracted. The seeds from male sterile plants serve as female parent for subsequent seed production programmes.

The male parent is maintained as such in hybrid seed production block because seeds collected from male parent serve as pure male seeds and can be stored for subsequent seed production programmes.

Cytoplasmic Male Sterility

This type of male sterility is determined by the cytoplasm. Since the cytoplasm of a zygote comes primarily from egg cell, progeny of such male sterile plants would always be male sterile. The CMS can be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in successive generations of backcross programme. After 6-7 backcrosses, nuclear genotype of male sterile line would be almost identical to the recurrent pollinator strain.

Utilization of CMS

Cytoplasmic male sterility can be maintained by crossing a male sterile line (*A line*) with pollinator strain (maintainer line) used as a recurrent parent in back cross programme since the nuclear genotype of the pollinator is identical with that of new male sterile line. Such a male fertile line is called the maintainer line or *B* line as it is used to maintain the male sterile line. The CMS can be utilized for hybrid seed production in those vegetables where vegetative part is of economic value, e.g. onion, carrot, radish, cole crops etc.

Limitations of CMS

Cytoplasmic male sterility is sensitive to environmental factors, e.g. A line may be completely male sterile in one set of environment and may have

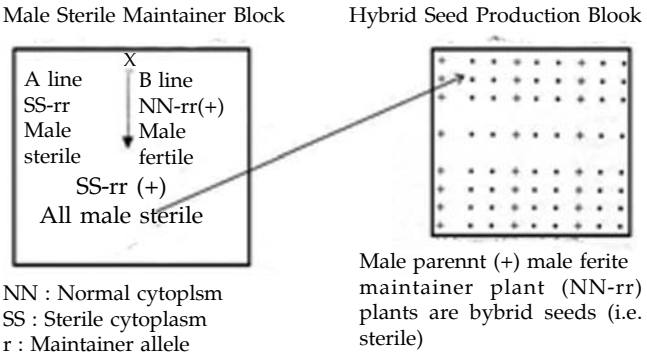


Fig. 2. General scheme of hybrid seed production utilizing CMS

partial fertility in another set of environment. This phenomenon may lead to mixture of selfed seed in an otherwise hybrid seed. Certain diseases or disorders are associated with a particular type of cytoplasm which leads to genetic vulnerability, e.g. T-cytoplasm is associated with southern corn blight in cotton. Continuous incorporation of small amount of male parent cytoplasm through each backcross during maintenance may lead to partial or complete breakdown of male sterility.

Cytoplasmic Genic Male Sterility (CGMS)

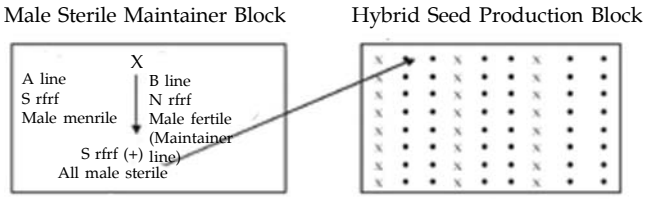
This is a case of cytoplasmic male sterility where a nuclear gene for restoring fertility in male sterile line is known. The fertility restorer gene, *RR*, is dominant and is found in certain strain, of the species or may be transferred from a related species. The sterility factor is determined by interaction of nuclear genes and cytoplasm but none of them singly can control sterility. This type of sterility is reported in carrot, onion, chilli, capsicum and *Brassica napus*.

New male sterile line may be developed following the same procedure as cytoplasmic system, but the nuclear genotype of the pollinator strain in such a transfer must be *NN-rr* otherwise the fertility would be restored.

Utilization of CGMS

The CGMS can be maintained by crossing a cytoplasmic male sterile line (*SS-rr*) or *A* line with the pollinator strain (*NN-rr*) used as recurrent parent in the backcross programme since the nuclear genotype of the pollinator is identical with that of new male sterile line (Fig.3). Such a male fertile line is known as maintainer line or *B* line as it is used to maintain the male sterile line.

For hybrid seed production, 2-3 rows of line *A* (*SS-rr*) are alternated with 1 row of line *C*, which is generally expected to *NN-RR*. The seed is harvested from *A* line for use as commercial hybrid seed. The line *C* may have genotypes *NN-rr*, *NN-Rr*, *SS-Rr*, *SS-RR*, but the hybrid developed by using any of the first three



N : Normal cytoplasm
S : Sterile cytoplasm
rf : Non-restorer allele
Rf : Restorve allele

Male parent (x) male fertile restorer plant (NRRD), female parent (%) male sterile plant (srfrf) seed harvested from male sterile (%) plants. This COMS system is used where seed is of economic value

Fig. 3. General scheme of hybrid seed preduction utilizing CGMS

genotypes as sterile and can be used where seed is not a commercial product but where seed is important, pollen parent should have genetic constitution NN-RR. The main advantage of CGMS system over GMS is that we can get 100% male sterile plants for direct use as female.

Chemically Induced Male Sterility

Male sterility as originated as a result of spontaneous mutations. In some species, male sterility has been derived from wild species through interspecific hybridization. It can also be induced through various chemicals. The chemicals which are used for induction of male sterility are referred to as male gametocides. The main difference between mutagen induced male sterility and gametocide induced male sterility is that the former is heritable, and the later is non-heritable. (Table 2).

Gametocide induced male sterility has three main advantages:

It is a rapid method to develop male sterile line. This method is less labourious and less expensive than backcross method, and there is no need to maintain *A,B* and *R* lines. The gametocide which has to be used as female parent in the development of hybrid can be treated with male gametocide to suppress pollen formation, resulting in induction of male sterility.

An ideal gametocide should have the following properties (Chopra, 1985):

- It should be selective in inducing male sterility without affecting ovule fertility.
- It should give consistent results.
- It should be economical and its method of application should be simple.
- It should be safer for the use and should have minimum side effects on plant growth.

Male gametocides have following main drawbacks (Sneep and Hendriksen, 1979):

- Pollen abortion is incomplete and erratic.
- Treatments are effective only at a specific stage of crop growth and effect is short lived.

Table 2. List of gametocides found effective in vegetables

Male gametocide	Vegetables on which found effective
Napthelene Acetic Acid (NAA)	Cucurbits
Gibberellins	Onion, lettuce
Maleic Hydrazide	Tomato, cucurbit, onion
FW 450	Tomato, groundnut, sugarbeet
Ethrel	Sugarbeet

- Ovule fertility is also adversely affected which leads to low seed setting.
- Present male gametocides have several harmful side effects, such as deformation of leaf, stunting of plant growth and sometimes wilting.
- High cost of gametocides and their repeated applications make their use uneconomical.

The effectiveness of large-scale application of male gametocides in practical plant breeding is not yet established. This area needs further intensive research to reach concentrate conclusions.

Transgenic Male Sterility

From the beginning of 1990s, new genetic approaches have been proposed and implemented to develop male sterility systems through genetic transformation. The gene responsible for inducing male sterility is generally used from microorganisms. This type of male sterility comes under genetic male sterility and is heritable. The ability to design new molecular strategies and their successful execution has been possible because of the isolation, cloning and characterization of anther or pollen-specific genes and promoter sequences. These genes are expressed in pollen themselves or cells and tissues that directly or indirectly support pollen development, such as tapetum, filament, anther wall etc. The gene *barnase* is the first transgene that was used to produce male sterility by Mariani *et al*. (1990). It has an effective fertility restoration system in *barstar*. For commercial exploitation, a dominant genetic male sterility system must have following important features:

- Efficient fertility restoration system.
- Easy maintenance of male sterility.
- Easy elimination of male fertile plants from male sterile line.
- Lack of adverse effects on other traits.
- Stable male sterile phenotype over different environments and in genetic backgrounds.
- Satisfactory performance of F_1 hybrids.

The *barnace-baratar* transgenic male sterility system appears to satisfy above mentioned requirements. It generally does not influence the heredity or expression

of the original good characters and thus avoids the linkage of harmful characters or distant hybridization separation in traditional cross hybridization breeding.

MALE STERILITY IN VEGETABLE CROPS

Pollen Abortion by Cytotoxic Genes

A pollen or anther tapetum-specific promoter, a cytotoxic gene and a tanscription terminator can be constructed to be a chimeric gene and used to transform plants. The specific expression of cytotoxin can selectively destroy the organs or tissues related to pollen development and thus block the development process and lead to male sterility. The RNase has a function of digesting RNAs. Two genes encoding RNase-*barnase* and *RNase T1*, have been cloned. The RNase gene and a specific promoter can be linked and transferred into plants to obtain male sterile plants. This method was first used by Mariani *et al*. They fused *TA29*, the tapetum-specific promoter isolated from tobacco anthers, with *barnase* gene from *Bacillus amyloliquefaciens* and with *RNase T1* gene to be introduced into tobacco and oilseed rape through genetic transformation.

Under the control of *TA29* gene promoter, chimeric RNase gene expression within the anther selectively destroyed the tapetal cell layer that surrounded the pollen sac, prevented pollen formation, and led to male sterility. The transgenic plants were normal in all respects except failure to produce functional pollen. Up to now, some specific promoters of plant anthers and pollen have been cloned and they can be linked to any cytotoxic gene to be used for creating male sterile plants. The *barnase* gene is still the most widely used cytotoxic gene in creating male sterile plants with the best effect by genetic engineering.

Antisense RNA or RNAi to Silence Relevant Gene Expression of Pollen Development

Pollen development is a very complicated process involving many genes. If a certain gene expression in pollen development was inhibited artificially, male sterility may be caused. As an inherent sequence-specific RNA degradation mechanism, post-transcriptional gene silencing can silence target genes in plants. Antisense RNA and RNA interference (RNAi) can both specifically reduce or silence the expression of target genes and thus produce individual plant losing the gene function.

There are other genes involved in pollen development, such as actin gene, *DAD1* gene encoding phospholipase A1. The antisense actin gene which was transferred into tomato and plants with abnormal and non-viable pollen cells were obtained. The action content of the transgenic plant pollen was significantly reduced, while the pistil was not affected (Yan *et al*. 1999). Flowering Chinese cabbage and broccoli transformed with the antisense *DAD1* gene showed male sterility.

Leading to Male Sterility by Early Degrading Callose

The specificity of callose enzyme synthesis and secretion in tapetum plays a decisive role in normal development of pollen. In normal conditions, after a microspore has formed the exine, the callose wall is degraded by β-1,3-dextranase (*i.e.* callose enzyme) secreted from the tapetum. Then the microspores move into the anther cells and this process is strictly time-limited. Curtis *et al*. linked the β-1,3-dextranase gene under the tapetum-specific promoter, and transformed lettuce using *Agrobacterium*-mediated transformation method to cause degradation of developing microspore callose wall and finally led to male sterility of transgenic plants. But the control microspores developed normally and were fertile. It was indicated that the degradation of callose could cause male sterility which made it possible to produce hybrid F_1 seed of lettuce.

Transferring Bacterial Genes or other Genes to Prevent Microspore Development

Microspore development needs a large number of physiological and biochemical materials and lacking one may influence its development. Schmülling *et al*. linked *rolC* gene (interfering with the metabolization of phytokinin) from *Agrobacterium rhizogenes* A4 with *CaMV35S* promoter and constructed a chimeric gene to transform potato, tobacco and *Arabidopsis*. The transgenic plants had pollen losing vitality and became male sterile. Analysis indicated that microspore development was influenced by *rolC* gene expression significantly and finally led to male sterility. The excessively expression of *rolC* gene restrained the accumulation of polyamines in anthers, and the insufficient of polyamines was related to male sterility.

In recent years, researches on creating male sterile line and restorer line of plants using genetic engineering have made significant progress. Many approaches of creating male sterile lines have been developed and applied successfully to many crops and some approaches have been used to produce hybrid seeds. Low transformation rate, non-100% sterility, unstable sterility and difficult restoration of fertility are all existing problems, needing further researches and resolutions. As almost all the transgenic male sterile lines developed till data are genic male sterility, the scope of research should be expanded. With the further researches on molecular biology of pollen development and improvement of biotechnology, the approaches creating male sterile lines using genetic engineering will become simpler, faster and more effective. They will be widely used to promote the heterosis utilization of vegetables and other crops and play more and more important roles in vegetable breeding.

MALE STERILITY IN VEGETABLES

Solanacious Vegetables/

Tomato

Hybrids are very popular in tomato (*Lycopersicon esculentum* Mill.), especially in the developed world and appear to be the primary reason for high productivity. An important factor limiting more extensive use of hybrid tomatoes is high cost of hybrid seed, which is produced through manual emasculation and pollination. Incorporation of male sterility can avoid emasculation and reduce the labour necessary for F_1 hybrid seed production, and thus reduce cost of F_1 hybrid seed. There are five types of male sterility in tomato but only pollen abortive type (sporogenic) and functional sterility have been exploited for hybrid seed production. Other male sterility mechanisms are associated with defective fruit development in hybrid progeny (Atanassova, 1999).

In pollen abortive type, pollen-grains are non-functional, whereas in functional type, pollen-grains are functional but anther dehiscence prevents self-fertilization. Functional male sterility can be maintained by manual pollination and offers production of homozygous stand of female parent. The pollen abortive type is maintained by backcrossing homozygous male sterile plants (*msms*) with heterozygote male fertile (*Msms*) plants. The progeny segregates into male fertile and male sterile in a 1:1 ratio. For commercial exploitation of male sterility, it is important that the sterility genes are transferred into horticulturally desirable and superior combining parents.

GMS

More than 55 male sterile (*ms*) alleles causing sporogenous, structural and functional sterility have been reported (Kaul 1988). Chromosomal locations of some genes is also known, Masudha *et al*. (1998) reported three induced male sterile mutants. Two stemen less mutants, *viz*. stemen less-1 (*sl*-1) and stemen less -2 (*sl*-2) produce flowers without stemens when grown at higher temperature, while those grown at low temperature produce flowers with abnormal stemens and often with viable pollen. Similarly in *ms*-15 and *ms*- 33 mutans, low temperature (< 30° C) is reported to be associated with fertility restoration (Sawhney 1983).

The *ms* - 14 locus has been mapped on RFLP marker and with the help of identified flanking markers, ~ 610 kb of YAC clone possessing *ms* -14 locus has been cloned through map based cloning technique (Gorman *et al*. 1996). Further, cloning and charecterisation of translation product of *m*- 14 gene , would be helpful in designing molecular strategies to develop transgenic male sterility system across the species (Gorman and McCormick 1997) (Table).

Chromosomal location of some male sterile genes in tomato (Kaul, 1998)

Table 3. Chromosomal location of some male sterile genes in tomato (Kaul, 1998)

Chromosome	ms gene
1	ms-6, ms-32
2	ms-2, ms-5, ms-10, ms-15, ms-26, ms-35
3	ms-9
4	Sl
6	ms-16, ms-33
8	ms-8, ms-17
10	ms-31
11	ms-3, ms-7, ms-12, ms-14, ms-42

CMS

Through protoplast fusion of *Lycopersicon esculentum* with S*olanum acaule* and *S. tuberosum*, CMS cybrid palnts has been isolated with different flower morphology than tomato. Recently, sterile cytoplasm from *L. peruvianum* has been transferred into the *L. pennellii*. Subsequently, CMS *pennellii* has been successfully crossed with esculentum. This hybrid provides basis for the development of CMS system in tomato derived from sterile cytoplasm of *peruvianum* (Petrova *et al*. 1999). However, practical utility of CMS would depend upon the identification of restorer gene in tomato. Incorporation of parthenocarpy trait in parental line would be the other alternative for desired amount of fruit set without restorer gene in the F_1 derived from CMS.

Chilli

The genetic male sterility is an important pollination control mechanism which is exploited commercially for hybrid seed production in chilli (*Capsicum annuum* L.). More than a dozen monogenic recessive male sterile mutants have been identified either in natural population or induced through mutagenesis (Shifriss and Frankel 1969). However, very little information is available on allelism of these male sterile alleles. The induced male sterile gene (*mc*-509) was found allelic to *msk* allele isolated spontaneously, which was later renamed *ms*-10 (Pochard 1970). The *ms*-10 gene is linked with taller plant height, erect growth and dark purple anthers (Das *et al*. 2001). Shifriss and Frankel (1969) found spontaneous genetic male sterile plants in the cultivar 'All Big' of capsicum. Male sterility was determined by a single recessive gene. Shifriss and Rylsky (1972) discovered a second gene encoding

genetic male sterility in the cultivar 'California Wonder' of capsicum. It too, was found to be inherited as a single recessive gene. Dhall and Cheema suggested that the sterility discovered in 1969 may be described as *ms*-1 and second as *ms*-2.

MS-12 line, carries genetic male sterility (GMS) controlled by recessive gene (*msms*). The male sterile line (MS-12) was developed by transferring sterility gene (*ms*-509, renamed as *ms*-10) from capsicum (imported from France) into cultivar 'Punjab Lal' through backcrossing (Singh and Kaur 1986). By using this male sterile line (MS-12), PAU has released two chilli hybrids, *viz*. CH-1 (Hundal and Khurana, 1993) and CH-3 (Hundal and Khurana 2001), showing heterosis of 80-100% and out-yielded all the recommended chilli varieties. The male sterile line (female), MS-12, is common in both these hybrids. The male parents are Ludhiana Local Selection (LLS) and Selection-2530 (S- 2530) in CH-1 and CH-3, respectively. On the basis of their high yield potential, multiple disease resistance and quality attributes, their acceptance has been very fast and, consequently, the acreage under chilli increased about 3-fold in the last 6-7 years in Punjab State and is likely to increase further in the near future. There is great acceptance of CH-1 in other states especially Haryana and Rajasthan.

The CGMS in chilli was first reported by Peterson (1958) in an introduction of *C. annuum* from India (PI-164835), however, it has not been exploited commercially, because of instability under fluctuating conditions, particularly temperatures and a low rate of natural cross-pollination in cultivated hot pepper (Kumar *et al*. 2007). In cytoplasm from Peterson's source, the pollen fertility has been found to be restored under 25°C and 17°C day and night temperature, respectively (Shifriss, 1997). The instability of sterility expression is attributed to the interaction between the temperature and sterility modifier genes. It has been observed that at low temperature, meiotic breakdown is either completely stopped or delayed, resulting in pollen fertility. Like EGMS system, in this CGMS system, seed increase of male sterile line can be done in cool season and hybrid seed can be produced during late summer when expressivity of sterility is absolute (Shifriss, 1997).

The World Vegetable Centre, Taiwan, has identified two CGMS lines (A lines) in chilli, *i.e*. CCA-4759 and CCA-4757, which were found to be reliably sterile under conditions of night temperatures less than 15°C (Liu and Gniffke 2004). A hybrid 'Jingla No 2' of chilli was developed by crossing CMS line 181 A with the restorer line 98199 (Geng *et al*. 2005). In the recent past in India, chilli CGMS lines (CCA-4261) have been introduced at IIVR from The World Vegetable Centre, which are utilized directly or indirectly to produce CGMS based hybrid, *i.e*. Kashi Surkh. A total of 9 sets of A and B lines are being maintained at IIVR and 5 promising CGMS based hybrid combinations, *viz*. A2 × Pusa Jwala, A3 × Pusa Jwala, A2 × Pant C1, A3 × Japani Longi and A7 × Pant C1 have been identified. At IIHR, Bangalore three chilli hybrids, *viz.* Arka Meghana, Arka Sweta and Arka Harita are developed using the CGMS lines developed at the institute. PAU, Ludhiana is also working on utilization of CGMS in chilli (Table).

Table 4. Reported male sterility genes in chilli (Muthukumar 2013)

Male sterile gene	Nature of origin	Parental source of origin
ms-1	Spontaneous mutant	All Big
ms-2	Spontaneous mutant	California Wonder
ms-3	Irradiation induced mutation	Pasardijishka Kapia 794
ms-4	Irradiation induced mutation	Pasardijishka Kapia 794
ms-6, ms-7, ms-8	Irradiation induced mutation	Hungary
ms-9	Irradiation induced mutation	-
ms-10	EMS induced mutation	France
ms-11	EMS induced mutation	-
ms-12	Spontaneous mutant	Gambo
ms-13	Mutant	CA-452-1
ms-14	Mutant	Kalyanpur selection
ms-15	Mutant	CA-960
msc-1	Spontaneous mutant (China)	-
msk-2	Spontaneous mutant (Korea)	-

Reported Male sterility genes in chilli (Muthukumar 2013)

Brinjal

Functional male sterility in brinjal has been reported by several authors. In contrast to different classes of functional male sterile mutants in tomato, only one type of mutant is included in brinjal in which the viable pollens-grains are trapped inside the anther locule because the anthers fail to dehisce due to lack of pore

development at the tip. This is controlled by a major recessive gene.

Cucurbitaceous Vegetables

The cucurbit vegetables have larger size of male and female flowers allow as to follow other systems of pollination control strategies. Most of the genetic male sterile mutants in cucurbits are monogenic recessive. There are several male sterile types are identified, but commercial exploitation is still lacking. Among the cucurbits male sterility is commercially exploited and utilized in musk melon.

Five male sterile genes (*ms*-1, *ms*-2, *ms*-3, *ms*-4, and *ms*-5) have been identified in melon (*Cucumis melo* L.) and all of them are recessive and non-allelic. In a greenhouse study (24°C night, 32°C day), it has been observed that male-sterile plants in *ms*-1 and *ms*-2 progenies are difficult to identify as the aberrant flowers are observed on genetically fertile siblings and thus the expression of these genes is unstable (McCreight 1984), which could lead to genetic impurity in F_1 hybrid seed. In India, male-sterile gene *ms*-1 was introduced in 1978 and used to release two commercial cultivars Punjab Hybrid (Nandpuri *et al*. 1982) and 'Punjab Anmol' (Lal *et al*. 2007). This was the first evidence of exploitation of *ms*-1 gene for heterosis breeding in melon. Due to the instability of this *ms*-1 gene in our subtropical field conditions, the seed production of these hybrids has posed numerous problems consistently (Dhatt and Gill 2000).

Kumar *et al*., 2008, reported that the lines *ms*-1 and *ms*-2 are phenotypicaly unstable and this will reduce the genetic purity of hybrid seed. It is safer to exploit *ms*-3, ms-4 and *ms*-5 genes for developing genetically pure hybrids. Despite the complex maintenance process and additional labour requirement to remove fertile segregants in hybrid seed production field, production of male sterile based hybrid seed is economical than the seeds produced by manual emasculation (Table 5).

The first male sterility in Watermelon, *Citrullus lanatus* (Thunb.) was reported by Watts (1962) who found a male sterile mutant in the X2 generation of 'Sugar Baby' irradiated with gamma rays. The mutant was described as a glabrous male sterile (*gms*) due to the associated lack of hairs on the plant foliage (Watts, 1962). Glabrousness and male sterility were inherited together as a single recessive nuclear gene, suggesting very close linkage or a pleiotropic effect of the locus involved (Watts, 1967). The *gms* gene not only disrupts the male reproductive function, but also reduces female reproduction (Watts, 1967). Therefore, there has been little commercial application of the *gms* gene (Zang *et al*., 1994).

A second male sterile mutant was reported by Zang *et al*., (1990). Two spontaneous male sterile mutants were found in a self-pollinated population of a commercial cultivar 'Nongmei 100' in China in 1983. These mutants were sib-mated and used to develop breeding line G17AB, which has desirable agronomic traits (Zang *et al*. 1990). In contrast to the *gms* trait, the Chinese male sterile line contains no gross morphological differences between sterile and fertile plants (Zang *et al*. 1990). Female fertility of the Chinese male sterile mutant is normal and there is stability for male-sterile and female-fertile characters despite varying environmental conditions (Zang *et al*., 1994). The Chinese male sterile mutation was inherited as a

Table 5. Male sterility genes in musk melon (Muthukumar 2013)

Male sterility	Inheritance	Source of origin
ms-1	Single recessive gene	-
ms-2	-do-	La Jolla 40460
ms-3	-do-	PI 321005
ms-4	-do-	Bulgaria 7
ms-5	-do-	Charentais

Table 6. Male sterility genes in water melon (Muthukumar 2013)

Male sterility	Inheritance	Source of origin
ms-1	Single recessive gene	Nongmei 100
ms-2	-do-	Kamyzyakskii
ms-3	-do-	Yellow Doll × Cream of Saskatchewan
ms-8	-do-	Gamma irradiation of Sugar Baby

Cucurbita species (Muthukumar 2013)

Male sterility	inheritance	*Cucurbita* sp.	Source of origin
ms-1	Single recessive gene	Winter squashte	Golden Hubbard
ms-2	-do-	Summer squash	Eskandarany
ms-3	-do-	Winter squash	-

Cucumber (Muthukumar 2013)

Male sterility	inheritance	Source of origin
ms-1	Single recessive gene	Black Diamond
ms-2	-do-	Burpless Hybrid

single recessive nuclear gene (Zang *et al*. 1990), and assigned the gene symbol, *ms*. The cytological phenomenon associated with male sterility in the Chinese male sterile line is the lack of the tapetum, and tetrads that do not form (Zang *et al*. 1994).

A third occurrence of male sterility was observed as a mutant in a breeding line, unrelated to the glabrous male sterility and Chinese male sterility in 1998 by Yang *et al*. in Korea. This line originated from a cross between the commercial hybrid cultivar 'Fiesta' (Syngenta) and a breeding line with high quality, HL229 (Table 6).

An off type was detected in ridge gourd which was characterized by production of rudimentary male flowers in racemes, but no fruit set after self pollination. However fruit set was observed when pollinated using staminate flowers of the monoecious cultivars (Pradeep kumar *et al*. 2007).

Okra

In India, commercial production lf F_1 hybrid in okra [*Abelmoschus esculetus* (L.) Moench.] is done by hand emasculation and hand pollination which is a tedious process that takes 70% of the time and labour in cultivation. GMS line MS-1 identified by IIHR, is being used for development of a commercial F_1 hybrid. Male sterility in okra is controlled by a pair of single, recessive genes when present in the homozygous (ms1ms1) condition and can be utilized by hybrid seed production. In okra, male sterility has not been observed in nature, but, has been induced by gamma radiation (Dutta, 1971). The gene was stable, not being influenced by environmental factors. Anthesis was normal but anther dehiscence was partial. Microsporogenesis was normal upto the tetrad stage. Hence, a great future for hybrid seed production is envisaged in a heterosis breeding programme.

Cole Crops

In cole (*Brassica oleraceae* L.) crops, F_1 hybrids are advantages especially in uniform maturity, high early and total yield, better curd/ head quality with respect to compactness, colour, resistance to insect- pests, diseaaes and heat tolerance. There are two pollination control mechanisms are working in cole crops such as self incompatibility and male sterility, which are used in the commercial hybrid seed production of crops. However, SI system has several disadvantages like, possibility of sibs in the hybrids and multiplication of SI parents through tedious bud pollination. SI is commercially employed for hybrid development in Indian cauliflower. The climate change is anticipated to pose a threat to such hybrids, as SI, being thermosensitve, breaks and increases percentage of selfs in hybrid seed lots (Kalia 2008). In such situation, male sterility system offers a better alternative for hybrid development in these crops.

Genic male sterility has been reported in most of the cole crops (Nieuwhof 1961, Dickson 1970). In brusselsprout (Johnson, 1958), cabbage (Nieuwhof, 1961) recessive genes (*ms*) controlling male sterility has been reported. However, in broccoli (Duneman and Grunewaldt, 1987), cabbage (Fang *et al*. 1997) and cauliflower (Crisp and Tapsel 1993) dominant gene(s) controlling male sterility has been also reported. A dominant male sterile gene *Ms-cd1* was identified as a spontaneous mutation in a spring cabbage line 79-399-3. This male sterility is well utilized to produce commercial hybrid cabbage seed production in china.

In Brassica *oleraceae* L. first CMS system was developed by Pearson (1972) through inter-specific hybridization between *B. nigra* and *B. oleraceae var italica*. Back crosses were also made between the amphidiploids and cabbage cultivar Green Globe and from these materials Pearson established two CMS systems, *viz*. petaloid and vestigial anther male sterility. Flowers of petaloid male sterile plants were less attractive to the pollinating insects, since pistils were enlarged, malformed and were lacking in nectarines (Pearson, 1972). In vestigial anther types, although flowers were smaller, normal and with functional nectarines, homozygous plants could not be recovered even after six generations of backcrossing in broccoli (Dickson 1975).

Ogura Cytoplasm

The CMS has been reported in an identified cultivar of Japanese radish by Ogura (1968) and first alloplasm was introduced by introgression of this sterility cytoplasm to *Brassica oleraceae* genome through repeated backcrosses with broccoli (Bannerot *et al*. 1974). Later Dickson, 1975 and Hoser- Krauze, 1987 transferred it from broccoli to cauliflower. In India this sterile cytoplasm from broccoli was transferred and established in three different maturity genetic backgrounds of Indian cauliflowers *viz*. early, mid and mid late through hybridization. The curd yield is increased 40-75% compared to SI system from different maturity groups (Kalia 2008). Recently Ogura based CMS lines developed in snowball cauliflower *vir*., Ogu1A, Ogu2A and Ogu3A for hybrid development in cauliflower. In India, IARI regional station Katrain develops two Cabbage hybrids H-64 & KCH-4 using cytoplasmic male sterility.

Anand Cytoplasm

The 'Anand' cytoplasm derives from the wild species *B. tournefortii*. It was transferred from *B*. rapa to *B.oleraceae* through cybridization process. The presence of 'Anand' chloroplasts with a *B. oleracea* nucleus did

not result in cold temperature chlorosis, as seen in Ogura CMS plants (Cardi and Earle, 1997).

Polima cytoplasm

Polima cytoplasm is originated from B. rapa. It was transferred from B. rapa to broccoli through protoplast fusion.

ROOT CROPS

Carrot

Cytoplasmic male sterility in carrot (*Daucus carota* L.) can occur in two morphologically distinct phenotypes.

Brown Anther Type

The brown anther (*ba*) male sterility was first discovered in the cultivar Tendersweet and reported by Welch and Grimball in 1947. The results of Hanshe and Gabelman (1963) and Banga *et al*. (1964) suggested that expression of the brown anther sterility was due to a homozygous recessive locus *Ms5* or a dominant allele for *Ms4*, but dominant allele of either of the two complimentary loci would restore the fertility.

This sterility has also been found in several other cultivated and wild carrot sources. The male sterile plants forms normal anthers but development is halted as anthers fail to continue to produce mature pollen, remain rudimentary and turn brown. This CMS was the first type used for developing hybrid carrot varieties, particularly early carrot hybrids but it is frequently unstable. It is most widely utilized for Nantes hybrid seed production in Europe.

Petaloid Male Sterility

Petaloid sterility is commercially used for hybrid seed production in the world. It is homeotic mutation. This is manifested as the replacement of stamens with petals (white petaloidy) or both stamens and petals with green bract like structures (green petaloidy) (Kitagawa *et al*. 1994). It is stable across a wide range of environments through flowering and seed production.

CMS system has been established for the first time in Asiatic carrot germplasm in India at IARI. The first public sector tropical carrot hybrid Pusa Vasudha has been developed by IARI, New Delhi. This male sterility utilized and developed a first in temperate carrot hybrid Pusa Nayanjyothi, at IARI Regional Station, Katrain. CMS was introduced in to carrot breeding materials, provides a very efficient tool for mass scale pollination control.

GUM CMS Type

This male sterility is originated from wild species D. carota ssp. gadecae. The flowers of the regular GUM type had neither petals nor stamens. They consisted only of the reduced sepals and the unmodified central gynoecium with two styles (Muthukumar, 2013).

Radish

As in all other cruciferae members, Cytoplasmic male sterility is reported in radish also. Ogura cytoplasm was first identified in Japanese radish and is controlled by single recessive gene and this is one which is extensively studied and is used for the production of hybrid seeds in radish **(***Raphanus sativus* L.). Male sterility expressions in the most of the populations have been reported to be stable, except on some population reversible temperature effect has been reported by Nieuwhof (1990).

NWB Cytoplasm

The new CMS obtained from the novel male sterile radish line NWB CMS which was originated from South Korea. This is characterized by anthers are more yellowish with some visible pollen. These male sterile phenotypes are more advantages for attraction of insect pollinators (Muthukumar, 2013).

Bulb Crops - Onion

Pollination control used to be a major obstacle to the production of hybrid seeds in onion. The onion umbel contains hundreds of perfect flowers and, although out crossing is encouraged by protandry, mature pollen and receptive stigmas are present at the same time in the densely packed inflorescence. Large scale emasculation is not practical at the field level. At present, CMS is used commercially to produce hybrid seed of onion (*Allium cepa* L.).

First CMS plant was reported within the progenies of an onion cultivar Italian Red (Jones and Emsweller, 1936) and male sterility was under the control of single recessive nuclear restorer locus (Jones and Clarke 1943).

The first CMS source in onion was CMS-S type. This CMS source is more likely an alien cytoplasm and differs from N cytoplasm for many polymorphisms in the chloroplast and mitochondrial genome. The CMS-S system has been widely used because of its stability in various environments. The CMS line (*S ms/ms*) and its near isogenic maintainer line (*N ms/ms*) are essential to breed the F_1 hybrid using the CMS system.

A new sterile cytoplasm was identified (T-cytoplasm) in French cultivar Jaune Paille des Vertus. Now it is commonly utilized in Europe and Japan. The male fertility restoration is controlled by a single dominant locus (A-) or dominant alles at two complimentary loci (B-C-) (Scheweisgyth 1973).

Worldwide more than 50% onion varieties currently cultivated are F_1 hybrids derived from

S-cytoplasm. In India, the work gained momentum in the eighties at IIHR (Bangalore), IARI (New Delhi) and MPKV (Rahuri). At IARI, male sterility was found in a commercial variety Pusa Red. Only two hybrids, Arka Kirtiman and Arka Lalima have been released by IIHR after development of CMS lines along with the maintainer.

CONCLUSION

The research on male sterility in vegetables is a never ending process due to rapid advancement of molecular technique and their implementation. Substantial progress has been made in understanding the mechanism of male sterility in selected vegetable crops. In fruit bearing vegetables like tomato, brinjal, chilli, muskmelon etc., identification and utilization of functional male sterility are more attractive. This is because unlike in sporogenous genic male sterility (50,50 ratio of male sterile and male fertile plants), 100% male fertile progenies can be obtained after forced selfing of male sterile plants, since functional pollen can be extracted from non-dehising anther. The attempts should be made to collect GMS lines of important vegetables at one place, so that these can be evaluated against sensitivity to temperature or photoperiod. This helps in the identification of EGMS lines, which are like functional sterility, believed to be having more potential for the production of commercial hybrid seeds.

In India, research on transgenic male sterility system was initiated in selected vegetables but our first priority should be utilization of existing and established but unexploited male sterility systems especially in chilli, onion, tomato, muskmelon etc. this will not only promote adoption of hybrid vegetable technology by economizing the cost of hybrid seeds but also provide basic material and scope for the development of more efficient male sterility system in respective vegetable crops.

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Genetic diversity, heritability, genetic advance and correlation coefficient in cape gooseberry (*Physalis peruviana*) under temperate environment

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ABSTRACT

Genetic variability, heritability and correlation coefficient were studied in 20 genotypes of cape gooseberry (*Physalis peruviana L.*). Wide range of variability was observed in a number of fruits/plant (26-73), fruit weight (6.51-19.1g), fruit length (20.42-29.82 mm), fruit diameter (23.06-34.22 mm), per cent juice content (56.2-63.17), fruit yield (199.67-1145.03 g/plant/if), total soluble solids (6.92-9.71° B), acidity content (0.28-0.67%), and ascorbic acid content (19.54- 24.36 mg/100 g edible part). Heritability was recorded in the range of 71.1% (TSS content) to 99.7% (fruit weight). Genetic advance range was recorded between 0.56 (fruit yield per plant) and 454.2 (fruit weight). Genotypic coefficient of variation ranged from 2.99 (TSS content) to 38.27 (weight of fruit) while phenotypic coefficient of variation ranged from 3.54 (TSS content) to 38.28 (weight of fruit). Significant and positive genotypic and phenotypic correlation co-efficient were observed in a number of fruits/plant and fruit yield/plant. Fruit weight showed high genotypic and phenotypic coefficient of variation with fruit length, diameter and yield/plant. However non-significant negative correlation coefficient were observed in a number of fruits/plant with fruit weight, fruit length, fruit diameter, and juice percentage. For selecting promising and high-yielding genotypes of cape gooseberry emphasis should be given on fruit length, fruit diameter and fruit weight.

Key Words: Genetic diversity, High-yielding, Qualitative attributes, Correlation coefficient Heritability, Genetic advance.

Cape gooseberry (*Physalis peruviana* L.), grouped under small fruits, is usually cultivated as an annual crop but in the absence of frost it can be perennial. Pollination in cape gooseberry is predominantly autogamous, although some degree of outcrossing can occur in the presence of wild or pollination insects like bees (Mc Cain, 1993). Fruits are yellow-orange berries, 1-3.5 cm diameter, very juicy, aromatic with a peculiar bittersweet flavour, enclosed by the acrescent epicalyx, which gives them the shape of bladder. The fruit can be eaten as raw, dessert, or as an appetizer, for dish decoration in cakes and for making jam (NRC 1989). It is rich in vitamin A, B_1, B_2, B_{12}, C and poly phenols (Branzati and Manaresi 1980; Sarangi *et al*. 1989). In spite of its great potential in respect of production, nutrient value and market acceptability has not been exploited fully as commercial cultivated fruit. Since, it is a self-pollinated, its crop with narrow genetic base, precise study on cape gooseberry germplasm available in natural habitat shall boost up the pace in its improvement through various devices. To evolve improved cultivars with better quality, information on association of quantitative and qualitative characters are of immense value in selection. A large number of local cultivars/genotypes of cape gooseberry are under cultivation in North-West Himalayan region of India. However, information on performance of these cultivars and extent of genetic variation for different quantitative and qualitative characters in agroclimatic situation of temperate region is lacking. Qualitative parameters are directly associated to consumers to whom lastly the produce is likely to be disposed off. Heritability, genetic advance, coefficient of variance and correlation coefficient for a character are hurdle in crop improvement if they are slow or negligible in progress. Cape gooseberry crop is well acclimatized in Jammu and Kashmir, India (Singh *et al*. 2011) and these regions

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have several strains which need to be identified, compared, selected and further improved for commercialization. Keeping in view, present investigation was aimed to assess 20 selections of cape gooseberry for their quantitative and qualitative attributing characters and observe the extent of genetic variation in temperate region for further improvement of its genetic stock.

MATERIALS AND METHODS

The present study was carried out at the Research Farm of Central Institute of Temperate Horticulture (CITH), Srinagar, During 2009-10 to 2010-11. Twenty selections (CITH CGB Sel. 1- CITH CGB Sel.2, CITH CGB Set.3, CITH CGB Sel. 4, CITH CGB Sel. 5, CITH CGB Sel 6, CITH CGB Sel. 7, ... CITH CGB Sel 20) were taken for the study. Plants from nursery were transplanted during May 2010 at a spacing of 30cm × 30cm. Avats cane/sprinkler was used to water the plants and no training was done. The experiment was conducted in a randomized block design replicated three times. The pooled data of two years were analyzed as per the method of Gomez and Gomez (1984). Plant height (cm) was measured as per the standard methods. Fruit length was measured from the point of pedicel attachment to the calyx end. The diameter of fruits was measured at highest perimeter. Fruit weight was measured using electronic balance. Juice of the fruit was analyzed for major chemical composition.

Chemical parameters, viz. acidity was determined by titration to pH 8.1 with 0.1M NaOH solution and expressed as percentage (AOAC, 1984). The total soluble solids (TSS) were determined with Atago digital refractrometer calibrated using distilled water and expressed as °Brix at 22 °C. Ascorbic acid (mg/100 g edible part) was determined by employing the method described by Ruck (1963). Heritability, genetic advance, coefficient of variance and, correlation coefficient were worked out as per the method given by Al-jibouri *et al*. (1958).

RESULTS AND DISCUSSIONS

Analysis of variance showed significant variation among genotypes for all the physico-chemical characters (Table 1). Maximum number of fruits/ plant (73.00) was recorded in CITH CGB Sel-16, while minimum fruits/plant 26 was recorded in CITH CGB Sel-13 with the overall mean 52.73 and coefficient of variation of 4.36 %. However, yield/plant (g) ranged from 199.67-1145.03 (g) and maximum yield/plant (g) recorded highest in CITH CGB Sel-20, followed by CITH CGB Sel-9 and minimum in CITH CGB Sel-4 with over all mean 576.71g and coefficient of variation of 0.40 %. The moderate range of variation may be attributed to genetic make-up of individual seedling tree, variation in soil condition, age and environmental conditions.

Maximum fruit weight (19.10 g) was observed in CITH CGB Sel-3, followed by CITH CGB Sel-12 (16.44 g) and CITH CGB Sel-20(15.33 g), while minimum fruit weight was recorded in CITH CGB Sel-4 (6.51g). Of the 20 selections, 9 bears fruit weight more than 60 g, which are one of the best criteria for their selection. Fruit length varied from 20.42 mm (CITH CGB Sel-5) to 29.82 mm (CITH CGB Sel-3) with overall mean 23.66 and coefficient of variation of 4.58%. However, Fruit diameter varied from 23.06 mm (CITH CGB Sel-16) to 34.22 mm (CITH CGB Sel-12).

There was wide variability in fruit weight. The variation in fruit weight was because of genetic behaviour of different cultivars or genotypes with bigger or smaller sizes varying with weight. Wide variability was also recorded in fruit juice percentage which ranged from 56.20 (CITH CGB Sel-17) to 63.17% (CITH CGB Sel-14) with overall mean 60.10 and coefficient of variation 1.90. CITH CGB Sel -16, CITH CGB Sel-20 and CITH CGB Sel-3 can be taken in improvement programme to increase number of fruits/ plant, fruit yield and fruit weight in commercially important varieties. Great range among physical parameter was observed which needs further critical observation and selection for desirable traits like number of fruits, fruit weight, fruit diameter and fruit length.

Wide variation was recorded in chemical composition of fruits of all the genotypes. The TSS ranged from 6.92 (CITH CGB Sel-10) - 9.71°Brix (CITH CGB Sel-1) with mean of 8.58 °Brix and coefficient of variation 2.06 %. Acidity in fruits has an important role in determining consumer acceptability and market appeal. Acidity ranged from 0.28 % (CITH CGB Sel-2) to 0.67% (CITH CGB Sel-19) with mean of 0.51% and low coefficient of variation (1.31%). Ascorbic acid content was recorded maximum (24.36 mg/100 g edible parts) in CITH CGB Sel- 9 and minimum (19.54 mg/ 100 g edible part) in CITH CGB Sel-5.

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were high for fruit yield/plant (38.28, 38.27 %), fruit weight (32.89, 32.06%), number of fruits/plant (27.11, 26.76%) which suggested greater phenotypic and genotypic variability among accessions and sensitiveness of attributes for making further improvement by selection. Very narrow difference between PCV and GCV for other traits suggested their relative resistance to environmental alteration. The PCV was higher than the respective GCV for all characters denoting environmental factors influencing their expression to some degree or other.

Table 1. Quantitative and qualitative trait variability in cape gooseberry

Treatment	No. of fruits/ plant	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Juice (%)	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg/100g edible part)	Fruit yield/plant (g)
CITH CGB Sel-1	40	15.15	27.17	31.22	60.77	9.71	0.533	19.57	606.26
CITH CGB Sel-2	57.66	14.2	23.63	33.35	59.41	8.16	0.28	22.72	825.58
CITH Sel-3	36.33	19.1	29.82	34.19	62.17	8.76	0.477	21.24	686.83
CITH CGB Sel-4	32	6.51	20.44	24.49	61.19	8.62	0.527	21.31	199.67
CITH Sel-5	57.33	9.27	20.42	26.63	59.35	9.14	0.477	19.54	548.57
CITH CGB Sel-6	61	8.14	22.1	24.35	61.33	9.25	0.563	24.31	496.53
CITH Sel-7	38.66	9.86	22.13	26.36	60.51	9.06	0.617	22.68	386.44
CITH CGB Sel-8	45.66	10.17	23.07	27.02	58.2	9.3	0.56	22.71	464.33
CITH Sel-9	67.66	13.52	24.16	28.98	61.1	8.77	0.513	24.36	924.81
CITH CGB Sel-10	64	7.4	22.22	23.56	61.67	6.92	0.447	19.61	467.06
CITH CGB Sel-11	60.33	7.9	20.79	24.22	60.13	8.75	0.533	21.21	484.02
CITH CGB Sel-12	42.66	16.44	28.84	34.22	62.77	8.43	0.583	22.68	715.67
CITH CGB Sel-13	26	13.47	25.42	27.63	61.32	8.3	0.587	19.63	347.86
CITH CGB Sel-14	60.66	7.03	22.54	23.42	63.17	7.29	0.473	21.2	428.71
CITH CGB Sel-15	36	9.37	22.28	25.36	58.14	8.15	0.587	22.7	337.99
CITH CGB Sel-16	73	7.63	22.25	23.06	58.23	9.28	0.533	21.23	552.95
CITH CGB Sel-17	62	11.29	21.71	28.47	56.2	9.13	0.477	22.66	684.45
CITH CGB Sel-18	58	8.72	22.36	24.72	58.43	9.22	0.477	19.55	511.18
CITH CGB Sel-19	63	11.51	23.42	27.69	57.01	8.11	0.67	22.79	721.08
CITH CGB Sel-20	72.66	15.33	28.45	31.51	61.07	7.31	0.423	19.55	1145.03
CD (5%)	3.81	1.35	1.79	1.08	1.9	0.29	0.011	0.301	3.83
CV	4.36	7.34	4.58	2.36	1.9	2.06	1.3	0.84	0.4

The heritability in broad sense ranged from 71.10% for Juice to 99.7% for yield/plant. Such high values of heritability for number of fruits/plant, fruit weight, fruit diameter, TSS, acidity and ascorbic acid clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable.

The genetic advance as per cent of mean (genetic gain) varied from 7.51 to 57.44%. High estimates of genetic gain were obtained for fruit yield/plant (454.2%) and number of fruits/plant (28.89%). These characters also exhibited high values of GCV which portrayed that these are controlled by additive gene and phenotypic selection for their improvement could be

Table 2. Estimate of genetic parameters for economic characters in cape gooseberry

Character	Range	Mean + SE	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Heritability (broadsense) (%)	Genetic advance (%)	Genetic advance % of mean
No of fruits/ plant	26-73	52.73 + 5.29	27.11	26.76	97.4	28.89	54.39
Fruit weight (g)	6.51-19.1	11.10 + 5.33	32.89	32.06	95	7.14	30.30
Fruit length (mm)	20.42-29.82	23.66 + 0.66	12.46	11.58	86.4	5.24	19.04
Fruit diameter (mm)	23.06-34.22	27.52 + 1.17	13.4	13.19	96.8	7.35	12.12
Juice (%)	56.2-63.17	60.10 + 60.10	3.54	2.99	71.1	1.12	13.05
TSS (°Brix)	6.92-9.71	8.58 + 8.58	8.94	8.7	94.6	4.15	554.26
Acidity (%)	0.28- + 0.67	0.516 0.516	16.33	16.28	99.4	0.17	0.78
Ascorbic (mg/100g edible part)	19.54-24.36	21.56 + 21.56	7.47	7.42	98.7	3.27	0.56
Fruit yield/plant (g)	199.67-1145.03	576.71 + 1.33	38.28	38.27	99.7	454.2	4091.8

achieved by simple selection. Genetic advance as % of mean was also calculated and it ranged between 0.56 and -4091.8, and maximum value was obtained for fruit yield/plant and lowest for ascorbic acid content. Golani *et al*. (2007) also reported high magnitude of heritability for fruit weight in several diverse accessions of tomato. High estimates of heritability coupled with low GCV and genetic gain were observed for fruit length, fruit diameter, juice (%) TSS, titrable acidity and ascorbic acid which might be attributed to non-additive gene action controlling its expression and simple selection would not be rewarding. Nevertheless this could be improved by development of hybrid varieties or utilization of trangressive segregants in heterosis breeding programme.

The present results endured to those of Kamruzzahan *et al*. (2000) and Mohanty (2003) in tomato. The major causes underlying association are either due to pleiotropic gene action or linkage or both. The phenotypic correlation includes a genotypic and environmental effect, which provides information about total association between the observable characters. The phenotypic correlations were normally of genetic and environmental interaction which provided information about the association between two characters. Genotypic correlation provided a measure of genetic association between the characters and normally used in selection, while environmental as well as genetic architecture of a genotype plays a great role in achieving higher yield combined with better quality.

The genotypic and phenotypic correlation for fruit yield and its component in cape gooseberry are presented in Table 3 and the data revealed a significant and positive genotypic and phenotypic coefficient of variation between number of fruits/plant and fruit yield/plant (0.563*). Fruit weight showed highly significant genotypic and phenotypic correlation coefficient with fruit length (0.926**), fruit diameter (0.971**) and yield/plant (0.644**). Similar findings were also reported by Naidu (2001) and Jogi (2007) in tomato. Fruit length showed highly genotypic and phenotypic correlation coefficient with fruit diameter (0.834**) and fruit yield (0.571*). Fruit diameter is significantly positively associated with fruit yield/plant. However, juice, TSS and ascorbic acid are non - significantly associated. Non-significant positive genotypic and phenotypic coefficient of variation also observed in number of fruits with ascorbic acid content, fruit weight and juice percentage, TSS and ascorbic acid, fruit length and juice percentage, fruit diameter with juice percentage and TSS with ascorbic acid content. Non-significant negative genotypic and phenotypic correlation coefficient observed for number

Table 3. Genotypic and phenotypic correlation among important characters in capegoose berry

Character		No. of fruits/ plant	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Juice (%)	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg/100 g edible part)	Fruit yield/plant (g/plant)
No. of fruits/ plant	G	1.00	0.237	-0.208	-0.202	-0.260	0.203	0.348	0.07	0.555
	P	1.00	-0.220			-0.195	-0.187	-0.340		
Fruit (g)	G	1.00	0.940**	0.968**	0.237	0.030	-0.122	0.012	0.644**	
	P	1.00	0.838**	0.928**	0.199	0.024	0.129	0.013	0.628**	
Fruit length (mm)	G	1.000	0.845**	0.498	-0.124	0.048	-0.135	0.571*		
	P	1.000	0.780**	0.337	0.129	-0.045	-0.113	0.531		
Fruit diameter (mm)	G	1.000	0.201	0.024	0.246	0.080	0.653**			
	P	1.000	0.165	0.019	-0.240	0.078	0.643**			
Juice (%)	G	1.000	-0.299	-0.084	-0.168	-0.012				
	P	1.000	-0.382	-0.108	-0.150	-0.017				
TSS (°Brix)	G	1.000	0.255	0.189	-0.169					
	P	1.000	0.248	0.188	-0.164					
Acidity (%)	G	1.000	0.279	-0.384						
	P	1.000	0.277	-0.382						
Ascorbic acid (mg/100 g edible part)	G	1.000	0.099							
	P	1.000	0.098							
Fruit yield/plant (g/plant)	G	1.00								
	P	1.00								

*, Correlation is significant at 0.05 level
**, Correlation is significant at 0.01 level

of fruits/plant with fruit weight, fruit length, fruit diameter, juice percentage, TSS and acidity. Fruit weight showed negative association with acidity however fruit length with TSS, acidity, ascorbic acid.

Conclusively, all the physical parameters showed significant variability and provide great opportunity in breeding programme to get desired variety. Similarly, chemical characters also reflected potential variation. Heritability, genetic advance, coefficient of variance and correlation coefficient analysis showed encouraging results. Therefore, emphasis should be given on number of fruits/plant, fruit yield/plant, fruit length; fruit diameter and fruit weight for improvement in fruit yield of cape gooseberry for further improvement programme and transfer desired traits into an elite cultivar.

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Evaluation of combiners for resistance to early blight disease using interspecific crosses in tomato (*Solanum lycopersicum*)

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ABSTRACT

The study was undertaken by using nine genetically diverse tomato (*Solanum lycopersiam* L.) genotypes wherein five susceptible cultivars, viz. CO-3, Punjab Chhuhara (PBC), DT-10, Sel-7 and DVRT-2, were crossed by four resistant accessions of wilds species, *viz. S. habrochaites* (EC-520061 and H-88-78-1), *S. glandulosum* (WIR-3928) and *S. pimpinellifolium* (EC-521080) to determine the best general combining ability (GCA) and specific combining ability (SCA) combiners for resistance to early blight disease, yield and quality traits in tomato. All the 20 hybrids along with their parents showed both additive and non-additive gene interaction for GCA and SCA variances. Based on GCA estimates it was identified that the best general combiners, for (EC-520061, Sel-7, PBC and CO-3) PDI, PH, NPB and NFPP; for AFW and FYPP for (H-88-78-1, DT-10, CO-3 and WIR-3928); (EC-520061, DVRT-2, EC-521080, Sel-7, WIR-3928). for TSS, AA, LP and TP. The best specific combiners were Sel-7 × H-88-78-1 for PDI; DVRT-2 × WIR-3928, DT-10 × EC-520061, CO-3 × WIR-3928, DT-10 × H-88-78-1 and CO-3 × WIR-3928 for PH, NPB, NFPP, AFW and FYPP and Sel-7 × WIR-3928, Sel-7 × EC-521080, DT-10 × EC-521080 and PBC × EC-520061 for TSS, AA, LP and TP, respectively. These best combiner parents and crosses could be utilized in further resistance breeding programme.

Key Words: Tomato, Interspecific crosses, Early blight, Resistance, Quality traits

Tomato (*Solanum lycopersicum* L.) is a highly self-pollinated and annual herbaceous crop having either determinate or indeterminate growth habit with hairy stem and acute leaves (Singh *et al.* 2013a). This Tomato is a rich source of antioxidants (mainly lycopene and β-carotene), Vitamin A, Vitamin C and minerals like Ca, P and Fe in diet (Saleem *et al*. 2013). Natural genetic variation in tomato and its other wild relatives have given a genetic treasure trove of genes that produce vitamin A, vitamin C, anthocyanin, and other antioxidants which have various medicinal values (Lequatra *et al*. 2005 and Ellinger *et al*. 2006).

This crop has suffered from a number of biotic stresses like fungi, bacteria, viruses and nematodes (Lukyanenko, 1991). Among the fungal diseases, early blight is one of the important diseases after Tomato Leaf Curl Virus (ToLCV) caused by *Alternaria solani* and has been counted as substantial yield losses in each tomato cropping season (Singh *et al*. 2012). The symptoms of early blight were characterized by Barksdale and Stoner (1977). Sanitation, crop rotation and fungal spray practices have limited success to prevent tomato crops by this disease and availability of resistant cultivar would reduce the use of routine fungicide (Horsefall and Heuberger 1942).

Breeding for early blight disease resistance requires efficient screening techniques, and appropriate breeding strategies to transfer resistant genes into improved genetic backgrounds. Moreover, plants with late maturity, indeterminate growth habit, or low-yielding ability appear to be more resistant (Barksdale 1971, Barksdale and Stoner 1977, Gardener 1984 and Singh *et al.* 2012a, b). In previous studies, a number of resistant lines and hybrids were identified for early blight disease and these line can be used to be quantitatively inherited for the breeding lines (Barksdale 1971, Maiero *et al*. 1989 and Barksdale and Stoner, 1977). In addition, red pigment (lycopene) in tomato fruit does not develop at temperatures above 30°C while in mid-summer it does not produce good quality fruit. This type of fruits may

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be small, flabby, cracked, orange instead of red, and off-flavour.

Although tomato is self-pollinated crop, combining ability helps in diagnosis of additive and non-additive gene action would in turn lead a breeders to select desirable tomato (Angadi *et al*. 2012). Information about magnitude of GCA in parents and specific combining ability SCA in F_1s crosses is imperative for crop improvement programmes (Sprague and Tatum, 1942) because GCA reveals the existence of additive gene effects while SCA reveals non-additive gene effects. Judicious application of information relevant to standard heterosis and SCA are fruitful for selecting best hybrids for desired traits (Yadav *et al*. 2013). The present experiment was carried out to identify best combiners in tomato for resistance to early blight disease and associated yield and quality traits using interspecific crosses.

MATERIALS AND METHODS

A total 20 F_1s crosses were made by crossing between five determinate, high-yielding and susceptible lines, e.g. CO-3, PBC (Punjab Chhuhara), DVRT-2 (Kashi Anupam), Sel-7 (Hissar Arun) and DT-10 of *Solanum lycopersicum* into four indeterminate, taken high number of fruits, poor yielding and resistant accessions of wild species, viz. *Solanum habrochaites* (EC-520061), *S. pimpinellifolium* (EC-521080), *S. glandulosum* (WIR-3928) and *S. lycopersicum* derivative of *S. habrochaites* f. *glaboratum* (H-88-78-1). These interspecific hybrids were made in line × tester matting design through manual pollination during main growing season in 2007. The 20 F_1s along with 9 parents were taken for experiment and evaluated at Indian Institute of Vegetable Research (IIVR), Varanasi, India, under natural environment during October-February 2008. About 21 days old seedlings were transplanted in a complete randomized block design. Each replication has 30 plants at regular spacing 45 cm (plant-to-plant) and 60 cm (row-to-row). The recommended dose and methods of mannuring and fertilizers were used. There were no any application of fungicides during cropping season to check the fungal attack.

For data observation, randomly selected five plants from middle row of each replication, avoiding the border row, were tagged before flowering. The observations were recorded for only those horticultural and quality traits which were affected by early blight disease, e.g. plant height (PH) in centimeter, number of primary branches (NPB), number of fruits/plant (NFPP), average fruit weight (AFW) in gram and fruit yield/plant (FYPP) in kg, ascorbic acid (mg/100g), lycopene (mg/100g), phenol (mg/100g). The disease symptoms were regularly observed at 7 days interval on appearance of disease. First data were taken at 15 days after transplanting. The symptoms of disease were scored on 0-5 scale with some modification (Pandey *et al*. 2003). The per cent disease incidence (PDI) was calculated by the following formula:

[PDI = (total number of diseased plants/total number of observed plants) × 100]

Total soluble solids (TSS) was estimated by Erema hand Refractometer using fruit juice. The ascorbic acid (mg/100g) content was estimated titrimaticaly by using 2, 6, dichloro endophenol dye method of Rangana (1986), while lycopene (mg/100g) was evaluated by using acetone and petroleum ether optical density was taken at 503nm with the help of Spectrophotometer Ranagana (1986), total phenol (mg/100g) was also estimated by using the standard of Rangana (1986) sample crushed with 80% ethanol and centrifuge, filtrate was heated at 70°C to evaporate ethanol. Further Folin and Ciocalteu's reagent (FCR) was used and O.D. taken at 650nm. Entire process was repeated at three times and mean value was taken as well.

The mean data was subjected to combined analysis of variance combining ability analysis was carried out according to Kempthorne (1957); Singh and Chaudhary (1979) and Singh *et al*. (2013a) fixed effect model using the following formula:

$$[X_{ijk} = \mu + G_i + G_j + S_{ij} + E_{ijk}]$$

where, μ= general mean, g_i= GCA effect of i_{th} line (female parent), g_j=GCA effect of j_{th} tester (male parent), Sij= SCA effects of hybrids with the i_{th} lines and j_{th} tester, e_{ijk}= error associated with the i_{th} observation at the plot, i= 1, 2-- (numbers of line), j=1, 2----1 (numbers of tester), k= 1, 2, 3---r (numbers of replication).

RESULTS AND DISCUSSION

Analysis of variance for combining ability (general and specific) was significant for all the traits (Table 1). Significant difference were observed in line, testers and line vs tester interaction among the all traits, indicating both type of additive and non-additive gene interaction and played a significant role in appearance of these traits on P<0.001 and P<0.05 level. Similar results were proposed by Saleem *et al*. (2009) and Hannan *et al*. (2007). The value of σ^2GCA female was lessar than that of σ^2GCA male for all the characters except number of primary branches (NPB), it means male parent was dominant on female for all characters but NPB was expressed dominancy of female parents on relative male. Whereas, σ^2GCA female and male was less than σ^2SCA for all characters but NPB of σ^2GCA female and PDI and PH of σ^2GCA male was observed more than σ^2SCA (Table 1), indicating predominance of both types of additive and non-additive gene action for the characters and may be

NPB of female and PDI and PH of male was dominant on their hybrids (Table 1). As per results the ratio of σ^2SCA was > 1 for all characters except PDI (0.17) and FYPP (0.73) and the degree of dominance (σ^2D/ σ^2A)1/ 2 was < 1 for all characters except PDI (2.29), showed predominance of additive type gene action and also presented that the F_1s hybrids were moderate for both disease and fruit yield. This finding was supported by Maiero *et al*. (1990) and Hannan *et al*. (2007).

The general combining ability is an indication of additive genetic variance for disease, yield and quality traits. In the present study, all the female and male parents showed highly positive significant value for per cent disease incidence (PDI) except PBC and DT-10 (-0.03), Sel-7 (-0.19) and EC-520061 (-2.52). These parents were found for negatively significant and a good combiner for disease resistant because negative significant PDI value is an indication of high disease resistant capacity of parents (Barksdale, 1971, Pandey *et al*. 2003, Singh *et al*. 2012 and 2013c).

The plants of EC-520061 were highest (47.55 cm) followed by Punjab Chhuhara (18.80 cm) and CO-3 (14.05 cm) and showed positively significant value. The good combiners for plant height were studied by Avdikos *et al*. (2011) and Singh and Asati (2011). While the parents, CO-3, EC-520061 and Punjab Chhuhara, were good combiners for number of primary branches as they showed positive and significance value, this may be possible by adopting highest plant height by these parents (Rai *et al*. 2003 and Singh and Asati, 2011). Maximum number of fruits /plant (NFPP) was observed in EC-520061 (62.93), followed by CO-3, Punjab Chhuhara and EC-521080.

These parents were good combiners for number of fruits/lant due to high and positively significant GCA value. For average fruit weight (AFW), H-88-78-1 and DT-10 was highly positively significant and good combiner. These two characters, viz. NFPP and AFW, are responsible for increase or decrease in fruit yield (Kumar *et al*. 2010, Prabuddha *et al*. 2008, Singh and Asati, 2011 and Singh *et al*. 2013a). In fruit yield, highest FYPP was recorded in CO-3, followed by WIR-3928, DT-10 and EC-520061 and depicted high and positive significant GCA value and good combiners for FYPP with an agreements of Kumar *et al*. (2010) and Prabuddha *et al*. (2008). However, in respect of quality characters,b parents EC-520061, EC-521080, DVRT-2 and DT-10 showed positively significant value for total soluble solids (TSS), while ascorbic acid (AA) was highest and significant in EC-521080, Sel-7, DT-10 and WIR-3928, a similar study for good combiner of TSS and AA has been reported by Bhatt *et al*. (2001). Lycopene (LP) is a good antioxidant in tomato and produce red colour pigment. In present study, WIR-

Table 1. Analysis of variance for combining ability and A and D component of genetic variance for EB (PDI), yield and quality traits in tomato

Source↓	Replication	Lines effect	Testers effect	Line vs tester	Error	σ^2GCA for parents		σ^2SCA	σ^2A/	σ^2D	$\frac{\sigma^2D}{\sigma^2A}$
D.F. →	2	4	3	12	38	Female	Male				
PDI (%)	0.01	0.27**	42.99**	0.53**	0.006	-0.02	2.83	0.17	0.39	0.17	2.29
PH (cm)	17.27	2808.82**	22253.48**	884.93**	10.39	160.32	1424.57	285.71	220.59	285.72	0.77
NPB	8.47	33.98**	30.47**	6.99**	1.52	2.25	1.57	1.9	0.55	1.9	0.29
NFPP	63.15	12326.04**	48327.42**	8637.85**	11.08	307.35	2645.97	2849.33	411.13	2849.33	0.14
AFW (g)	24.87	155.46**	786.64**	167.57**	7.79	-1.01	41.27	46.44	5.56	46.44	0.12
FYPP (kg)	0.41	1.19**	1.02**	0.92**	0.16	0.08	1	0.73	0.15	0.73	0.21
TSS (B0)	0.43	3.30**	17.32**	2.30**	0.09	1.15	2.3	8.03	1.18	5.03	0.23
AA (mg /100g)	0.25	47.43**	68.03**	20.67**	0.37	2.23	3.16	6.77	0.77	6.78	0.11
LP (mg/100g)	5.043	148.29**	4009.36**	233.12**	0.48	-7.07	251.75	77.52	33.76	77.52	0.44
TP (mg/100g)	0.65	2369.59**	4709.07**	662.77**	0.54	142.24	269.75	220.71	58.27	220.71	0.26

* Significant at P<0.05 level.

** Significant at P<0.01 level; PDI= per cent Disease Incidence; PH= plant height; NPB= number of primary branches; NFPP= number of fruits/plant; AFW= average fruit weight; FYPP= fruit yield per plant; TSS= total soluble solids; AA= ascorbic acid; LP= lycopene; TP= total phenol

Table 2. General combining ability (male and female parents) and specific combining ability (F_1 hybrids) for EB (PDI), yield and quality traits

Character	PDI (%)	PH (cm)	NPB	NFPP	AFW (g)	FYPP (kg)	TSS (B0)	AA (mg /100g)	LP (mg/100g)	TP (mg/100g)
GCA in female parents (lines)										
CO-3	0.04	14.05 **	1.78 **	45.67 **	-1.67	0.41 **	-0.1	-0.23	-2.76 **	1.86**
PBC	-0.03	18.80 **	1.45 **	17.17 **	-2.59	0.003	-0.50**	-0.73 **	-4.27 **	-14.86**
DT-10	-0.03	-10.78 **	-2.39 **	-5.83 *	6.33 **	0.18	0.20 *	1.55 **	3.86**	-14.04**
Sel-7	-0.19 **	-14.62 **	-0.47	-22.59 **	-0.83	-0.2	-0.40**	2.22 **	0.22	14.72**
DVRT-2	0.22 **	-7.45 **	-0.38	-34.42 **	-1.25	-0.39 **	0.80**	-2.81 **	2.94 **	12.32**
SE (line)	0.03	1.52	0.33	2.73	1.53	0.13	0.09	0.17	0.21	216.56
GCA in male parents (testers)										
EC-520061	-2.52 **	47.55 **	1.77 **	62.93 **	-5.52 **	0.18	1.38 **	-1.88 **	-24.18**	26.38**
H-88-78-1	0.59 **	-46.58 **	-1.70 **	-73.67 **	10.02 **	-0.18	-0.98 **	-1.65 **	4.18**	-5.71**
WIR-3928	1.15 **	2.62	0.17	-5.13 *	0.62	0.26 *	-0.71 **	1.01 **	10.05 **	-10.07**
EC-521080	0.77 **	3.58 *	-0.23	15.87 **	-5.12 **	-0.26 *	0.30 **	2.52 **	9.95 **	-10.59**
SE (tester)	0.03	1.36	0.29	2.44	1.37	0.11	0.08	0.15	0.19	193.69
SCA in F_1 crosses (hybrids)										
CO-3 × EC-520061	-0.06	3.28	-1.18	-31.60**	1.27	-0.36	-0.83 **	2.07 **	2.83 **	6.14**
CO-3 × H-88-78-1	0.37 **	-5.58	0.28	-36.67**	0.73	-0.03	0.39 *	-3.08 **	-6.25 **	3.46 **
CO-3 × WIR-3928	-0.29 **	1.22	0.75	80.13**	-1.87	0.75**	0.72**	0.18	8.61**	-7.29 **
CO-3 × EC-521080	-0.03	1.08	0.15	-11.87*	-0.13	-0.36	-0.29	0.84 *	-5.18 **	-2.30 **
PBC × EC-520061	-0.15 **	-4.13	-1.85**	46.90**	1.52	0.32	0.63 **	0.02	3.39**	23.45**
PBC × H-88-78-1	0.15 **	7.67*	-0.38	42.17**	-10.02**	0.03	-0.14	-0.05	5.25**	-13.34 **
PBC × WIR-3928	-0.01	-23.87**	0.75	-62.03**	8.05*	-0.01	-1.41 **	-0.73 *	-0.57	9.22**
PBC × EC-521080	0.02	20.33**	1.48*	-27.03**	0.45	-0.34	0.91 **	0.76 *	-8.07 **	-19.33 **
DT-10 × EC-520061	0.20 **	-6.55*	2.32**	11.90*	-5.73	0.02	0.53 **	0.18	-3.72**	-6.06 **
DT-10 × H-88-78-1	0.07	-7.75*	0.12	-19.83**	17.73**	0.56*	0.16	1.51 **	-14.0**	8.15**
DT-10 × WIR-3928	0.15 **	4.38	-1.42*	-51.03**	-4.53	-0.75**	-0.31	1.72**	3.59**	4.21 **
DT-10 × EC-521080	-0.43 **	9.92**	-1.02	58.97**	-7.47*	0.18	-0.39 *	-3.41**	14.19 **	-6.30 **
Sel-7 × EC-520061	0.18 **	6.95*	-0.93	-2.68	2.77	0.33	0.27	-3.66 **	0.17	-10.96 **
Sel-7 × H-88-78-1	-0.99 **	16.42**	1.87**	5.25	-4.43	-0.29	-1.31 **	2.89**	2.49**	-12.35 **
Sel-7 × WIR-3928	0.32 **	-13.78**	-0.33	-35.28**	-0.7	-0.67*	0.96 **	-2.73**	-4.52 **	4.17**
Sel-7 × EC-521080	0.49 **	-9.58 **	-0.6	32.72**	2.37	0.64*	0.08	3.49 **	1.86**	19.14 **
DVRT-2 × EC-520061	-0.18 **	0.45	1.65 *	-24.52**	0.18	-0.31	-0.60 **	1.39 **	-2.66 **	-12.57 **
DVRT-2 × H-88-78-1	0.41 **	-10.75 **	-1.88 **	9.08	-4.02	-0.26	0.89 **	-1.27	12.57 **	14.09 **
DVRT-2 × WIR-3928	-0.17 **	32.05 **	0.25	68.22**	-0.95	0.68*	0.03	1.55 **	-7.11**	-10.31 **
DVRT-2 × EC-521080	-0.06	-21.75**	-0.02	-52.78**	4.78	-0.11	-0.32	-1.68 **	-2.80 **	8.79 **
SE (hybrid)	0.06	3.04	0.66	5.47	3.07	0.25	0.19	0.34	0.43	433.12

* Significant at P<0.05 level.

** Significant at P<0.01 level; PDI= per cent disease incidence; PH= plant height; NPB= number of primary branches; NFPP= number of fruits/plant; AFW= average fruit weight; FYPP= fruit yield/plant; TSS= total soluble solids; AA= ascorbic acid; LP= lycopene; TP= total phenol; PBC= Punjab Chhuhara

3928, EC-521080, H-88-78-1, DT-10 and DVRT-2 were good combiner for LP and they exhibited high GCA value. Previously some studies have been done for lycopene in tomato (Agarwal and Rao 2000). For total phenol, parents EC-520061, Sel-7 and DVRT-2 revealed high positively significant GCA value and these parents can be utilized as good combiners in resistant breeding programme (Singh *et al*. 2010 and 2013b)

The specific combining ability is an indication of non-additive genetic variance for various traits in F_1s hybrids. For the per cent disease incidence (PDI) of early blight disease was low and negative and significant in the crosses of Sel-7 × H-88-78-1 (-0.99), followed by DT-10 × EC-521080 (-0.43) and CO-3 × WIR-3928 (-0.29), DVRT -2 × EC-520061 and DVRT-2 × WIR-3928 in the range of -0.01 (PBC × WIR-3928) to -0.99 (Sel-7 × H-88-78-1). Earlier this was studied that negative and least value of disease incidence indicated to resistance capacity by these hybrids (Singh *et al*. 2013a, b, c). In plant height (PH), the crosses like, DVRT-2 × WIR-3928 (32.05), Punjab Chhuhara × EC-521080 (20.33) and Sel-7 × H-88-78-1 (16.42), however, for number of primary branches (NPB), cross combinations DT-10 × EC-520061, Sel-7 × H-88-78-1, DVRT-2 × EC-520061 and Punjab Chhuhara × EC-521080 showed maximum SCA value and good specific combiner. This study was supported by Rajput (1998) and Avdikos *et al*. (2011).

The indeterminate and maximum PH and more NPB may be indicated to the least infection of disease in tomato plants (Singh *et al*. 2012 and 2013c). For number fruits/plant (NFPP) highest positive and significance value were showed by crosses, CO-3 × WIR-3928 (80.13), DVRT-2 × WIR-3928 (68.22), DT-10 × EC-521080 (58.97), followed by crosses Punjab Chhuhara × EC-520061, Punjab Chhuhara × H-88-78-1, Sel-7 × EC-521080 and DT-10 × EC-520061, respectively and for average fruit wait (AFW) maximum and minimum range in cross combinations DT-10 × H-88-78-1 (17.78) to CO-3 × EC-521080 (-0.13) were good specific combiners for average fruit weight. The NFPP and AFW are representing to high and low yield in crops this study was an agreements of Rai *et al*. (2003) and Singh *et al*., 2013b.

In present study, maximum and minimum fruit yield were in PBC × WIR-3928 (-0.01) and DT-10 × WIR-3928 (-0.75) but most of crosses showed low and negative significant SCA value. These theories of low yielder varieties have positive relation from resistance capacity of crops (Pandey *et al*. 2003, Rai *et al*. 2003 and Singh *et al*. 2013a,b). In quality traits, cross combinations, Sel-7 × WIR-3928, Punjab Chhuhara × EC-521080, DVRT-2 × H-88-78-1, CO-3 × WIR-3928, Punjab Chhuhara × EC-520061, DT-10 × EC-520061 and CO-3 × H-88-78-1 were highly positive and significant SCA value for total soluble solids and the crosses like Sel-7 × EC-521080, Sel-7 × H-88-78-1, CO-3 × EC-520061, DT-10 × WIR-3928, DVRT-2 × WIR-3928, DT-10 × H-88-78-1, DVRT-2 × EC-520061, CO-3 × EC-521080 and Punjab Chhuhara × EC-521080 showed high value for ascorbic acid content.

The similar finding for TSS and ascorbic acids in tomato F_1s were reported (Rai *et al*. 2003 and Kaur *et al*. 2002). However, lycopene content (LP) was found in using crosses in the range of 0.17 (Sel-7 × EC-520061) to 14.19 (DT-10 × EC-521080) as good specific combiners, these finding were depicted high positive SCA value for lycopene content (Garg *et al*. 2008). In present study, cross combinations, viz. Punjab Chhuhara × EC-520061, Sel-7 × EC-521080, DVRT-2 × H-88-78-1, Punjab Chhuhara × WIR-3928, DVRT-2 × EC-521080, DT-10 × H-88-78-1, CO-3 × EC-520061, DT-10 × WIR-3928, Sel-7 × WIR-3928 and CO-3 × H-88-78-1 showed high value for total phenol and good specific combiners. The resistant plants had more total phenol content (Radhey *et al*. 1985 and Singh *et al*. 2010).

Thus, it was concluded that both GCA and SCA showed additive and non-additive gene action for parents and their crosses, respectively.The best general combiners for PDI (EC-520061 and Sel-7); for PH were (EC-520061 and PBC); for NPB and NFPP (EC-520061 and CO-3); for AFW (H-88-78-1 and DT-10); for FYPP (CO-3 and WIR-3928) and for TSS, AA, LP and TP (EC-520061, DVRT-2, EC-521080, Sel-7, WIR-3928). Whereas, best crosses, viz. Sel-7 × H-88-78-1 for PDI; DVRT-2 × WIR-3928, DT-10 × EC-520061, CO-3 × WIR-3928, DT-10 × H-88-78-1 and CO-3 × WIR-3928 showed best specific combiners for PH, NPB, NFPP, AFW and FYPP, respectively. Whereas, in quality traits like, TSS, AA, LP and TP showed best specific combiners for the crosses, Sel-7 × WIR-3928, Sel-7 × EC-521080, DT-10 × EC-521080 and PBC × EC-520061, respectively. Thus, best combiner parents and crosses could be used for desirable segregates and in breeding programme for improving respective characters.

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Morphological characterization and floral biology of parental lines of pumpkin cv. Pusa Hybrid 1

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ABSTRACT

Morphological characterization and floral biology of parental lines of pumpkin cv. Pusa Hybrid 1 were studied during summer seasons of 2008 and 2009 at Seed Production Unit Farm, IARI, New Delhi. The parental lines were evaluated for 40 phenotypic characters (3 plant, 2 stem, 3 tendril, 7 leaf, 4 flower, 19 fruit and 2 seed), following NBPGR descriptor list at vegetative, flowering and fruit maturity stages. Parental lines showed considerable variation in early plant vigour, plant growth habit, petiole length, leaf size, leaf blotches and number of primary branches, node number at which first female flower appears, days to 50% flowering, sex ratio, fruit colour, fruit shape, blossom-end shape, ridges on fruit, rind thickness, fruit weight, length, breadth, flesh thickness and 100-seed weight. Flower anthesis began between 3.00 and 4.00 AM with peak reaching between 5.00 and 6.00 AM in both parental lines. The flower closure was initiated at 8.00 AM and finally closed between 11.00 and 12.00 noon in both the male and female flowers. The flowers remained open for 3 h 30 min in male and female parents. However, there was a period of overlapping in flower anthesis of both sexes, which coincides with the peak of pollen viability and pollinator activity. Pollen viability was about 97.64 - 98.55% in freshly-opened flowers but decreased to 74.73% after 48 h and crashed to 23.40% after 96 h of its storage under ambient conditions. Pollens-giains stored under refrigerated conditions (4° °C temperature and RH - 40%) maintained 63% viability after 96 h of storage. Stigma receptivity, measured by fruit set percentage, was noted from 12 h before anthesis, highest being at the time of anthesis.

Key Words: Morphological characterization, Floral biology, Time of anthesis, Pollen viability, Stigma receptivity, Pumpkin

Pumpkin occupies a prominent place among vegetables owing to its high productivity, nutritive value, good storability, long period of availability and better transport potential. It is extensively grown in rainy and summer seasons in all parts of India. Seed production of cucurbits including pumpkin in India is still dominated by locally available open-pollinated cultivars. Seventy percent area of gourds and pumpkin is covered by local cultivars (Dey *et al*. 2003).Hence, there is a great scope to improve the local cultivars through breeding and replace them with improved varieties and hybrids for that, morphological characterization of available genotypes is necessary. Successful hybridization programme requires knowledge about floral biology such as time of anthesis, time of anther dehiscence, pollen viability and duration of stigma receptivity. There is a need to standardize the techniques like suitable time of pollination, by keeping in mind the stigma receptivity and pollen viability to increase hybrid seed yield with better quality so that the cost of seed production can be reduced.

Varieties and hybrids gain acceptance only when farmers get genetically pure seed of high standards. For this purpose, each cultivar should be properly defined with suitable descriptors, so as to maintain its identity in seed production through field inspection and certification. Morphological characterization and floral biology of parental lines of pumpkin cv. Pusa Hybrid 1 have not been studied extensively in India although studies were reported on pumpkin in Nigeria (Agbagwa *et al*. 2007) and on other cucurbit species. Hence, present study was undertaken to characterize parental lines on the basis of qualitative and quantitative morphological characters and to study floral biology of parental lines for efficient and successful hybrid seed production in pumpkin.

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MATERIALS AND METHODS

The seed material of parental lines of Pusa Hybrid 1 were obtained from Division of Vegetable Science, IARI, New Delhi. The seeds were sown in plug trays (1½" cell size). The and 25 days old seedlings were transplanted at a spacing of 3.5m × 1m in field during summer of 2008 and 2009. The field experiment was laid out in a randomized block design with three replications. All the recommended cultural practices were followed. Qualitative and quantitative morphological characters listed by NBPGR, New Delhi, descriptors for pumpkin (Srivastava *et al*. 2001) were used for characterization. Plant morphological characteristics were recorded under field condition for all the characters at different stages of crop growth.

Parental lines were evaluated for a total of 40 phenotypic characters (3 plants, 2 stems, 3 tendrils, 7 leaves 4 fower, 19 fruits and 2 seeds) at vegetative, flowering and maturity stages. The vegetative characters of five randomly selected and tagged plants in each replication were used to differentiate the parental lines based on visual assessment. Fourth leaf from top of the main stem and fully expanded flowers were used in measurements. A minimum of five mature fruits from each parental line were used for recording the data.

Observations on floral biology of the two parental lines commenced at the beginning and continued until completion of flowering period. Anthesis of male and female flowers was monitored. The time of anthesis, duration of anthesis (difference in the time of initiation and termination of anthesis) in flowers of each parental line, pollen viability of pollen parent and stigma receptivity of seed parent were investigated.

For pollen viability, pollen-grains were collected from five randomly selected flowers of pollen parent at the time of anthesis and stored at ambient (30.8°C ± 3°C temperature, 61.8% ± 5% RH) and refrigerated conditions (4°C temperature, 40% RH). Pollen viability was tested at 0 h (immediately after collection), 24 h, 48 h and 72 h and 96 has suggested by Prakasha (1953) by placing pollen-grains on a slide and adding a drop of 1 per cent acetocarmine stain. After 5 minutes, pollen viability was recorded under 40 × magnification binocular microscope. Four random microscopic fields in each case were studied. Pollen-grains which took deep stain were considered viable, whereas flaccid, unstained, and irregular ones were taken as non-viable. Pollens-grains viability (%) was calculated as number of viable pollen-grains/total number of pollen-grans × 100 per each microscopic field.

The receptivity of stigmas was determined by pollinating pistillate flowers (at 12 h) before anthesis and at anthesis. The results were calculated on fruits set basis. The number of fruits set was recorded 7 days after pollination. The stigma receptivity was calculated as number of fruits set/number of flowers pollinated × 100 and expressed in percentage.

RESULTS AND DISCUSSION

Vegetative Characters

The seed parent is vigorous in growth habit with a long-running vine, which is moderately strong and covered with coarse pilose hairs. The pollen parent is a medium viny, good in early plant vigour. The number of primary branches that arise from the main vine had marginally higher values in seed parent (10.50) than pollen parent (9). They climb by means of several coiled proximally branched tendrils. The stem is moderately hard, hollowed, sharply or smoothly 5-angled, pilose on ribs but glabrous in grooves. The leaves are large in seed parent, whereas medium in size in pollen parent, cordate, shallowly 5-lobed, with white blotches in seed parent and without light yellow blotches in pollen parent and pilose (Table 1). The leaf petiole was longer in seed parent (18.65 cm) than in pollen parent (12.95 cm) (Table 3).

Table 1. Vegetative characters of parental lines of pumpkin cv. Pusa Hybrid

Character	Seed parent	Pollen parent
Early plant vigour`	Very good	Good
Plant growth habit	Long viny	Medium viny
Stem pubescence	Pubescence	Pubescence
Stem shape	Angular	Angular
Tendril	Present	Present
Tendril type	Coiled	Coiled
Tendril branching	Branched	Branched
Leaf margin	Multifid	Multifid
Leaf shape	Cordate	Cordate
Leaf size	Large	Medium
Leaf pubescence nature	Intermediate	Intermediate
Leaf pubescence density	Intermediate	Intermediate
Leaf blotches	Present	Absent

Floral Characters

Cucurbita moschata is monoecious with unisexual flowers. The male flowers, which are more numerous and appear earlier than the female ones, are borne on a pedunculate raceme or sometimes solitary on a very long peduncle. The racemes bear 10-15 flower buds, each with a tubular receptacle at the end of the pedicel. The seed parent took 61 - 65 days while the pollen parent took 56-60 days to reach 50% flowering. The

Table 2. Floral and fruit characters of parental lines of pumpkin cv. Pusa Hybrid 1

Character	Seed parent	Pollen parent
Sex type	Monoecious	Monoecious
Peduncle surface	Hard corky	Hard corky
Peduncle shape	Angular grooved	Angular grooved
Fruit shape	Elliptical	Flattened
Immature fruit skin colour	Light green	Green
Mature fruit skin colour	Yellowish	Yellowish
Fruit skin colour Pattern	Striped	Striped
Fruit skin colour intensity	Light	Intermediate
Fruit skin luster	Intermediate	Intermediate
Stem-end fruit shape	Depressed	Depressed
Blossom-end fruit shape	Pointed	Flattened
Skin hardness of the fruit	Soft	Intermediate
Fruit ridge (rib) shape	Superficial	Grooved
Mature flesh colour	Golden yellowish	Golden yellowish
Seed lustre	Intermediate	Intermediate

Table 3. Vegetative characters of parental lines of pumpkin cv. Pusa Hybrid

Character	Seed parent	Pollen parent
Number of primary branches	10.50	9.00
Petiole length (cm)	18.65	12.95
Node number at which first female flower appears	12.93	8.24
Days to 50% flowering	63.00	58.00
Sex ratio (female : male flowers)	1:10	1:9
Peduncle length (cm)	8.60	5.60
Number of ridges (ribs) per fruit	19.00	16.00
Flesh thickness(cm)	4.10	3.40
Fruit length (cm)	21.70	11.00
Fruit breadth (cm)	19.60	17.80
Fruit weight (kg)	3.45	1.77
100-seed weight (g)	7.10	6.80

first female flower starts to appear at higher nodes in seed parent (12.93) compared to pollen parent (8.24) and female flowers show variation in morphology where the ovary in seed parent is elliptical in shape and oval in pollen parent. The sex ratio (female : male flowers) was recorded 1: 10 in seed parent and 1: 8 in pollen parent (Table 3).

Fruit Characters

Weight of fruits differed from 3.44 kg in seed parent to 1.77 kgin pollen parent (Table 3). A distinct difference was observed for fruit shape. Round shape was the character of pollen parent, while seed parent had elliptic fruits (Table 2). Although there was arelatively low variation for ground colour of skin, Intensity of colour was quite varying with light in seed parent to intermediate in pollen parent. At marketable stage, the rind colour of fruits was light green in seed parent and green in pollen parent with striped fruit skin colour pattern. The fruits were intermediate in fruit skin lustre with depressed stem end fruit and pointed blossom-end in seed parent and flattened in pollen parent.

Fruit ridges were superficial in seed parent with 19 ridges and grooved in pollen parent with 16 ridges. At marketable stage, Fruit skin was soft and intermediate in seed parent and pollen parent respectively. But at maturity stage, Fruit skin colour turnes to golden yellow in both parental lines. The peduncle of fruit was hard corky, angular, grooved, measuring a length of 8.60 cm in seed parent and 5.60 cm in pollen parent. The colour of flesh was golden yellow in both parents. Thickness of flesh was more in seed parent, recording as 4.10 cm and 3.40 cm in pollen parent. The fruits were bigger in size in seed parents measuring 21.70 cm in length and 19.60 cm in breadth. The fruit length in pollen parent was 11.00 cm and breadth was 17.80 cm. The seed of female parent was marginally higher in 100 seed weight (7.1g) than male parent (6.8g) with intermediate seed luster (Table 3).

Floral Biology

Flowering in pollen-grains and seed parent began 42 - 45 days after planting (67-70 days after sowing) and 48 -50 days after planting (73-75 days after sowing) respectively with the male flowers emerging first and predominating in number throughout the flowering period followed by female flowers7 days later (Table 2). The total flowering period was 45 - 60 days and 30 - 45 days in pollen and seed parent respectively. In both male and female flowers, opening of flower commenced between 3.00 and 4.00 AM and continued up to 8.00 AM (Table 4). Flowers in both sexes opened by outward stretching of corolla and closed by wilting and spiral winding of apical part of corolla. Peak in flower anthesis (63.22 % and 65.42 %) was achieved

Seed parent Pollen parent

Fig. 1. Vegetative characters of parental line of Pusa Hybrid 1

Seed parent Pollen parent

Fig. 2. Floral characters of parental line of Pusa Hybrid 1

between 5.00 and 6.00 AM in both seed and pollen parental lines respectively. Flower closure in both sexes was initiated at about 8.00 AM and complete closure took place between 11.00 AM and 12.00 noon. The duration of flower anthesis was about 3 h 30 min and closure duration was about 3 - 4 h in both the male and female flowers.

Pollen Viability

The pooled data presented in Table 5 indicate that the pollen storage conditions influenced the viability of pollen grains. The pollen stored in refrigerator recorded higher viability over that in the ambient conditions. The pollen viability fell down rapidly under ambient conditions with increasing storage period of pollen grains. The pollen viability decreased from an initial value of 97.64 % at anthesis to 89.48% and 74.73 % at 24 h and 48 h respectively. As the storage period progressed, the viability crashed to 23.40% at 96 h after anthesis under ambient storage. On the other hand, the decline in pollen viability was gentle under refrigerated conditions. The initial pollen viability was 98.55 %

Seed parent Pollen parent

Fig. 3. Fruit characters of parental line of Pusa Hybrid 1

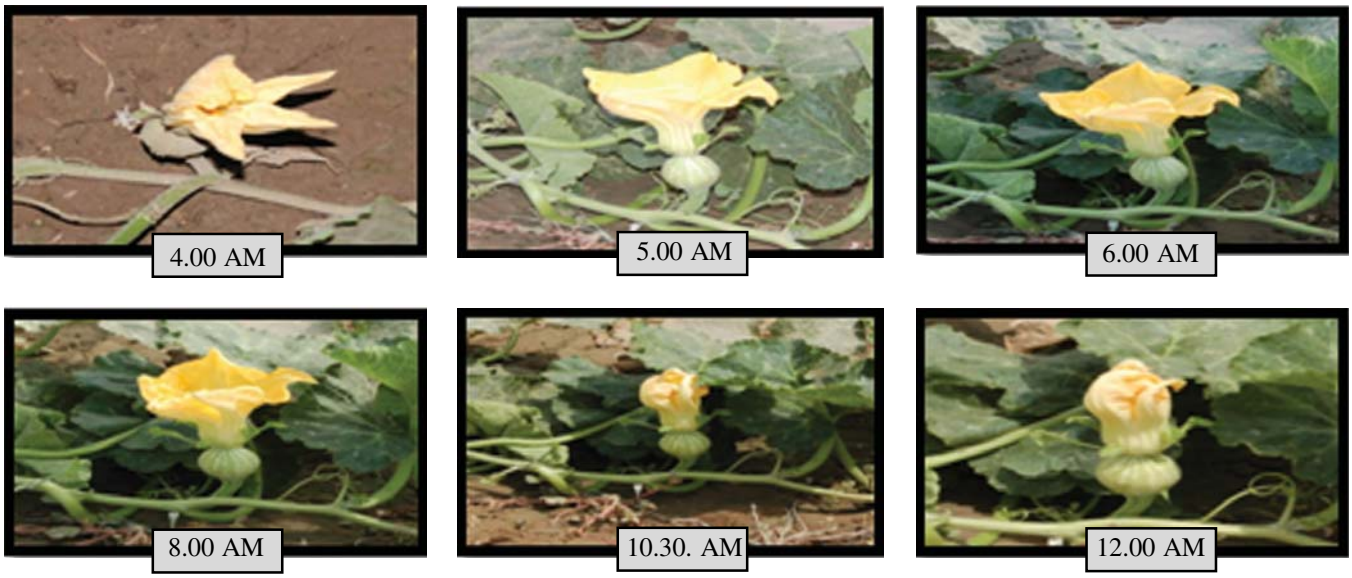

Fig. 4. Anthesis of female flower

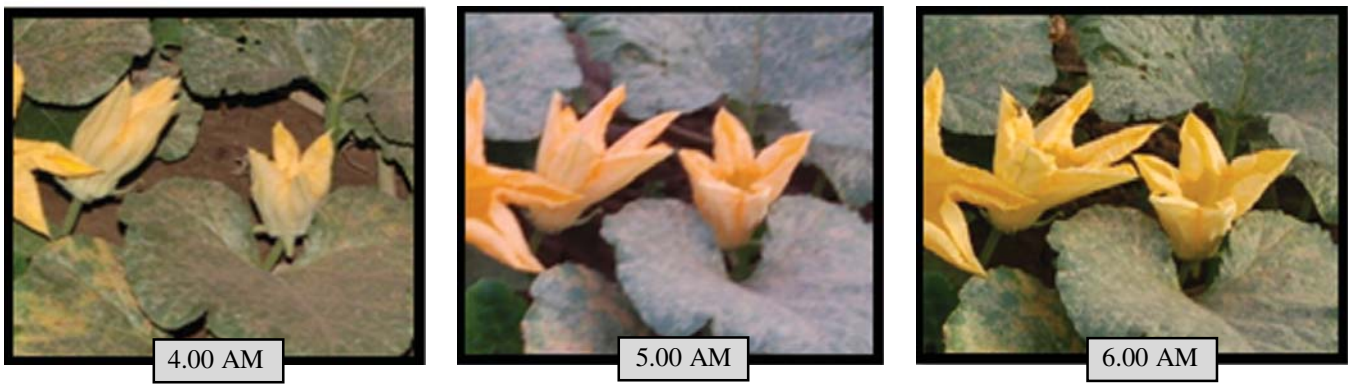

Fig. 5. Anthesis of male flower

Table 4. Time of anthesis in male and female flowers of pumpkin cv. Pusa Hybrid.

Date / time	Percentage of flower opened at different hours (AM)									
	3.00		4.00		5.00		6.00		7.00	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Mean	0.49	0.28	4.66	4.46	16.60	15.55	63.22	65.42	14.90	14.58
Sd±	0.38	0.35	0.68	1.23	2.74	2.05	4.43	2.17	2.53	3.24

Table 5. Pollen viability at different hours from anthesis in pollen parent of pumpkin cv. Pusa Hybrid-1.

Time (hr)	Pollen Viability (%)			
	Ambient@	Refrigerated #	Significance	Sd±
0	97.64	98.55	NS	1.52
24	89.48	94.97	NS	2.35
48	74.73	87.85	*	2.82
72	49.65	76.52	*	2.08
96	23.40	62.99	**	2.40

** significance at 1% level,
* significance at 5% level, NS - Non Significant
@Average temperature - 30.80 C and RH - 61.8 %,
Average temperature - 40 C and RH - 40 %

which has gradually fallen to 94.97 %, 87.85 %, 76.52 % and finally to 62.99 % on storage for 24, 48, 72 and 96 h respectively.

Stigma Receptivity

In the pooled data revealed that lower fruits set (%) was observed at 12 h before anthesis (9.16%), whereas pollination at anthesis resulted in relatively higher fruit set (50.60%). Thus, the stigma was being receptive 12 h before anthesis. (Table 6).

The parental lines of pumpkin cv. Pusa Hybrid - 1 showed variation in the morphological characters. Early plant vigour, plant growth habit, leaf size and number of primary branches were the important vegetative viability was gentle under refrigerated conditions. They might be due to genetic make up of parents and also due to soil, environmental, cultural and nutritional factors during crop growth. Considerable variation was observed for floral characters among parental lines and the differences showed by parental lines for floral characters are due to genetic background of the parental lines and also influenced by cultural and environmental conditions or genotype x environment interaction in the expression. The fruit characters viz., fruit colour (at immature stage), shape, blossom end shape, ridges on fruit, rind thickness, fruit weight, fruit length, fruit breadth, flesh thickness and 100 seed weight showed diversity between parental lines. The fruits of seed parent were larger in size than that of the pollen parent. The seed was bolder in female parent. Reasons attributed for such differences in fruit characters among parental lines are due to genetic background of parental lines.

Cucurbita moschata is predominantly monoecious, self-compatible with unisexual flowers. Flowering in this species began with the appearance of male flowers followed by female flowers 7 days later. However, male flower predominated throughout the experimental period with a ratio of 1:9 female to male flowers/ plant which is contrary to the findings of Nepi and Pacini (1993) in *C*. pepo, where early formation of male flowers was reported with female predominating afterwards. Although flowering in male and female flowers began at different times, peaks of flowering in both sexes almost overlapped, favouring female receptivity

Table 6. Stigma receptivity of seed parent in pumkin cv. Pusa Hybrid-1

	Fruit set (%)					
	2008		2009		Pooled	
	12 h before anthesis	At anthesis	12 h before anthesis	At anthesis	12 h before anthesis	At anthesis
Mean	9.82	56.90	8.50	44.30	9.16	50.60
Sd±	4.49	15.30	7.65	7.85	3.66	4.55

(Richards 1986 Nepi and Pacini 1993). Incomplete opening, withering or shrivelling of flowers was common in first week of flowering in both sexes. Fruit abortion was also common among flowers that developed at the early and later weeks of flowering. Tangmitcharoen and Owens (1997) suggested that pollination success might be more in flowers developing early and during the peak of flowering period than in those that develop at the end of flowering season.

The beginning of anthesis was marked by the opening of flowers, irrespective of type of pollination. The end of anthesis, on the other hand, occurs with the closing of the flower, ending pollen viability and stigma receptivity. Closing of flower in pumpkin occurs when pollen viability and stigma receptivity is decreasing and similar results were also reported by Nepi and Pacini (1993) in *C. pepo.* In *C. moschata*, there is a decrease in pollen viability beginning from 7.00 AM on the day of opening. It is noteworthy that appreciable overlap in anthesis occurs between the sexes.

Viability of pollen decreased rapidly at room temperature (30.8° C ± 3°C temperature and RH - 61.8 %). The viability of pollen is known to be inversely proportional with temperature and relative humidity. High humidity levels above 60% were found to be unfavourable for pollen viability (Ghatnekar and Kulkarni 1978) as it causes swelling and bursting of pollens with increased possibilities of fungal and bacterial infection (Vasil 1958). Extent of fungal and bacterial infection and catabolic processes were not studied in the present investigation but loss of viability at room temperature may be attributed to high catabolic processes (Linskens 1964, Ghatnekar and Kulkarni 1978). Low temperature below 10°C is known to keep the metabolic activities at minimum. Coupled with low humidity, the catabolic processes might have been very slow and thus the period of viability was enhanced under refrigerated conditions (4° C temperature and RH - 40%). The studies by Kerhoas *et al*. (1986) and Nepi&Pacini (1993) showed that pollen grains of *C. pepo* when exposed lose so much water that causes the decline in viability. This probably depends on the fact that, unlike most poollen, it does not dehydrate before anthesis and is therefore very vulnerable. This observation in *C. pepo* is similar to pumpkin, where pollen grains were highly viable at first, but progressively decreased from 97.64% to about 23.40% 96 h after anthesis at ambient conditions.

The higher fruit setting percentage in the flowers pollinated at anthesis than at 12 hours before anthesis indicates that stigma was receptive at 12 hours before anthesis. Nandpuri and Singh (1967) reported that stigma was receptive from 36 hr before anthesis and remained so till 60 hr after anthesis in bottle gourd. But maximum receptivity was reported on the day of anthesis. Similar findings were observed in pumpkin (Agbagwa *et al*. 2007), spine gourd (Vahab 1993, Kale *et al*., 2003, Dubey *et al*. 2007), pointed gourd (Sachan *et al* 1998), snake gourd (Hasanuzzaman *et al*, 2004).

Therefore, it was concluded that genetic purity of parental lines in seed multiplication as well as in hybrid seed production could be maintained by monitoring the production programme. This study on floral biology of parental lines could be utilized in hybrid seed production by pollinating the pistillate flowers at the time of peak anthesis, *i.e.* between 5.00 and 6.00 AM with either fresh pollen-grains or pollen-grains stored for 24 and 48 h in refrigerated conditions for better fruit setting, development and ultimate seed yield.

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Effect of foliar application of Ca, Zn, Fe and B on growth, yield and quality of papaya (*Carica papaya*) cv. Taiwan Red Lady

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ABSTRACT

The field experiment was conducted to find effect of foliar application of Ca, Zn, Fe and B on growth, yield and quality of papaya (*Carica papaya* L.) cv. Taiwan Red Lady at Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, during 2008-11 with eleven different treatments. The foliar application of calcium nitrate 1000 mg/ℓ + borax 30 mg/ℓ + zinc sulphate 200 mg/ℓ + ferrous sulphate 200 mg/ℓ (T_{10}) was followed 60, 90 and 120 days after planting. The plant height, stem girth as well as leaf area with total yield and quality parameters were studied. Borax 30 mg/ℓ was relieved earliness in initiation of flowering (days) of papaya cv. Taiwan Red Lady.

Key Words: Flowering, Foliar, Growth, Papaya, Quality, Yield

Papaya (*Carica papaya* Linn.) is wonder fruit crop of tropical world. It gives higher production of fruits and an income next to banana (Singh 1990). In Gujarat, it is cultivated in 15.3 thousand ha with a total production of 832.9 thousand tonnes and average productivity of 54.3 tonnes/ha (NHB 2010. Micronutrients Ca, Zn, Fe and B are not only essential but they are equally important like other micro and macronutrients. These micronutrients also help in uptake of major nutrients and play an active role in plant metabolic processes (Das 2003). Presently, farmers in Gujarat are much interested in cultivation of Taiwan Red Lady variety of papaya due to gynodioecious in nature and also ease and convenient raising. The production technology of papaya is known and farmers harvest higher fruit yield, but main problem lies in its nutrients deficiency which needs to be standardized. Proper growth and quality of papaya fruits depend largely on micronutrients, therefore effect of foliar application of Ca, Zn, Fe and B on papaya cv. Taiwan Red Lady was studied.

MATERIALS AND METHODS

The experiment was conducted at the Regional Horticultural Research Station, Navsari Agricultural University, Navsari, during 2008-2009 and 2009-2010. The seeds of papaya (cv. Taiwan Red Lady) were sown in small-sized plastic polythene bags and seedlings were raised with all possible care. Forty-five days old seedlings of uniform size and vigour were planted during last week of August in the pits of 30 cm × 30 cm × 30 cm size at a distance of 2 m in both directions. Soil application of fertilizers (200g N, 200g P and 250g K/ plant) was done in four equal splits at two months interval starting from transplanting of seedlings.

The experiment was laid out in a randomized block design with treatments replicated three times. The treatments were T_1, calcium nitrate 500 mg/*l*, T_2, calcium nitrate 1000 mg/*l*, T_3, borax 15 mg/*l*, T_4, borax 30 mg/*l*, T_5, zinc sulphate 100 mg/*l*, T_6, zinc sulphate 200 mg/*l*, T_7, ferrous sulphate 100 mg/*l*, T_8, ferrous sulphate 200 mg/*l*, T_9, calcium nitrate 500 mg/*l* + borax 15 mg/*l* + zinc sulphate 100 mg/*l* + ferrous sulphate 100 mg/*l*, T_{10}- calcium nitrate 1000 mg/*l* + borax 30 mg/*l* + zinc sulphate 200 mg/*l* + ferrous sulphate 200 mg/*l* and T_{11}, the control (water spray). The application or spray was done 60, 90 and 120 days after planting. The data were recorded on different growth characters, viz. plant growth and stem girth (90, 120 and 150 DAP) and leaf area at one day and 15 days before and after first and third spray. In yield attributes and quality parameters, the data were recorded at ripening stage

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during both the years. Sugar and acidity contents of fruits were estimated as per the A O A C (1997). The data of both individual years and pooled over two years were analyzed separately adopting analysis of variance method as suggested by Panse and Shukhatme (1967). The data were pooled.

RESULTS AND DISCUSSION

Plant height and stem girth (Table 1) are considered to be important factors to judge the vigour in papaya crop. The robust vegetative growth is an essential prerequisite for higher yield. The total leaf area was almost identical in all the treatments, except T_{11}, where the effect has been due to size of individual leaf. Therefore, treatment favorable affecting vegetative growth showed higher leaf area. The foliar spray of calcium nitrate 1000mg/*l* + borax 30mg/*l* + zinc sulphate 200mg/*l* + ferrous sulphate 200mg/*l*, *i.e.* T_{10}, resulted in maximum plant height, stem girth as well as leaf area. Results pertaining to above growth attributes could be due to improved photosynthetic activity and respiration of plants as influenced by Ca, Zn, Fe and B.

Through addition of calcium (Ca), cell wall strength and thickness increased. Calcium is a critical part of cell-wall that produces strong structural rigidity by forming cross-links within pectin polysaccharide matrix. With rapid plant growth, structural integrity of stems that hold flowers and fruits as well as the quality of fruits produced, is strongly coupled to calcium availability (Easterwood, 2005). The effect of zinc sulphate on enhancing the vegetative growth may be ascribed to presence of zinc in structure of tryptophan which is the precursor of auxin. Thus, combination of boron and zinc increased metabolic activities which lead to increased plant metabolites responsible for cell division, cell elongation and plant growth. Fe is also necessary for vital plant metabolic function such as chlorophyll synthesis, various enzymatic reactions, respiration and photosynthesis. Boron regulates metabolism involved in translocation of carbohydrates, cell-wall development and RNA synthesis.

These findings are in conformity with above mentioned growth parameters have also been reported by Veena and Lavania (1998) and Singh *et al*. (2010) in papaya. On the contrary, initiation of flowering (days) (Table 1) was utmost advanced (required minimum days) under the treatment receiving borax @ 30mg/*l* (T_4-93.40 days). Second best treatment T_6 (zinc sulphate @ 200 mg/*l*) also take minimum days (97.60 days) for flowering in papaya. The earliness in flowering might be due to boron regulates metabolism involved in translocation of carbohydrates, cell-wall development and RNA synthesis and it also increased the phenolic compounds which regulate polar auxin transport (Ram and Bose 2000). These results are in conformity with the findings of Veena and Lavania, 1998), Singh *et al*. (2010) and Modi (2010) in papaya.

The data revealed that the increase in size (diameter and length) of fruit with maximum yield obtained when papaya plants were sprayed with calcium nitrate 1000mg/*l* + borax 30mg/*l* + zinc sulphate + 200mg/*l* + ferrous sulphate 200mg/*l* (T_{10}) (Table 2). Size of fruits and yield of plant are cumulative effect of various attributes as affected by micronutrients through higher rate of cell division and enlargement, photosynthesis and increase in enzymatic activities as well as involvement of zinc in biosynthesis of auxin.

The foliar spraying of calcium nitrate 1000mg/*l* + borax 30mg/*l* + zinc sulphate + 200mg/*l* + ferrous sulphate 200mg/*l* thrice at one month interval from 60 DAP, significantly increased the number of fruits/plant, resulting in higher yield. Further, boron application might have enhanced the translocation of metabolism from source (leaf) to sink (fruit) and increased accumulation of dry matter within the fruits, resulting into higher yield. Zinc, regulating the semi-permeability of cell walls, thus mobilizing more water into fruits resulted in increased in fruit size. Similar findings were also observed by Veena and Lavania (1998), Kavitha *et al*. (2000) and Singh *et al*. (2010) in papaya.

The physiological loss in weight (PLW) reduced in foliar application of calcium nitrate 1000mg/*l* + borax 30mg/*l* + zinc sulphate 200mg/*l* + ferrous sulphate 200mg/*l* (T_{10}) which helped in higher firmness of fruits (Table 3). Similar findings were noted by Veena and Lavania 1998 and Kavitha *et al*. (2000a). Moreover, higher fruit firmness which resulted in increased shelf life, maximum sugar percentage and ascorbic acid with minimum titrable acidity were significantly influenced by combine foliar application of calcium nitrate 1000mg/*l* + borax 30mg/*l* + zinc sulphate 200mg/*l* + ferrous sulphate 200mg/*l* (T_{10}) on papaya cv. Taiwan Red Lady.

It might be due to adequate amount of zinc improving auxin content and it also acts as a catalyst in oxidation-reduction processes in plants. Besides, it also helped in other enzymatic reactions like transformation of carbohydrates, activity of hexokinase and formation of cellulose, and change in sugar are considered due to its action on zymohexose. The reduction of titrable acidity of papaya fruits through application of different levels of calcium, zinc, ferrous, boron and their different combinations might be due to positive effect of boron and zinc in conversion of acids into sugars and their derivatives by reaction involving glycolytic path way or be used in respiration of both (Singh *et al*., 2010). Kavitha *et al*. (2000) also reported the increase of ascorbic

Table 1. Effect of Ca, Zn, Fe and B on growth attributes of papaya cv. Taiwan Red Lady (pooled data)

Treatment	Plant height (cm)			Stem girth (cm)			Leaf area (cm^2)			Initiation	
	90 DAP	120 DAP	150 DAP	90 DAP	120 DAP	150 DAP	One day before first spray	Fifteen days after first spray	One day before third third	Fifteen days after third spray	of flowering (days)
T_1	79.71	94.84	102.84	18.80	26.02	31.77	430.10	595.25	887.44	987.01	123.10
T_2	82.16	96.70	104.68	19.12	26.56	32.90	464.77	659.14	929.37	1061.12	121.10
T_3	84.14	102.37	111.51	20.51	29.23	34.80	420.59	627.54	898.23	1014.56	100.25
T_4	85.31	106.55	114.10	21.31	29.84	35.36	529.15	719.68	1018.50	1200.99	93.40
T_5	84.55	104.33	112.12	21.05	29.56	35.03	448.60	641.27	913.17	1040.14	110.80
T_6	92.56	117.70	132.41	22.74	31.88	37.96	498.27	697.30	976.03	1156.88	97.60
T_7	82.83	99.74	108.11	19.24	27.43	33.81	482.71	674.44	930.16	1099.57	118.50
T_8	83.24	100.68	109.50	19.57	27.72	34.53	572.31	678.00	949.55	1117.50	113.40
T_9 -T_1 + T_3 + T_5 +T_7	90.73	112.23	121.94	21.98	30.43	36.50	476.99	666.03	921.80	1089.53	107.90
T_{10} -T_2 + T_4 + T_6 +T_8	98.58	123.40	136.20	23.85	33.18	39.46	491.95	785.95	1090.89	1248.10	103.40
T_{11}	68.40	82.46	94.41	18.64	23.02	29.05	438.38	580.78	873.09	971.31	128.40
S Em ±	2.74	3.60	4.36	0.56	0.76	0.92	19.16	23.06	25.53	31.77	1.44
C D at 5 (%)	7.81	10.23	12.38	1.59	2.16	2.61	NS	65.56	72.57	90.32	8.59
C V (%)	8.65	9.47	10.48	7.42	7.19	7.22	10.98	9.48	7.40	7.99	7.47

Table 2. Effect of Ca, Zn, Fe and B on yield parameters of papaya cv. Taiwan Red Lady (pooled data)

Treatment	Number of fruits/plant	Average weight of fruit (g)	Fruit yield/plant (kg)	Fruit yield (tonnes/ha)	Fruit diameter (cm)	Fruit length (cm)
T_1	19.32	1.07	20.73	51.83	18.51	20.60
T_2	20.17	1.09	22.15	55.38	19.06	20.77
T_3	21.27	1.19	25.37	63.44	19.66	21.56
T_4	22.36	1.29	28.91	72.28	20.61	22.93
T_5	21.75	1.22	26.59	66.48	19.82	21.95
T_6	22.99	1.32	30.57	76.42	21.09	23.64
T_7	20.03	1.11	22.44	56.10	20.17	22.54
T_8	21.14	1.15	24.39	60.97	19.46	21.36
T_9 -T_1 + T_3 + T_5 +T_7	21.81	1.26	27.65	69.14	19.95	22.29
T_{10} -T_2 + T_4+ T_6 +T_8	23.45	1.37	32.31	80.76	21.86	25.39
T_{11}	18.89	1.03	19.63	49.07	17.30	19.12
S Em.±	0.66	0.04	1.41	3.55	0.50	0.65
C D 5 (%)	1.87	0.10	4.03	10.08	1.42	1.86
C V (%)	8.49	8.53	15.19	15.18	6.90	8.04

Table 3. Effect of Ca, Zn, Fe and B on quality of papaya cv. Taiwan Red Lady (pooled data).

Treatment	PLW (%)	Fruit firmness (kg/cm^2)	Shelf-lif (days)	Total sugar (%)	Reducing sugar (%)	Titrable acidity (%)	Ascorbic acid (mg/100g)
T_1	19.31	5.67	5.12	6.12	4.26	0.035	20.19
T_2	15.56	6.76	6.06	6.30	4.77	0.021	21.04
T_3	17.77	6.56	5.86	6.66	4.57	0.027	20.41
T_4	11.75	6.98	6.71	6.83	5.01	0.015	22.00
T_5	18.20	5.95	5.67	6.91	4.48	0.031	20.28
T_6	14.97	6.86	6.25	7.10	4.85	0.020	21.72
T_7	18.80	5.88	5.40	6.46	4.31	0.032	20.22
T_8	13.83	6.86	6.41	6.65	4.89	0.017	21.77
T_9 -T_1 + T_3 + T_5 + T7	16.01	6.62	5.96	7.00	4.74	0.027	20.53
T_{10} - T_2 + T_4 + T_6 + T_8	10.63	7.17	6.95	7.49	5.27	0.014	23.23
T_{11}	20.37	4.02	3.86	5.69	4.07	0.038	19.89
S Em ±	0.59	0.19	0.18	0.17	0.13	0.0013	0.42
C D (5 %)	1.67	0.53	0.50	0.48	0.38	0.0036	1.21
C V (%)	9.64	8.05	8.18	6.83	7.95	13.40	5.52

acid content after application of zinc, boron and ferrous which might be due to conversion of sugars into ascorbic acid in papaya.

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Standardization of forcing schedule in cultivars of narcissus *(Narcissus pseudonarcissus)* under midhill conditions of Himachal Pradesh

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ABSTRACT

An experiment was conducted to standardize the forcing scedule for cultivars of narcissus (*Narcissus pseudonarcissus* L.) at the Department of Floriculture and Landscaping, University of Horticulture and Foresty, Nauni, during 2010-11. There was a wide range in diversity in flower shape, size, colour and flowering duration in different cultivass. The forcing schedule for cultivars was followed in the field of the experimental farm. Generally, programmed bulbs sprouted earlier (24.40 days) than the non-programmed ones (43.60 days). Among cultivars, Scilly White was earliest (24.77 days) to sprout, whereas Carlton' took maximum time (43.67 days) for sprouting. Flowering in programmed bulbs occurred earlier (46.85 days) compared to non-programmed ones (61.63 days). Scilly White was earliest to flower (33.17 days), whereas Ice Follis took maximum number of days (78.33 days) to flower.

Key Words: Forcing schedule, Cultivars, Midhill conditions, Flower shape, Size, Flowering duration

Narcissus (*Narcissus pseudonarcissus* L.) is the most admired garden plant in the world. It is considered one of the heralds of spring, planted in autumn season and burst up in spring season. It is also known as 'queen of spring flowers'. These are grown in beds and borders, rock gardens, in grass and woodlands, outdoor mass plantation in formal and informal locations, lawns and wild gardens and in pots. They are also excellent cut flowers when used alone. Narcissus and daffodils belong to family Amaryllidaceae. These are originated from Northern Hemisphere, i.e. Europe, especially Spain and Portugal, France, Switzerland, former Yugolslavia and North Africa with some exceptions of bunch flowered (*N. tazetta* L.) narcissi which are found across Iran, China and Japan. In the present investigations, efforts were made to standardize forcing schedule(s) of four narcissus cultivars growing under midhill conditions of Himachal Pradesh.

MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Department of Floriculture and Landscaping, College of Horticulture, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, during 2010-11. Located at 1276 m above mean sea-level at a latitude of 30°52'N and longitude of 77°11'33"E, the climate of this area is typically semi-temperate. Bulbs of cultivars, Scilly White, Nauni Local, Ice Follies and Carlton were harvested on 29 May 2010 and kept at prevailing temperature for 10 days. Thereafter, one half of the bulbs was subjected the temperature sequence given below (programmed) and other half stored at prevailing conditions till planting (un-programmed). The bulbs were kept in BOD at 17°C on 7 June 2010. These bulbs were shifted to cocopeat in shallow trays for rooting on 7 September 2010. Thereafter, rooted bulbs and those stored at prevailing conditions were planted under polyhouse conditions on 7 October 2010.

The field used for experimentation was ploughed to a depth of 30-50 cm to get a fine tilth and it was that area where sunlight remained for the whole day. A basal dose of fertilizers and manure comprising 5 kg FYM, 25 g N, 62.5 g P_2O_5 and 62.5 g K_2O/m^2 were incorporated in the soil. Half dose of CAN was applied at the time of planting and the remaining half 40 days after planting. The nitrogen was supplied in the form of calcium ammonium nitrate, P_2O_5 in the form of

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single superphosphate and K_2O as muriate of potash at the time of field preparation, respectively. The bulbs of were treated with Dithane M-45(0.2%) and Bavistin (0.1%) before planting. Bulb were planted one-and-a-half times the size of bulbs into the soil.

Irrigation after planting and mulching was done with the help of dry grass. Mulching was tremendously helpful for narcissus to conserve moisture. Planting distance of bulb-to-bulb was 10 cm and row-to-row-was 20 cm. The experiment was laid out under polyhouse during October 2010 in a randomized block design replicated thrice. The field was irrigated depending upon requirement of plants because moisture is required in all the stages of growth. In addition, NPK(19:19:19) @ 1 ml/liter at weekly intervals before bud formation and multi-K @ 1 ml/litre during bud formation was also applied at fortnightly intervals.

RESULTS AND DISCUSSIONS

The growth cycle of narcissus is dependent upon temperature and moisture cycles. It emerges quickly in the spring, flowers and survives for lengthy period in hot summers in vegetative stage. The *N. pseudonarcissus* and *N. cyclamineus* require an absolute cold treatment for further floral differentiation, development and rapid emergence, whears *N. tazetta* does not require cold. All these three species require warm temperature for floral initiation and differentiation which occur before harvesting and continue afterwards.

At harvesting, narcissus has an almost completely formed flower (Hartsema 1961), the flower being initiated in May shortly after the mother bulb flowers. The bulbs require warm and cool-warm temperature sequence for growth and development (Rees 1972). When bulbs are lifted from the field, they have partially formed flower parts. Hence, while the initial warm temperature helps to complete flower development, the subsequent low temperatures are required for stalk and leaf elongation and warm temperature for flowering (De Hertogh 1974). The data indicated that in general, programmed bulbs sprouted earlier (24.40 days) than non-programmed ones (43.60 days).

Among cultivars, Scilly White was earliest (24.77 days) to sprout, whereas Carlton took maximum time (43.67 days) for sprouting. Interaction between cultivars and treatments revealed earliest sprouting of programmed bulbs of Scilly White (15.33 days). In contrast to this non-programmed bulbs of Carlton took maximum time (52.53 days) for sprouting (Table 1). Further, all cultivars showed earlier sprouting of programmed bulbs over non-programmed ones.

The data recorded on appearance of 'goose neck stage' showed that it appeared earlier (45.35 days) in programmed bulbs than non-programmed (61.40 days) (Table 2). Among cultivars, Scilly White exhibited earliest (31.87 days) appearance of goose neck stage, whereas Ice Follis was the last (77.10 days) to reach at this stage.

Interaction between cultivars and treatments revealed earliest appearance of goose neck stage in programmed bulbs of Scilly White (21.93 days). This stage was observed after maximum days of planting of non- programmed bulbs of Ice Follis.

Table 1. Effect of programming on number of days taken to sprout by different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	34.80	52.53	43.67
Scilly White	15.33	34.20	24.77
Nauni Local	19.40	41.47	30.43
Ice Follis	28.07	46.20	37.13
Mean	24.40	43.60	

$CD_{0.05}$ for: Programming treatments : 0.97
Cultivars : 1.38
Cultivars × treatment : 1.94

Table 2. Effect of programming on days taken to goose neck stage by different cultivars of narcissus (days)

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	63.00	74.20	68.60
Scilly White	21.93	41.80	31.87
Nauni Local	23.67	48.20	35.93
Ice Follies	72.80	81.40	77.10
Mean	45.35	61.40	-

$CD_{0.05}$ Programming treatments = 0.29
Cultivars = 0.41
Cultivar × treatments = 0.58

Table 3. Effect of programming on number of flowers/ scape by different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	1.00	1.00	1.00
Scilly White	7.20	6.80	7.00
Nauni Local	5.80	3.80	4.80
Ice Follies	1.20	1.20	1.20
Mean	3.80	3.20	--

$CD_{0.05}$ Programming treatments =0.11
Cultivars =0.15
Cultivar × programming treatments = 0.21

In general, number of flowers per scape were observed more in programmed bulbs (3.80) than non-programmed ones (3.20) (Table 3). Cultivar Scilly White showed maximum flowers on (7.00) the scape whereas, Carlton showed the minimum. (1.00) Interaction between cultivars and treatments also revealed maximum flowers per scape in Scilly White (7.20) programmed bulbs. Further, minimum flowers per scape (1.00) was found to be at par with Ice Follies (1.20) irrespective of the programming treatment.

As far as duration of flowering is concerned, it was found more in programmed bulbs (12.30 days) than non-programmed bulbs (10.35 days) (Table 4). Among cultivars, Scilly White recorded maximum (13.50 days) duration of flowering as compared to minimum (10.13 days) in Nauni local Interaction data also revealed maximum duration of flowering of programmed bulbs of Scilly White (14.60 days). Minimum duration of flowering (9.60 days) of Carlton and Ice Follies non-programmed bulbs was found to be at par with 'Nauni Local' in the same treatment.

Table 4. Effect of programming on duration of flowering (days) by different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	11.73	9.60	10.67
Scilly White	14.60	12.40	13.50
Nauni Local	10.47	9.80	10.13
Ice Follies	12.40	9.60	11.00
Mean	12.30	10.35	

$CD_{0.05}$	Programming treatments	= 0.12
	Cultivars	= 0.17
	Cultivar × treatments	= 0.23

Table 5. Effect of programming treatments on number of days to flowering on different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	65.60	76.00	70.80
Scilly White	23.20	43.13	33.17
Nauni Local	24.20	45.13	34.67
Ice Follies	74.40	82.27	78.33
Mean	46.85	61.63	--

$CD_{0.05}$	Programming treatments	= 0.15
	Cultivars	= 0.22
	Cultivars and programming treatments	= 0.30

The data shows that flowering in programmed bulbs occurred earlier (46.85 days) as compared to non-programmed ones (61.63 days). (Table 5). Scilly White was earliest (33.17 days) to flower whereas Ice Follis took maximum number (78.33days). of days to flower. Interaction between cultivars and programming treatments revealed that programmed bulbs of Scilly White (23.20 days) took minimum number of days to flower. In contrast non-programmed bulbs of Ice Follis took maximum number of days to flower (82.27 days).

The scape length was shorter in (27.65 cm) programmed bulbs as compared to to non-programmed bulbs (27.92 cm) Nauni Local (30.30 cm) was found to be at par with Scilly White (29.80 cm) (Table-6). In contrast, minimum scape length was observed in Ice Follis, and Carlton (25.60 cm). Interaction between cultivars and programming treatments depicts that maximum scape length of 33.60 cm was noticed in Nauni Local which was found to be at par with Scilly White (30.60 cm). However, minimum scape length was observed in programmed bulbs of Ice follis (22.20 cm) which was found to be at par with Carlton (24.20 cm).

Table 6. Effect of programming treatments on scape length (cm) on different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	65.60	76.00	70.80
Scilly White	23.20	43.13	33.17
Nauni Local	24.20	45.13	34.67
Ice Follies	74.40	82.27	78.33
Mean	46.85	61.63	--

$CD_{0.05}$	Programming treatments	= 2.17
	Cultivars	= 3.07
	Cultivars × programming treatments	= 4.34

Table 7. Effect of programming treatments on flower size (cm) on different cultivars of narcissus

Cultivar	Programmed bulbs	Non-programmed bulbs	Mean
Carlton	6.48	6.76	6.62
Scilly White	4.40	4.10	4.25
Nauni Local	3.40	3.14	3.27
Ice Follies	9.10	8.76	8.93
Mean	5.84	5.69	

$CD_{0.05}$	Programming treatments	= 0.36
	Cultivars	= 0.05
	Cultivars × programming treatments	= 0.71

There was maximum flower size in programmed bulbs as compared to non-programmed ons (5.69 cm). (Table 7). Maximum flower size (8.93 cm), was observed in Ice Follies, whereas minimum (3.27 cm). in case of cv. Nauni Local Interaction between cultivars and programming treatments depicts that maximum flower size was observed in Ice Follis (9.10 cm) when bulbs were programmed which was found to be at par with the same cultivar when bulbs were not programmed (8.76 cm). Minimum flower size (3.40 cm) was noticed in Nauni Local

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Performance of aonla *(Emblica officinalis)* cultivars for growth, yield and quality in semi-arid condition

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ABSTRACT

An experiment was conducted to find out the performance of different commercial varieties, viz. Krishna, Kanchan Chakaiya, NA-6, NA-7, NA-10 and Anand-1 of aonla (*Emblica officinalis* Gaertn) under semi-arid condition during 2005 and 2006 at KVK, Ujwa, New Delhi. Observations on growth parameters were taken during June, whereas fruit characters were recorded at the time of harvesting in December. The plant height was maximum (3.46m) in NA-7 and minimum (2.1m) in Anand 1. Canopy spread of the tree in East-West and North South directions were 2.75-4.0 and 4.20-3.0m, respectively. The maximum plant girth (125 cm) was recorded in NA 6. Length of determinate shoot varied from 7.6 to 16.6cm and the range of leaf size was 1.3 to 1.8 cm in different varieties. Maximum fruit size (3.92 cm × 4.60 cm) was observed in Krishna and minimum (3.31 cm × 3.80 cm) in Kanchan. However, fruit weight varied from 30.16 to 48.3g depending on cultivar. It was maximum (48.3g) in Krishna and minimum (30.16g) in Kanchan. Total pulp weight was maximum (45.6g) in Krishna and minimum (28.77g) in Kanchan. The size of stone was maximum (2.45 ×1.48 cm) in Krishna. There were significant variation in stone weight and different cultivars, which varied from 1.39 to 2.34 g. Besides, there were significant variation in yield, vitamin C, juice content, TSS, dry-matter content and acidity in different cultivars. However, overall performance of NA 7, Chakaiya, NA 6 and Krishna were promising for cultivation in hot arid ecosystem.

Key Words: Aonla, Cultivars, Growth, Yield, Quality, Semi-arid condition, Fruit character, Plant ginth, Fruit size

Aonla or Indian gooseberry (*Emblica officinalis* Gaeitn), a member of family Euphorbiaceae, is originated in Tropical South-East Asia, particularly South India (Ferminger 1947; MonTon 1960). In India, it is widely grown in Uttar Pradesh, Gujarat, Rajasthan, Madhya Pradesh and Tamil Nadu. It bears two types of shoots. On the basis of growth characteristics; they are categorized as long or indeterminate and short or determinate (Bajpai, 1965). The new determinate shoots emerged out during first week of April (Bajpai 1965, Ram 1971), while flowering takes place in axil of leaves. However, there are two prominent cropping seasons in South India, i.e. July–August and April May (Naik 1963). The fertilized ovary of aonla remains dormant for three-and-a-half months and resume growth in August after the onset of mansoon. The fruits mature during November-December under hot arid ecosystem of Rajasthan (Shukla *et al.* 2004).

Aonla has gained momentum under hot arid region because of its hardy nature, prolific bearing, potential fruit crop and capacity to grow under various adversities (Shukla *et al.* 2002). After development of new cultivars, viz Krishna, Kanchan NA 6, NA 7, NA 10, Anand 1 and Anand 2, its crop emerged as remunerative fruit crop in India (Dhandar and Shukla 2003). Since all the cultivars of aonla did not performing equally well under the hot arid ecosystem, the yield and quality attributes due to extremes of high temperature and high wind velocity are not food for fruit setting. Keeping in view, an experiment was conducted to assess the performance of different varieties and to select varieties for cultivation under hot arid ecosystem.

MATERIALS AND METHODS

The experiment was conducted during 2005 and 2006 to evaluate the performance of commercial varieties, viz. Krishna, Kanchan, Chakaiya, NA 6, NA 7, NA 10 and Anand 1. Planting was done at a distance of 8 m × 8 m during 1996 and uniform cultural practices were followed for all cultivates. The data on growth parameter were taken during June, whereas on fruit

characters it was recorded at the time of harvesting. Height was measured with the help of ranging rod in metre. Data presented are mean value of two years. The experiment was performed in a randomized block design with three replications using M-stat package. The mature fruits were taken randomly from all direction of plants from each variety and data were recorded for fruit characters. Size of fruits and stone were measured with the help of Vernier callipere. The TSS was determined with the help of hand refactometer. Titrable acidity was estimated against N/10 NaOH and vitamin C was estimated through standard dye solution as per Rangana (1986).

RESULTS AND DISCUSSION

There was maximum plant height (3.45m) in NA 7, followed by NA-6 (3.21m), NA-10 (3.11m), Krishna (3.10m) and Anand 1 (2.1m) (Table 1). Canopy spread in East West direction varied from 2.75 to 4.0m. The maximum spread in East West direction was noted in NA 7 (4.0m), followed by 3.25m in NA 6, 3.20m in NA 10 and minimum being in Anand 1 (2.75m). Canopy spread in North South direction was also maximum in NA 7 (4.20m), followed by Kanchan (3.5m), NA 6 (4.00m), NA 10 (3.20m), Anand 1 (3.10m), whereas it was minimum in Krishna (3.0m). The maximum girth (125 cm) was recorded in NA 6, followed by Krishna and NA 7 (112.5 cm), and minimum being in Chakaiya (80 cm). Length of determinate shoot varied from 7.6 to 16.6cm in different varieties (Table 1). The maximum length (16.6 cm) was observed in NA 6, followed by NA 7 (15.9 cm), NA 10 (12.3 cm) and Anand 1 (10.5 cm), and minimum in Kanchan (7.6 cm). Leaf size varied from 1.3 to 1.8 cm depending on cultivars with maximum (1.8 cm) in NA 6, followed by NA 7 (1.7 cm), Chakaiya (1.6cm), and NA 10 and Kanchan (1.3 cm).

Internodal length in determinate shoots varied from 0.31 to 0.81 cm. The maximum intermodal length (0.81cm) was observed in NA 6, followed by NA 7 (0.46 cm), NA 10 (0.42 cm) and Krishna (0.31 cm). Variation of plant growth characters in different cultivars is genetic feature of individual variety. Fruit size is an important component of yield. The maximum fruit length (3.92 cm) was in Krishna, followed by NA 6 (3.83 cm), Chakaiya (3.65 cm) and Kanchan (3.51 cm). The maximum fruit diameter (4.6cm) was observed in Krishna, followed by NA 6 (4.4 cm), Chakaiya (4.1 cm), NA 7 (4.0 cm), NA 10 and Anand 1 (3.8 cm). Fruit weight varied from 30.16 to 48.3g depending on cultivars. The maximum fruit weight was reported in Krishna (48.3 g), followed by NA 6 (40.8 g), NA 7 (36.52 g), Chakaiya (33.4 g) and Kanchan (30.16 g). Total pulp weight was found maximum in Krishna (45.96 g), followed by NA 6 (39.09 g), NA 7 (34.93 g) and Kanchan (28.77 g).

The pulp content varied from 94.15 to 95.89% depending on varieties. Size of stone with respect to length was found maximum in Krishna (2.45 cm), followed by NA 7 (2.01 cm), Chakaiya (1.96 cm) and minimum being in NA 10 (1.48 cm). Stone diameter was found maximum in NA 7 (2.15 cm), and minimum in NA 10 (1.39 cm). There was sufficient variation in stone weight in different cultivars which varied from 1.39 to 2.34 g, maximum being in Krishna (2.34 g) and minimum in NA 10 (1.39 g).The seed weight in aonla was found maximum (0.036 g) in Krishna, whereas minimum being in NA 7 (0.028g). Pulp : stone ratio was recorded maximum in Chakaiya (22.88) and minimum in Kanchan (19.03).The stone content (%) varied from 4.11 to 4.99% with maximum in Kanchan (4.99%) and minimum (4.11 %) in Chakaiya.

The fruit yield per tree was recorded maximum (105 kg) in NA 7. This may be due to more number of fruits per shoot. Pathak and Pathak (1983) also

Table 1. Mean vegetative growth performance of different anonla cultivars.

Cultivar	Length of Determinate Shoot (cm)	Leaf size (cm)	Inernodal length of determinate (cm)	Plant height (m)	Plant spread (m)		Plant diameter (inch)
					NS	EW	
Chakaiya	9.10 (6-13)	1.6	0.35	2.50	3.00	3.00	80.0
Krishna	8.4 (5-12)	1.5	0.31	3.10	2.80	3.00	112.5
Kanchan	7.6 (5-11)	1.3	0.38	2.91	3.00	3.50	90.0
NA-6	16.6 (7-30)	1.8	0.81	3.21	3.25	4.00	125.0
NA-7	15.9 (6-28)	1.7	0.46	3.45	4.00	4.20	112.5
NA-10	12.3 (4-16)	1.3	0.42	3.11	3.20	3.20	105.0
Anand-1	10.5 (4-13)	1.4	0.38	2.10	2.75	3.10	82.5
CD at 5%	2.37	0.43	0.08	0.27	1.27	0.32	13.76

Note : Data given in parenthesis is range value.

Table 2. Yield and yield-contributing attributes in different aonla cultivars

Cultivar	Fruit size (cm)		Fruit weight (g)	Pulp weight (g)	Pulp content (%)	Stone weight (g)	Stone size		Seed weight (g)	Pulp: stone ratio	Stone content (%)	Yield kg/ tree
	L	D					L	D				
Chakaiya	3.65	4.10	33.4	32.00	95.89	1.40	1.96	1.72	0.034	22.88	4.11	52
Krishna	3.7	4.60	48.3	45.96	95.15	2.34	2.45	1.92	0.036	19.65	4.85	45
Kanchan	3.6	3..95	31.25	29.69	95.01	1.56	1.82	1.68	0.031	19.03	4.99	35
NA 6	3.6	4.40	40.8	39.09	95.8	1.71	1.80	1.62	0.034	22.86	4.20	67
NA 7	3.5	4.00	36.52	34.93	95.64	1.59	2.01	2.15	0.028	21.98	4.36	105
NA 10	3.5	3.8	30.16	28.77	95.39	1.39	1.48	1.39	0.029	20.73	4.61	31
Anand 1	3.5	3.8	31.35	29.83	95.15	1.52	1.52	1.42	0.031	19.61	4.85	25
CD at 5%	0.97	1.06	4.35	5.29	0.58	0.48	0.57	0.51	0.014	3.25	1.81	12.12

Table 3. Quality attributes of different aonla cultivars

Cultivar	Juice content	Dry matter	TSS	Acidity	Vitamin C	TSS/acid
Chakaiya	73.65	16.4	18.00	2.04	594	8.82
Krishna	75.30	18.39	17.50	2.35	542	7.65
Kanchan	69.36	14.75	14.90	2.70	632	5.51
NA-6	74.39	17.58	19.30	2.43	559	7.94
NA-7	75.38	16.8	15.20	2.51	612	6.05
NA-10	66.35	14.02	15.40	2.02	678	7.62
Anand 1	71.69	14.93	16.60	2.14	556	7.75
CD at 5%	7.47	4.38	4.45	0.57	26.92	2.18

attributed high fruit yields in these cultivars due to production of larger proportion of female flowers. Juice content in different varieties varied from 66.35 to 75.38%, maximum being 75.38% in NA-7 and minimum in NA 10 (66.35%). Dry-matter content was reported highest (81.39%) in Krishna and lowest (14.02%) in Kanchan. TSS was found maximum in NA 6 (19.30%) and minimum in Kanchan (14.90%). Aonla growing in arid region with limited water tended to more accumulation of dry-matter content and lower moisture may result in higher TSS in fruits (Meghwal and Azam, (2004).

Acidity in fruits was recorded highest (2.70%) in Kanchan and minimum (2.04%) in Chakaiya. However, vitamin C was maximum (678 mg) in NA 10 and minimum (542 mg) in Krishna, whereas TSS: acid ratio was maximum (8.82) in Chakaiya and minimum (5.51) in Kanchan. The variations in physico chemical attributes including vitamin C in different cultivars have been also reported by Teaotia *et al.* (1968).

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Role of transposons for virulence of necrotrophic plant pathogen (*Botrytis cinerea*)

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ABSTRACT

The *Botrytis cinerea* is a heterothallic filamentous plant pathogenic ascomycete. Its control at the crop level is still very difficult to achieve. Population genetic analysis revealed a high level of genetic diversity and lack of gene flow among cryptic species of *B. cinerea*. Presence of transposable elements is found as an active part in dispersal and variability of the fungi. Presence or absence of transposons in *B*. cinerea were found to affect pathogenic potential of the fungus and might play a role in speciation. Research leading to more insight into the presence/absence of transposons can prompt innovative approaches to control *B*. cinerea.

Key words: *Botrytis cinerea*, Transposable elements, Genetic variation, Boty, Flipper

The *Botrytis cinerea* is a necrogenous saprophyte that causes grey mould on many economically important crops. It has been identified in more than 200 plant species, with no apparent host specificity (Riggotti *et al*. 2006). Modern phytopathological studies focus on genetic structure of pathogen populations for gaining insights into better control strategies. The complexity and variability in *B*. cinerea makes it difficult to control and reflect the existence of several distinct populations which have different characteristics. The *Botrytis cinerea* holds second position among top ten fungal pathogens as voted by plant mycologists (Dean *et al.* 2012). As a result of high cost of current control measures as well as the frequency of fungicide resistance, an increased effort is required to identify genetic resistance towards necrotrophic pathogens. The *B. cinerea* possess a variety of extrachromosomal genetic elements including the chromosomes of mitochondria, viruses, plasmids and transposable elements (Rosewick and Kistler 2000).

TRANSPOSONS

Transposable elements (TEs) are mobile, repetitive DNA sequences of genomes that can change the sequence, expression and chromosome rearrangements of genes (Zhao *et al.* 2009). The alterations caused by TEs can create a new phenotype due to blocked transcription of associated genes or alteration in the transcription process. The TEs were known to be activated by stress and silenced by epigenetic processes (De Lima Fávaro *et al.* 2005). Transposition as a response to environmental stress was proposed as an adaptive response of the genome (Mc Clintock 1984). Several transposons have been reported to show activity under abiotic or biotic stresses (Wessler 1996; Capy *et al.* 2000). Transposons act as insertional mutagens and genes altered in this way can be cloned as sequences that flank transposon insertion sites (Daboussi, 1996). Presence of transposons in pathogenic isolates and absence in non-pathogenic isolates indicates that populations may be marked by transposons. Transposons have been used to distinguish genetically divergent populations because they can mark specific genotypes that have a common ancestor (Dobinson *et al.* 1993; Kachroo *et al.* 1994; Shull and Hamer, 1996; Giraud *et al.* 1997; Zhu and Oudemans, 2000).

Transposable elements could be regarded as an efficient tool for molecular characterization of fungal pathogens. The TEs are ubiquitous in prokaryotic and eukaryotic organisms and are a common cause of spontaneous genetic changes that can have a wide

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range of effects on the biology of the host and on its evolution (Smith and Corces, 1991). There are two main classes of TEs (Daboussi 1996; Kidwell and Lisch 2001): class I (retroelements) are TEs that transpose by RNA mediated reverse transcription and class II (DNA elements) are TEs that transpose directly from DNA to DNA (Fig 1).

The DNA elements have characteristic inverted terminal repeats (ITRs) bordering an internal transposase-encoding sequence which functions only for DNA based transposition. Retroelements have been found in a number of fungal species: CfT-1 in *Cladosporium fulvum*, Foret 1 in *Fusarium oxysporum*, Tad in *Neurospora crassa*, Grasshopper, MGR583 and Maggy in *Magnaporthe grisea* (Dobinson *et al.* 1993; Farman *et al.* 1996a) and *boty* in *Botrytis cinerea* (Diolez *et al.* 1995). The DNA transposons have also been found in a number of fungal pathogens as Fot 1 and other DNA elements were identified in *F. oxysporum* (Daboussi *et al.* 1992; Daboussi and Langin 1994), Pot 2 and MGR586 in *M. grisea* (Kachroo *et al.* 1994; Farman *et al.* 1996b), Ant1 in *Aspergillus niger* and *flipper* in *B. cinerea* (Levis *et al.* 1997).

Boty contains 596 bp long terminal repeats (LTR) and one internal putative *gag pol* gene that encodes a polyprotein with sequences homologous to the reverse transcriptase (RT) and RNaseH (RH) domains of retroelement *pol* genes (Zhao *et al.* 2009). *Boty* is presumed to give rise to repeated sequences and may cause important evolutionary variations. Levis *et al.* (1997) isolated TE *flipper*, from *B. cinerea*. The element was identified as an insertion sequence within the coding region of the nitrate reductase (*niaD*) gene. The *flipper* sequence is 1842 bp long with perfect inverted terminal repeats (ITRs) of 48 bp and an open reading frame (ORF) of 533 amino acids, potentially encoding for a transposase; the element is flanked by the dinucleotide TA (Fig 2).

The *B. cinerea* was proposed to be a species complex (Giraud *et al.* 1997, 1999, Albertini *et al.* 2002, Muñoz *et al.* 2002, Fournier *et al.* 2003). Giraud *et al.* (1997) studied genetic diversity of *B. cinerea* using a range of markers including the presence or absence of TEs boty and flipper. *B. cinerea* appears to be composed of two subgroups, transposa and vacuma that are genetically isolated and occur in sympatry on the same host plants and in the same region (Giraud *et al.* 1997). Transposa contains the TEs boty and flipper, whereas neither of these can be amplified from strains of the vacuma population. Giraud *et al.* (1997; 1999) concluded the existence of two 'sibling species' vacuma and transposa, consistent with the restriction of transposons to one population. However, strains containing only the *boty* element have now been detected in Europe (De Miccolis

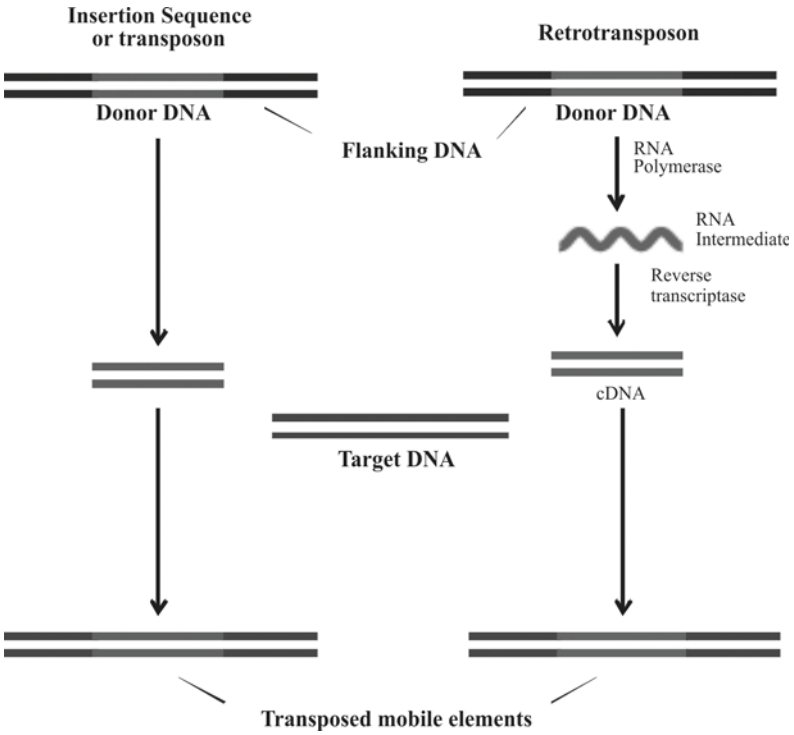

Fig. 1. Mechanism of transposition in two classes of transposons: insertion sequence or transposon (class I) and retrotransposon (class II)

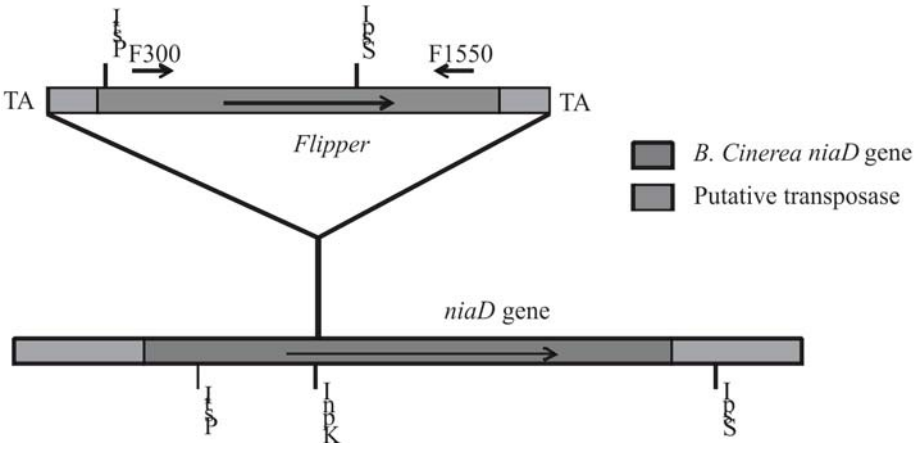

Fig. 2. Mapping of *flipper* insertion within *niaD* gene. The locations of primers used to clone the *flipper* element are indicated by arrows (*Courtesy:* Levis *et al.* 1997).

et al. 2003) and Chile (Muñoz *et al.* 2002), and strains containing only the flipper element have been detected in Europe (Albertini *et al.* 2002; De Miccolis *et al.* 2003).

Giraud *et al.* (1997) found highly significant differences in allelic frequencies between transposa and vacuma isolates. This extensive genotypic diversity indicated limited clonal propagation and a significant role for recombination. In a subsequent study, Giraud *et al.* (1999) examined isolates from various host plants showing statistically significant difference in spore size, spores of vacuma being slightly larger than transposa isolates. Transposa isolates were shown to be more virulent than vacuma isolates and changes in transposon type frequencies during crop development were possibly due to differences in their saprotrophic and pathogenic fitness. Thus, these observations supported the possibility of genetic differentiation between transposon types (Martinez *et al.* 2003, 2005).

Fournier *et al.* (2005) showed that genetic differentiation determined from multiple gene sequences was not concordant with either of the previously described transposon types (transposa or vacuma) and revised partitioning of *B. cinerea* into Group I and Group II phylogenetic cryptic species. These cryptic species have also been shown to coincide with resistance to the fungicide fenhexamid, and synonymously known as FenR (resistant) = Group I and FenS (sensitive) = Group II (Albertini *et al.* 2002). Group I isolates were resistant to fenhexamid, whereas Group II isolates were sensitive. Group I isolates were all vacuma type, whereas Group II isolates included both vacuma and transposa types. Fournier *et al.* (2003) reanalyzed data of Giraud *et al.* (1997) and confirmed the genetic distinctiveness of Groups I and II. To date, vacuma, flipper-only and boty-only transposon types have been detected with no transposa types in Group I and all transposon types have been detected in Group II (Giraud *et al.* 1999; Albertini *et al.* 2002; Fournier *et al.* 2003; Ma and Michailides, 2005). *B. cinerea* Group II has shown to be the predominant cryptic species. However, it was still unclear whether transposon types were genetically differentiated within this group (Fournier *et al*., 2005). Ma and Michailides (2005) examined isolates from a range of field crops and found no differentiation between transposon types using microsatellite primed or inter simple sequence repeat (ISSR) PCR markers. However, their study detected a small number of Group I isolates of the boty-only type. The presence of *B. cinerea* Group I and II cryptic species in Asia and Australia is unknown and knowledge of this status may be useful in determining global migration patterns.

Furthermore, it has not been determined if frequencies of transposon types differ in other populations, if transposons boty and flipper were associated with one another or if transposon types were genetically differentiated within *B. cinerea* Group I or II complex. This could be due to molecular markers that lack high levels of polymorphism such as in sequence analyses or studies that have insufficient sample size (Fournier *et al.* 2005). Since *boty* and *flipper* transposons can be found together within genomes or separately between genomes, the frequencies and association between transposon markers may elucidate genetic differentiation between populations from different geographic origins (Muñoz *et al.* 2002). The correlation of genetic differentiation with transposon type could indicate allopatric or sympatric mechanisms of convergent evolution in *B. cinerea*. This would thereby lay different control measures for different locations.

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Effect of sucrose and silver nitrate on flower quality, vase-life and correlation in gerbera *(Gerbera jamesonii)*

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ABSTRACT

An experiment was conducted to find out the response of silver nitrate on post-harvest life of cut flowers of gerbera (*Gerbera jamesonii* Bolus ex Hooker F). Six varieties, Rionegro (V_1), Manizales (V_2), Galileo (V_3), Tecala (V_4), Marinilla (V_5) and Pia (V_6); four levels of vase solution, tap water (C_1), silver nitrate at 25ppm (C_2), 50ppm (C_3), 75ppm (C_4) along with sucrose 4 per cent, respectively, were used under factorial completely randomized design with three replications. Significant differences in varieties of gerbera, vase solution and their interaction were found. The lower concentration of silver nitrate in vase solution found to be better for prolonged vase-life (13.66 days), increased fresh weight on second day (29.66g), fourth day (29.00g) and sixth day (31.00g) in Marinilla. Maximum acceptability of flower colour was obtained in Manizales, Marinilla and Pia. The-vase life of gerbera had positively and highly significant association with acceptability of cut flowers on 6th day (0.721), followed by acceptability of cut flowers on 4th day (0.712), fresh weight on 1st day (0.626) and fresh weight on 4th day (0.538), whereas it showed significant negative association with pH of holding solution (-0.438). Cultivars Marinilla, Manizales and Pia showed greater acceptability, better freshness, colour and improved vase-life in vase solution sucrose 4 per cent with 25ppm silver nitrate and was observed relatively better vase solution for gerbera cut flowers.

Key words: Gerbera, Sucrose, Silver nitrate, Vase-life, Correlation , Flower quality

Gerbera (*Gerbera jamesonii* Bolus ex Hooker F) is ideal for cut flower, beds, pots, border and rock gardening. It is in demand as cut flower in the world market and has a very good export potential because of its graceful appearance, hardiness and ability to withstand during transportation with long shelf-life. The vase-life of cut gerbera varies from 8 to 15 days and is available round the year. The post-harvest quality of any cut flower is affected by depletion of carbohydrate, water relation, temperature and relative humidity. Addition of sucrose and chemical preservatives to the holding solution is recommended to prolong the vase-life of cut flowers. There are many commercial preservatives of unknown formulations and are sold under brand names. However, basic components for enhancing vase-life of cut flowers are water, pH regulator, food source and biocide. The techniques of prolonging the vase-life of flowers will be a great asset to the growers and users. Therefore, an experiment was conducted to ascertain the role of silver nitrate and sucrose on flower quality and vase-life of its cut flowers.

MATERIALS AND METHODS

The experiment was conducted at Department of Floriculture, College of Horticulture and Forestry, Pasighat, during February 2013. Six varieties, Rionegro (V_1), Manizales (V_2), Galileo (V_3), Tecala (V_4), Marinilla (V_5) and Pia (V_6); four levels of vase solution, viz. tap water (C_1), silver nitrate at 25 ppm (C_2), 50ppm (C_3), 75ppm (C_4) along with sucrose (4 per cent), respectively,

Table 1. Average maximum and minimum temperature, relative humidity (RH) during the period of experimentation

Days in vase	Max. temp. (°C)	Min. temp. (°C)	RH
1	27.5	13.5	97
2	20.0	13.5	88
3	19.5	14.5	71
4	23.5	14.0	65
5	26.5	16.5	83
6	26.5	15.0	67
7	26.5	16.0	70
8	27.0	18.0	88
9	20.0	16.5	83
10	21.5	16.0	91
11	19.0	14.5	83
12	17.5	15.0	78
13	22.0	14.5	94
14	25.5	13.5	67
15	25.5	15.0	65

were undertaken for the investigation. The experiment was laid out in factorial completely randomized design with three replications. Gerbera flowers were harvested when outer 1-2 rows of disc florets became perpendicular to flower stalk. Flowers were immediately placed in water after harvesting and uniformly cut by keeping in water to avoid intrusion of air bubble in the xylem vessel. After cutting, these were placed in 500 ml conical flask containing 300 ml of different vase solutions which were prepared with distilled water.

The minimum and maximum temperature along with relative humidity inside the laboratory during the experiments was recorded (Table 1). The data were recorded on various parameters like fresh weight, pH of holding solution, vase-life: fading of 50 per cent of ray florets was considered the end of vase-life. Appearance: 1-9 hedonic scale suggested by Ranganna (1999), water uptake: on alternate days, weight of conical flask plus solution or without the flower was determined. The difference between the consecutive measurement of conical flask plus solution without flower represented the water uptake. The data collected were analyzed using statistical methods as suggested by Panse and Sukhatme (1995).

RESULTS AND DISCUSSION

The data revealed that vase solution containing sucrose and biocides improved vase-life of gerbera significantly (Table 2). Maximum vase-life (10.58 days)

Table 2. Effect of varieties and vase solutions on vase-life of cut gerbera and relative pH of vase solutions

Treatment Varieties	Vase-life	pH
Rionegro (V_1)	7.16	2.99
Manizales (V_2)	10.00	2.93
Galileo (V_3)	6.75	2.93
Tecala (V_4)	8.30	2.87
Marinilla (V_5)	10.58	2.86
Pia (V_6)	8.75	2.90
Mean	8.59	2.91
CD (P=0.05)	**0.70**	**0.04**
Concentration		
Tap Water (C_1)	6.94	3.10
$AgNO_3$ 25 ppm (C_2)	10.66	2.83
$AgNO_3$ 50 ppm (C_3)	8.78	2.85
$AgNO_3$ 75 ppm (C_4)	8.00	2.88
Mean	8.59	2.91
CD (P=0.05)	**0.99**	**0.05**
Interaction		
V_1C_1	7.00	3.08
V_1C_2	8.64	2.99
V_1C_3	6.30	2.95
V_1C_4	6.65	2.93
V_2C_1	7.32	3.04
V_2C_2	12.35	2.96
V_2C_3	10.65	2.88
V_2C_4	9.67	2.85
V_3C_1	7.00	3.13
V_3C_2	7.33	2.88
V_3C_3	6.66	2.87
V_3C_4	6.00	2.83
V_4C_1	6.68	3.13
V_4C_2	10.30	2.77
V_4C_3	8.65	2.79
V_4C_4	7.65	2.78
V_5C_1	7.63	3.11
V_5C_2	13.66	2.81
V_5C_3	10.65	2.79
V_5C_4	10.31	2.78
V_6C_1	6.00	3.13
V_6C_2	11.67	2.86
V_6C_3	9.67	2.84
V_6C_4	7.65	2.79
Mean	8.59	2.91
CD (P=0.05)	**1.40**	**0.07**

and reduced pH (2.86) in *cv.* Marinilla was obtained. The similar findings were observed by Prasanth *et al*. (2010), Prasanth and Chandershekhar (2007). Different concentrations of vase solutions had significant effect on vase-life and pH. Silver nitrate at 25 ppm showed enhanced vase-life (10.66) and reduced pH (2.83), followed by silver nitrate at 50ppm. The interaction

was found to be significant for vase-life and pH. Cultivar Marinilla in vase solution silver nitrate at 25 ppm was significantly superior for vase-life (13.66 days), followed by Manizales (12.35 days) and Pia (11.67 days), whereas decreased pH was obtained in Tecala (2.77). The vase solution maintained the better freshness of cut flowers of gerbera throughout the vase-life, especially during later part (8th, 10th and 12th day in vase) of vase-life. Low concentration of silver nitrate and sucrose in vase solution prolonged vase-life in gerbera was also reported by Vasudevan and Rao (2010).

The improvement in vase-life of cut flowers in 25 ppm silver nitrate ($AgNO_3$) solution might be due to that it is a very effective biocide, which completely inhibits the microbial growth. It is in conformity with the findings of Ketsa *et al.* (1995) who opined that $AgNO_3$ prevented microbial occlusion of xylem vessels in Dendrobium, thereby enhancing water uptake and increasing longevity of flowers. Awad *et al*. (1986) also attributed the beneficial effect of $AgNO_3$ in the vase solution to the production of Ag^+ ions, which might inhibit the rise of ethylene precursor, thereby enhancing the longevity of cut flowers. Sucrose is widely used in floral preservatives, which acts as a food source or respiratory substrate and delays the degradation of proteins and improves the water balance of cut flowers. Steinitz (1982) opined that addition of sucrose to the solution increased the mechanical rigidity of the stem by inducing cell wall thickening and lignification of vascular tissues.

The fresh weight was reduced as days increased for vase life except cv. Manizales, Tecala, Marinilla and Pia (Table 3). Cultivar Manizales showed highest fresh weight (24.33g) followed by. Marinilla (24.04g) and Tecala (23.12g) on first day. Fresh weight on 4th day and 6th day observed significantly higher in Manizales, followed by Marinilla. The vase solution containing sucrose 4 per cent and silver nitrate 25 ppm maintained maximum fresh weight at different days followed by silver nitrate at 50 ppm, respectively. The interaction was found to be significant during first, fourth and sixth days. Cultivar Marinilla in vase solution silver nitrate at 25 ppm showed increased fresh weight (29.66g, 29.32g and 31.00g, respectively).

The data revealed that as the day proceed for vase-life, water uptake by cut flower decreased but maximum water uptake was observed by *cv.* Pia on 2nd day (8.51g/flower) and 6th day (3.80g/flower), whereas on 4th day cv. Rionegro (5.40g/flower), 8th day Manizales (1.74g/flower), 10th day (0.94g/flower) and 12th day (0.52g/flower) in *cv.* Marinilla, respectively, showed maximum water uptake (Table 4). Higher concentration of silver nitrate in vase solution increased the water uptake during the entire study. This might be due to that $AgNO_3$ present in the holding solution inhibiting microbial population and reduced the blockage of vascular tissues. The stems of gerbera are highly prone to water stress. The blockage of the base of stem due to bacterial plugging results in decrease of water uptake. A very high level of turgidity is necessary for continuation of normal metabolic activities in the cut flowers.

Sucrose helps in maintaining the water balance and turgidity. Hence, addition of sucrose to holding solution might have lead to increased uptake of the holding solution. This was in conformity with the findings of Rogers (1973). The present investigation revealed that best holding solution for cut gerbera blooms would be a combination of silver nitrate and sucrose. The interaction was found to be non-significant except water uptake on second day. Cultivar, Pia, showed highest water uptake (9.24g/flower) on second day in vase solution of sucrose 4 per cent and silver nitrate at 50ppm which was at par with Marinilla (9.12g/flower) and Rionegro (9.16g/flower). The data on fresh weight and water uptake indicate that chemical treatments acted primarily by improving water uptake and consequently extending the vase-life (Amiri *et al.* 2009). Significant differences in water uptake and fresh weight in several cultivars of gerbera was also observed by Javad *et al*. (2011).

The fastest deterioration in flower colour occurred in Rionegro (4.16 score on 8th day in vase) and became unavailable on 10th day (Table 5). Maximum acceptability of flower colour was obtained in Manizales, Marinilla and Pia. The vase solution (sucrose 4 per cent) and silver nitrate (25 ppm) and silver nitrate (50 ppm) maintained flowers with acceptable colour even after 12 day. The interaction was found to be non-significant, however acceptable flower colour in cv. Marinila (4.85) and Manizales (4.35) was maintained in vase solution of sucrose 4 per cent and silver nitrate at 25 ppm even on 12th day.

Correlations studies were computed between ten pairs of characters (Table 6). The vase-life of gerbera had positively and highly significant association with acceptability of cut flower on 6th day (0.721), followed by acceptability of cut flowers on 4th day (0.712), fresh weight on 1st day (0.626) and fresh weight on 4th day (0.538), whereas it showed significant negative association with pH of holding solution (-0.438).

Fresh weight on 1st day showed highly significant positive correlation with fresh weight on 4th day (0.758), followed by acceptability of cut flowers on 4th day (0.465) and 6th day (0.426). Fresh weight on 4th day expressed highly significant positive correlation with acceptability of cut flower on 4th day (0.447), followed by acceptability of cut flowers on 6th day (0.392) and

Table 3. Effect of varieties and vase solutions on relative fresh weight (g) of cut gerbera

Treatment	Fresh weight 1st Day	Fresh weight 4th Day	Fresh weight 6th Day	Fresh weight 8th Day	Fresh weight 10th Day	Fresh weight 12th Day
Variety						
Rionegro (V_1)	22.04	21.42	21.08	20.65	20.50	0.00
Manizales (V_2)	24.33	26.25	24.67	25.04	24.42	25.18
Galileo (V_3)	16.96	15.83	15.40	14.00	0.00	0.00
Tecala (V_4)	23.12	22.30	22.45	25.24	24.65	0.00
Marinilla (V_5)	24.04	23.80	24.06	25.20	23.05	21.50
Pia (V_6)	21.42	21.54	21.25	22.57	20.82	13.00
Mean	21.98	21.85	21.48	22.12	22.68	19.89
CD (P=0.05)	**0.83**	**0.63**	**0.59**	**NA**	**NA**	**NA**
Concentration						
Tap water (C_1)	20.92	19.86	18.70	17.27	0.00	0.00
$AgNO_3$ 25 ppm (C_2)	23.69	23.81	24.09	24.92	24.69	18.10
$AgNO_3$ 50 ppm (C_3)	22.16	22.50	21.62	22.21	21.15	21.68
$AgNO_3$ 75 ppm (C_4)	21.15	21.28	21.52	24.07	22.20	0.00
Mean	21.98	21.85	21.48	22.12	22.68	19.89
CD (P=0.05)	**1.17**	**0.88**	**0.84**	**NA**	**NA**	**NA**
Interaction						
V_1C_1	21.17	19.50	18.00	0.00	0.00	0.00
V_1C_2	23.50	22.50	23.65	22.06	20.26	0.00
V_1C_3	22.33	24.17	23.00	0.00	0.00	0.00
V_1C_4	21.16	19.50	19.64	17.76	0.00	0.00
V_2C_1	21.66	22.82	20.50	0.00	0.00	0000
V_2C_2	22.67	24.80	24.50	23.07	25.38	25.47
V_2C_3	29.00	29.00	26.00	23.08	24.42	22.12
V_2C_4	24.00	28.00	27.63	26.76	22.76	0.00
V_3C_1	18.16	14.30	14.50	14.26	0.00	0.00
V_3C_2	16.32	15.32	14.50	12.26	0.00	0.00
V_3C_3	16.82	16.62	16.12	0.00	0.00	0.00
V_3C_4	16.50	17.00	16.45	0.00	0.00	0.00
V_4C_1	21.16	18.80	18.77	0.00	0.00	0.00
V_4C_2	23.84	23.65	23.20	22.43	24.41	0.00
V_4C_3	24.65	25.15	25.53	25.06	0.00	0.00
V_4C_4	22.82	21.63	22.30	26.01	0.00	0.00
V_5C_1	22.80	23.14	20.97	18.26	0.00	0.00
V_5C_2	29.66	29.32	31.00	34.56	27.76	18.27
V_5C_3	20.83	20.50	20.76	19.76	19.92	21.97
V_5C_4	22.80	22.50	23.50	25.26	20.76	0.00
V_6C_1	20.50	20.50	19.50	0.00	0.00	0.00
V_6C_2	26.16	27.50	27.66	29.08	23.40	11.62
V_6C_3	19.32	19.15	18.32	16.91	17.76	0.00
V_6C_4	19.66	19.00	19.50	19.51	0.00	0.00
Mean	21.98	21.85	21.48	22.12	22.68	19.89
CD (P=0.05)	**1.66**	**1.25**	**1.18**	**NA**	**NA**	**NA**

NA, not applicable due to absence of some treatments

Table 4. Effect of varieties and vase solutions on relative water uptake (ml) of cut gerbera

Treatment	Water uptake 2nd Day	Water uptake 4th Day	Water uptake 6th Day	Water uptake 8th Day	Water uptake 10th Day	Water uptake 12th Day
Variety						
Rionegro (V_1)	8.46	5.40	3.75	1.65	0.00	0.00
Manizales (V_2)	8.03	5.01	3.68	1.74	0.87	0.47
Galileo (V_3)	8.20	5.30	2.72	1.51	0.00	0.00
Tecala (V_4)	6.99	4.54	3.65	1.23	0.54	0.00
Marinilla (V_5)	7.76	4.91	3.54	1.38	0.94	0.52
Pia (V_6)	8.51	5.31	3.80	1.67	0.89	0.39
Mean	7.99	5.07	3.52	1.53	0.81	0.46
CD (P=0.05)	**0.55**	**0.42**	**NS**	**NA**	**NA**	**NA**
Concentration						
Tap water (C_1)	7.72	4.97	3.53	1.60	0.00	0.00
$AgNO_3$ 25 ppm (C_2)	7.88	5.03	3.42	1.43	0.69	0.45
$AgNO_3$ 50 ppm (C_3)	8.44	5.29	3.62	1.74	0.81	0.47
$AgNO_3$ 75 ppm (C_4)	7.93	5.02	3.49	1.35	0.92	0.00
Mean	7.99	5.07	3.52	1.53	0.81	0.46
CD (P=0.05)	**0.78**	**NS**	**NS**	**NA**	**NA**	**NA**
Interaction						
V_1C_1	8.71	5.72	3.69	0.00	0.00	0.00
V_1C_2	9.16	5.71	3.71	1.68	0.00	0.00
V_1C_3	7.70	4.91	3.45	0.00	0.00	0.00
V_1C_4	8.28	5.24	3.47	1.62	0.00	0.00
V_2C_1	8.62	5.29	3.65	2.05	0.00	0.00
V_2C_2	8.41	5.20	3.61	1.68	0.72	0.49
V_2C_3	7.91	4.96	3.48	1.83	0.81	0.41
V_2C_4	7.19	4.60	3.29	1.41	0.93	0.00
V_3C_1	7.71	5.06	3.53	1.33	0.00	0.00
V_3C_2	7.62	5.21	3.15	1.69	0.00	0.00
V_3C_3	8.90	5.59	3.81	0.00	0.00	0.00
V_3C_4	8.55	5.34	3.69	0.00	0.00	0.00
V_4C_1	6.03	3.99	3.78	0.00	0.00	0.00
V_4C_2	6.52	4.23	3.12	1.15	0.49	0.00
V_4C_3	7.78	4.99	3.50	1.46	0.00	0.00
V_4C_4	7.63	4.95	3.48	1.07	0.00	0.00
V_5C_1	7.28	4.66	3.00	1.50	0.00	0.00
V_5C_2	7.21	4.63	3.32	1.08	0.97	0.48
V_5C_3	9.12	5.59	3.80	1.78	0.74	0.52
V_5C_4	7.47	4.76	3.38	1.15	0.96	0.00
V_6C_1	7.95	5.09	3.53	0.00	0.00	0.00
V_6C_2	8.36	5.19	3.60	1.44	0.70	0.37
V_6C_3	9.24	5.69	3.70	1.97	0.97	0.00
V_6C_4	8.48	5.28	3.63	1.59	0.00	0.00
Mean	7.99	5.07	3.52	1.53	0.81	0.46
CD (P=0.05)	**1.10**	**NS**	**NS**	**NA**	**NA**	**NA**

NA, not applicable due to absence of some treatments; **NS** non-significant

Table 5. Flower colour of gerbera as affected in different varieties and vase solutions

Treatment	Acceptability 2st Day	Acceptability 4th Day	Acceptability 6th Day	Acceptability 8th Day	Acceptability 10th Day	Acceptability 12th Day
Varieties						
Rionegro (V_1)	9.00	7.73	6.24	4.16	0.00	0.00
Manizales (V_2)	9.00	9.00	7.81	6.15	4.76	3.75
Galileo (V_3)	9.00	7.24	7.08	4.50	0.00	0.00
Tecala (V_4)	9.00	7.07	7.01	4.94	4.00	0.00
Marinilla (V_5)	9.00	9.00	7.75	6.15	4.87	3.50
Pia (V_6)	9.00	9.00	6.25	5.43	4.83	2.50
Mean	9.00	8.17	7.02	5.22	4.62	3.25
CD (P=0.05)	**NS**	**NS**	**NS**	**NA**	**NA**	**NA**
Concentration						
Tap Water (C_1)	9.00	7.77	6.16	4.16	0.00	0.00
$AgNO_3$ 25ppm(C_2)	9.00	8.66	8.16	6.36	6.18	4.00
$AgNO_3$ 50ppm(C_3)	9.00	8.26	7.38	6.02	4.18	2.50
$AgNO_3$ 75ppm(C_4)	9.00	7.99	6.39	4.35	3.50	0.00
Mean	9.00	8.17	7.02	5.22	4.62	3.25
CD (P=0.05)	**NS**	**NS**	**NS**	**NA**	**NA**	**NA**
Interaction						
V_1C_1	9.00	7.38	6.30	0.00	0.00	0.00
V_1C_2	9.00	8.73	6.64	5.20	0.00	0.00
V_1C_3	9.00	7.74	6.00	0.00	0.00	0.00
V_1C_4	9.00	7.40	6.00	2.80	0.00	0.00
V_2C_1	9.00	9.00	6.65	4.78	0.00	0.00
V_2C_2	9.00	9.00	8.63	7.28	6.55	4.35
V_2C_3	9.00	9.00	8.30	6.44	4.20	2.85
V_2C_4	9.00	9.00	7.65	5.44	3.20	0.00
V_3C_1	9.00	7.73	8.30	3.78	0.00	0.00
V_3C_2	9.00	6.75	8.68	4.80	0.00	0.00
V_3C_3	9.00	7.38	7.32	0.00	0.00	0.00
V_3C_4	9.00	6.40	4.00	0.00	0.00	0.00
V_4C_1	9.00	4.73	5.34	0.00	0.00	0.00
V_4C_2	9.00	8.73	8.35	6.44	3.90	0.00
V_4C_3	9.00	7.72	7.67	4.45	0.00	0.00
V_4C_4	9.00	7.40	6.68	3.30	0.00	0.00
V_5C_1	9.00	9.00	6.00	3.45	0.00	0.00
V_5C_2	9.00	9.00	9.00	7.42	7.55	4.85
V_5C_3	9.00	9.00	8.35	6.78	3.54	1.85
V_5C_4	9.00	9.00	7.66	6.20	3.20	0.00
V_6C_1	9.00	9.00	4.34	0.00	0.00	0.00
V_6C_2	9.00	9.00	7.68	6.43	5.56	2.35
V_6C_3	9.00	9.00	6.65	5.78	3.90	0.00
V_6C_4	9.00	9.00	6.32	3.45	0.00	0.00
Mean	9.00	8.17	7.02	5.22	4.62	3.25
Chi Sq	**NS**	**22.89**	**NS**	**NA**	**NA**	**NA**
Sig	**0.00**	**0.001**	**0.00**			

NA, not applicable due to absence of some treatments; NS, non-significant

Acceptability of flower colour (1-9 scale) in vase; Like extremely-score-9, like very much-8, like moderately-7, like slightly-6, neither like nor dislike-5, dislike slightly-4, dislike moderatels-3, dislike very much-2 and dislike extremely-1.

Table 6 : Studies on different flower quality and vase life affecting parameters of gerbera

Variable	1	2	3	4	5	6	7	8	9	10
Fresh Weight 1st Day	1.00	0.758**	0.157	0.626**	-0.169	0.465**	0.426**	-0.181	-0.211	-0.156
Fresh Weight 4th Day		1.00	0.284*	0.538**	-0.210	0.447**	0.392**	-0.401**	-0.442**	-0.470**
Fresh Weight 6th Day			1.00	0.103	-0.075	0.147	0.115	-0.168	-0.172	-0.169
Vase-Life				1.00	-0.438**	0.712**	0.721**	-0.062	-0.115	-0.161
pH of vase-solution					1.00	-0.539**	-0.466**	-0.082	-0.046	0.100
Acceptability of cut flower 4th Day						1.00	0.739**	0.073	0.023	-0.228
Acceptability of cut flower 6th Day							1.00	-0.098	-0.139	-0.236
Water Uptake 2nd Day								1.00	0.978**	0.791**
Water Uptake 4th Day									1.00	0.784**
Water Uptake 6th Day										1.00

*, ** significant at 5 and 1 per cent

fresh weight on 6th day (0.284). However, it had significant negative correlation with water uptake on 6th day (-0.470), followed by water uptake on 4th day (-0.442) and 2nd day (-0.401). The pH of vase-life was found to have negative and significant association with acceptability of cut flowers on 4th day (-0.539) and 6th day (-0.466). However, significant positive association between acceptability of cut flowers on 4th day and 6th day (0.739) was noticed. Water uptake on 2nd day had significant positive correlation with water uptake on 4th day (0.978) and 6th day (0.791), respectively. The association between water uptake on 4th day and 6th day was found positive and significant (0.784). The correlation coefficients between rest pairs of the characters were non-significant either in positive or in negative directions.

Thus, results indicate that different varieties of gerbera, vase solution and their interaction was found to be better for enhanced vase-life. Cultivars Marinilla, Manizales and Pia in vase solution (sucrose 4 per cent) and silver nitrate (25 ppm) had greater acceptability, maintained better freshness, colour with improved vase-life and was observed relatively better vase solution for cut flowers of gerbera.

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Effect of INM on litchi *(Litchi chinensis)* for enhancing quality fruit production

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ABSTRACT

An experiment was conducted to find out the effect of integrated nutrient management (INM) on quality litchi *(Litchi chinensis Sonn.)* production at the National Research Centre on Litchi, Muzaffarpur, Bihar, during 2010-12. The treatments having Azotobactor (250 g/tree) with half of the recommended dose of fertilizers, 5 kg of vermicompost and 25 kg of FYM proved to be the most dynamic and suitable substrate recording maximum fruit yield and super quality grade (extra class). The wastage of fruits due to diseased/damaged category was also low in all the treatments having biofertilizers along with organic manures. The treatments having biofertilizers (Azospirillum, Azotobactor, Aspergillus, Trichoderma and Pseudomonas) with chemical fertilizers and organic manures, clearly indicated the possibility of reducing the dose of chemical fertilizers to the tune of 50 %, with quality fruit production in an economically-viable mode. The combined use of biofertilizers, chemical fertilizers and organic manures showed the overall improvement in physico-chemical characteristics of the soil. The judicious blend of organic and inorganic fertilizers with biofertilizers was encouraging, indicating that it is an eco-friendly approach for quality fruit production with enhanced productivity from 1.35 to 2.34 tonnes/ha in merely young bearing orchard.

Key words: INM, Quality fruit, Vermicompost, Azotobactor, Fertilizer, Biofertilizer

Litchi is an important commercial fruit crop belongs to family Sapindaceae and subtropical group of fruit crops (Menzel and Waite 2005). The commercial viability (including export potential) lies in its high quantum of quality production. The continuous declining health of litchi orchards due to poor orchard floor management and nutrient application are widening the gap of actual production and potential production mainly due to lack of balanced NPK nutrition and their dynamics in growth cycle (Lin *et al.*, 2001, Fan *et al.* 2005, Kumar 2010). The problems in growth, fruiting behaviour and fruit yield are being aggravated due to poor nutrient application and upsurge in the prices of chemical fertilizers. A low recovery of applied chemical fertilizers due to leaching, drainage and fixation as well as proving hazardous for soil health, it is of utmost importance to find out alternative sources in combination to reduce the use of chemical fertilizers. Biofertilizers are integral part of integrated nutrient management strategies in horticulture (Menzel, 2005). The reduced dose of chemical fertilizers along with organic and inorganic sources have been found to be reported by many workers in perennial fruit crops (Bould and Needham 1983, Menzel 1991, 2005, Panda 2006, Srivastava 2011), hence an experiment was conducted to evaluate the effectiveness of biofertilizers in combination with organic manures and chemical fertilizers to assess crop growth, fruit yield and quality as well as soil chemical properties along with savings in use of chemical fertilizers on litchi.

MATERIALS AND METHODS

The experiment was conducted at the research farm of NRC on Litchi, Mushahari, Muzaffarpur, Bihar, during 2010 - 2012 in eight-year old litchi orchard. The soil was characterized by low organic carbon (0.49%), low available N (198.00 kg/ha), available P_2O_5 (11.40

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kg/ha) and available K_2O (111.00 kg/ha) contents (Table 4). The experiment was laid out in a randomized block design with 11 treatments replicated thrice. The treatment included two units in one treatment. The plants of Shahi variety of litchi were used for the study. The cultures of Azospirillum, Pseudomonas, Azotobactor, Aspergillus and Tricoderma (colony forming units of 1010/g) produced by the company supplied at Muzaffarpur, were applied as per the recommended dose. The treatments were :

T_1 : 500 : 250 : 250g NPK/tree (control).
T_2 : T_1 + Zn (0.5%) + B (0.2%) as foliar twice (August and October).
T_3 : T_1 + organic mulching 10 cm thick.
T_4 : T_2 + organic mulching 10 cm thick.
T_5 : half of T_1 + 50 Kg FYM + Trichoderma (250 g).
T_6 : half of recommended dose of fertilizers + Azospirillum (250 g) + 50 kg FYM.
T_7 : half of recommended dose of fertilizers + Azotobactor (250 g) + 50 kg FYM.
T_8 : half of recommended fertilizers + Azotobactor (250 g) + 25 kg FYM + 5 kg Vermi compost.
T_9 : half of recommended dose of fertilizers + *Pseudomonas fluorecense* (250 g) + 50 kg FYM.
T_{10}: half of recommended dose of fertilizers + Trichoderma (250 g) + *Pseudomonas fluorecense* (250 g) + 50 kg FYM.
T_{11}: half of recommended dose of fert. + enriched with Aspergillus niger + 50 kg FYM.

The available NPK status was estimated by alkaline permanganate method (Subbiah and Asija 1956), Bray I method (Jackson 1973) respectively from the soil science laboratory of Rajendra Agricultural University, Samasitipur, Bihar. The analysis of variance was done using MSTATC programme following the method described by Cochran and Cox (1965).

RESULTS AND DISCUSSION

The data showed that there was maximum plant height (4.30 m) in the treatment T_{10}, *i.e.* half of recommended fertilizers + Trichoderma (250 g) + *Pseudomonas fluencese* (250 g) + 50 kg FYM, followed by treatment T_{11}, *i.e.* half of recommended dose of fertilizers + enriched with *Aspergillus niger* + 50 Kg FYM and T_6, *i.e.* half of recommended dose of fertilizers + Azospirillum (250 g) + 50 Kg FYM having tree heights 4.23 m and 4.20 m respectively (Table 1). The maximum girth (53.66 cm) of trees was recorded in the treatment T_8 : half of recommended fertilizers + Azotobactor (250 g) + 25 kg FYM + 5 kg vermicompost. It was also found no relation with the plant height with the tree spread measured in both directions (Table 1), where it was recorded maximum spread (5.26 m) in E-W direction in the treatment T_6 : half of recommended dose of fertilizers + Azospirillum (250 g) + 50 kg FYM, while it was maximum (4.73 m) spread in N-S direction for the treatment T_7 : half of recommended dose of fertilizers + Azotobactor (250 g) + 50 kg FYM. It was observed that vegetative growth was better in most of the treatments receiving biofertilizers.

The yield components were not profoundly influenced by different treatments. During 2010 (the initial year of experimentation), maximum fruit yield (13.50 kg/tree) was recorded in the treatment (T_{10}) receiving half of recommended fertilizers + Trichoderma (250 g) + *Pseudomonas fluencese* (250 g) + 50 kg FYM, during 2011 and 2012, the treatment T_8 receiving half of recommended fertilizers + Azotobactor

Table 1. Growth parameters and fruit yield under different treatments.

Treatment	Plant height (m)		Girth (cm)		Canopy E-W (m)		Canopy N-S (m)		Yield (kg/plant)			
	2010	2012	2010	2012	2010	2012	2010	2012	2010	2011	2012	Mean
T_1	3.20	4.07	32.40	49.00	3.00	4.70	3.50	4.33	11.00	14.50	17.00	14.66
T_2	3.00	3.97	35.60	50.66	3.00	4.76	3.90	4.38	08.50	13.00	19.00	13.50
T_3	3.20	4.13	41.60	49.00	3.70	4.71	3.30	4.46	12.00	14.50	18.00	14.83
T_4	3.20	4.06	45.50	51.00	3.70	5.06	3.50	4.56	08.50	16.00	22.00	15.50
T_5	2.80	4.00	34.90	48.00	3.30	4.76	3.30	4.60	11.50	17.00	29.00	19.16
T_6	2.80	4.07	37.90	49.00	3.60	5.26	3.60	4.43	07.00	15.50	24.00	15.50
T_7	3.10	4.13	36.40	52.00	3.60	4.50	3.40	4.73	12.50	17.50	26.00	18.66
T_8	3.00	4.16	32.60	53.66	3.20	4.98	3.20	4.68	10.50	21.50	39.00	23.66
T_9	2.90	4.03	28.80	49.00	3.40	4.51	3.80	4.60	10.50	14.00	29.00	17.83
T_{10}	3.30	4.30	35.60	51.66	3.00	4.98	3.60	4.56	13.50	17.50	20.50	17.16
T_{11}	2.90	4.03	38.50	51.66	3.70	4.73	3.60	4.66	11.00	16.00	24.00	17.00
CD (5%)	NS	NS	3.82	1.36	NS	NS	NS	NS	3.86	3.21	7.31	2.14

Table 2. Physico-chemical properties of fruits under different treatments

Treatment	Fruit length (cm)		Fruit diameter (cm)		Fruit weight (g)		TSS (°Brix)		Acidity (%)	
	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012
T_1	3.16	3.17	2.96	3.00	21.21	23.24	19.86	20.22	0.34	0.30
T_2	3.16	3.19	3.00	3.00	21.63	22.53	20.12	19.30	0.34	0.36
T_3	3.14	3.20	2.98	3.00	21.24	23.26	19.16	18.80	0.34	0.32
T_4	3.16	3.26	3.00	3.00	21.52	24.46	19.92	18.82	0.34	0.36
T_5	3.20	3.26	2.98	3.00	21.22	23.13	19.62	18.62	0.33	0.32
T_6	3.12	3.14	2.98	3.10	21.36	23.56	19.88	19.20	0.36	0.38
T_7	3.22	3.28	3.00	3.08	21.35	24.13	19.68	19.50	0.36	0.36
T_8	3.20	3.34	3.02	3.16	21.63	24.88	19.48	19.50	0.35	0.37
T_9	3.16	3.22	2.94	3.06	21.72	24.46	19.38	19.38	0.36	0.36
T_{10}	3.17	3.24	2.96	3.00	21.45	24.53	19.36	19.36	0.37	0.37
T_{11}	3.16	3.22	2.96	3.00	21.56	23.56	19.36	19.36	0.36	0.41
CD(5%)	NS	NS	NS	NS	NS	0.28	-	-	-	-

(250 g) + 25 kg FYM + 5 kg vermicompost recorded maximum fruit yield *i.e.* 21.50 kg/tree and 39.00 kg/tree. The fruit length and fruit width (diameter) did not show any significant differences but fruit weight was significantly influenced by different treatments. The fruit weight was maximum in the treatment (T_8) receiving half of recommended fertilizers + Azotobactor (250 g) + 25 kg FYM + 5 kg vermicompost.

The data showed pronounced trend in improved quality attributes in fruits during 2010-2012, particularly with the treatments having reduced dose of chemical fertilizers plus application of organic manures and biofertilizers (Table 2). The data showed significant but erratic trend in incresing fruit yield (Fig. 1). The profound effect of different treatments were only recorded when actual obtained yield was categorized into different quality grades. The maximum fruit yield (57.95%) under extra class was recorded in the treatment (T_8), *i.e.* half of recommended fertilizers + Azotobactor

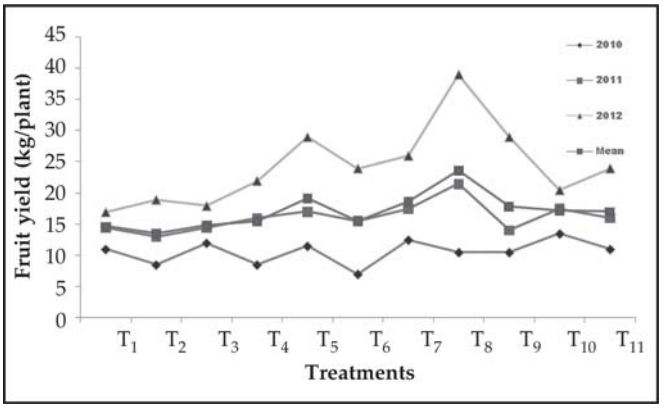

Fig. 1. Fruit yield (kg/plant) in different treatments

(250 g) + 25 kg FYM + 5 kg vermicompost (Table 3b), compared to the same recorded as 30.77 % during 2010 (Table 3a). This was mainly due high values recorded for fruit length (3.34 cm), fruit diameter (3.16cm) and fruit weight (24.88 g) which were also recorded maximum for the same treatment (T_8) (Table 2). The findings support to that of organic manures and biofertilizers along with chemical fertilizers (under INM) which offers an opportunity to enhance use of all major sources of plant nutrients in an integrated manners so as to get maximum economic yield with long-term sustainability in crop production (Menzel 1987, Panda 2006) systems without land.

The yield promoting effect may be ascribed to improved plant nutrition due to N fixation by Azotobactor as well as its facilitating effect in appreciable uptake of nitrate, ammonium, phosphate, potassium and iron as Azotobactor treatment excretes mainly ammonia in rhizosphere as root exudates in presence of organic manure which helps in nutrient uptake, resulting in more availability of N, P and K to plants. The growth promoting effects of plants hormones especially auxins and gibberellins, produced by biofertilizers stimulates root growth, branching and surface area of roots. As a result of this improved bearing habit arising from inoculation, plants take up more nutrients and water, resulting in higher quality fruit yield.

The effect of treatments on available N, P and K status in soil at the initial level during 2010 and at the end of experimentation, *i.e.* during 2012 pH level of soils of rhizosphere of trees receiving the treatments having conjoint use of organic manure and biofertilizers along with reduced dose of chemical fertilizers showed

Table 3a. Fruit yield (kg/plant) and quality categorization under different quality grades under various treatments during 2010.

Treatment	Fruit yield (kg/plant)	Categorization of fruit yield (kg/plant) under different quality grades			
	2010 (a)	Extra Class	Class-I	Class-II	Wastage
T_1	11.00	3.5 (31.81)	3.0 (27.27)	3.0 (27.27)	1.5 (13.63)
T_2	11.50	3.0 (26.09)	3.0 (26.09)	2.5 (21.73)	2.0 (17.39)
T_3	11.00	3.0 (27.27)	3.5 (31.82)	3.0 (27.27)	1.5 (13.64)
T_4	09.50	3.0 (31.57)	2.0 (21.05)	3.0 (31.57)	1.0 (10.52)
T_5	09.00	3.0 (33.33)	2.5 (27.78)	2.0 (22.22)	1.5 (16.67)
T_6	09.00	3.0 (33.33)	2.5 (27.78)	2.5 (27.78)	1.0 (11.11)
T_7	09.50	3.0 (31.57)	3.0 (31.57)	2.0 (21.05)	1.5 (15.79)
T_8	13.00	4.0 (30.77)	3.5 (26.92)	3.0 (23.08)	1.5 (11.54)
T_9	09.50	3.0 (31.57)	2.0 (21.05)	2.5 (26.32)	1.5 (15.79)
T_{10}	09.00	3.0 (33.33)	2.0 (22.22)	2.5 (27.78)	1.5 (16.67)
T_{11}	10.00	3.0 (30.00)	3.0 (30.00)	2.5 (25.00)	1.5 (15.00)

Table 3b. Fruit yield (kg/plant) and quality categorization under different quality grades under various treatments during 2012.

Treatment	Fruit yield (kg/plant)	Categorization of fruit yield (kg/plant) under different quality grades			
	2012 (b)	Extra Class	Class-I	Class-II	Wastage
T_1	17.00	7.1 (41.76)	4.3 (25.30)	3.7 (21.76)	1.8 (10.58)
T_2	19.00	7.0 (36.84)	4.8 (25.26)	5.1 (26.84)	2.1 (11.05)
T_3	18.00	8.0 (44.44)	4.0 (22.22)	4.7 (26.11)	1.3 (7.22)
T_4	22.00	11.0 (50.00)	4.5 (20.45)	5.9 (26.82)	1.6 (7.27)
T_5	29.00	13.0 (44.82)	7.7 (26.55)	6.5 (22.41)	1.8 (6.21)
T_6	24.00	13.0 (54.16)	5.2 (21.67)	3.7 (15.42)	2.1 (8.75)
T_7	26.00	13.6 (52.31)	6.5 (25.00)	4.1 (15.77)	1.8 (6.92)
T_8	39.00	22.6 (57.95)	10.2 (25.15)	4.6 (11.79)	1.6 (4.10)
T_9	29.00	14.0 (48.28)	8.0 (27.59)	5.4 (18.62)	1.6 (5.52)
T_{10}	20.50	10.0 (48.78)	5.2 (25.37)	3.5 (17.07)	1.8 (8.78)
T_{11}	24.00	12.0 (50.00)	6.0 (25.00)	4.4 (18.33)	1.6 (6.67)

Table 4. Physico-chemical properties of soil under different treatments.

Treatment	EC (dS/m)	pH	Organic C (%)	Available N(kg/ha)	Available P_2O_5(kg/ha)	Available K_2O (kg/ha)
Initial (2010)						
	0.38	8.20	0.49	198	11.40	111.0
Final (2012)						
T_1	0.34	8.00	0.44	201.4	18.52	218.2
T_2	0.34	8.20	0.51	209.2	16.10	226.4
T_3	0.33	7.18	0.51	212.2	16.20	244.4
T_4	0.36	7.60	0.56	244.6	17.15	286.4
T_5	0.40	7.60	0.54	258.4	9.55	240.6
T_6	0.35	7.06	0.56	244.2	9.85	240.4
T_7	0.38	7.08	0.59	279.2	8.85	240.2
T_8	0.42	7.08	0.58	284.2	12.57	220.4
T_9	0.42	7.96	0.54	240.8	11.15	220.2
T_{10}	0.38	7.68	0.54	252.2	16.45	196.8
T_{11}	0.39	7.80	0.56	248.2	8.65	186.2

the marked decline from highly alkaline to neutral side and availability of nutrients to trees in available form with better uptake and utilization (Table 4). Though the effect of treatment having only chemical fertilizers has not shown the appreciable improvement on the available N, P and K status of soil, but it was profoundly influenced by biofetilizers and organic manures, application, and was appreciably higher compared to initial status (Table 4).

The similar trend has also been reported by many workers (Gregorich *et al.* 1994, Chen *et al*. 2004, Panda 2006, Srivastava 2011) as integrated nutrient management system envisages the use of organic manures, biofertilizers and oraganic manures along with chemical fertilizers (inorganic). The combined use of biofertilizers with half of recommended dose of chemical fertilizers and organic manures as FYM along with vermicompost improved soil physical condition in best way for providing enhanced quantum of quality production as resultant of environmental manipulation for enhanced nutrient availability in rhizosphere (Hare Krishna, 2012).

Thus, it is concluded that treatment (T_8) comprising half of recommended fertilizer + Azotobactor (250 g) + 25 kg FYM + 5 kg vermicompost per tree gave maximum fruit yield (39.00 kg/plant), indicating the possibility of reduced use of chemical fertilizers to the tune of 50%. The improved quality of fruit yield with maximum category under extra class was also recorded under the treatment (T_8). The combined use of chemical fertilizers, organic manures and biofertilizers improved soil physical condition (soil health) and found feasible for orchard floor management with enhanced quantum of quality fruit production.

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The TEXT should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All symbols and abbreviations must be defined and used when necessary. Superscripts and subscripts should be clearly indicated. The units of measure should be metric.

Figures

The line drawings should be roughly twice of their final printed size. The photographs must have a scale bar, which should be defined clearly in the legend.

The map of India, if forming a part of the article, must conform to official maps released by the Survey of India, giving reference to the source of map.

The letter size in figures should be sufficiently large to be clear after reduction to print size.

Materials and Methods

Give relevant details of MATERIALS AND METHODS containing experiment given separately al design and techniques used. Cite standard work done. Statistical methods should be dealt in detail. Mean results with relevant standard error should be given rather than detailed data.

Results and Discussion

The RESULTS AND DISCUSSION should be combined to avoid the repetition. The Results should be supported by brief but adequate number of tables or graphs etc. Use metric unit system, e.g. km, m, cm etc.Tables and illustrations should be up to 20% of the text only. Both tables and illustrations should not repeat the same data.Cite adequate number of latest references. Old references should be avoided except standard ones.

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The REFERENCES should have author-year system. Give sir names of authors first, followed by their initials, year of publication, full title of article, name of the journal (without abbreviations; if you use abbreviations, write standardized abbreviations only), volume (No. in bold), issue No. (in parenthesis) and complete page-range. Ensure that names and year in the text correspond to those of given in the list of references. In case of books, give name of book, complete name of publisher and place of publication of books. For proceedings, complete details should be given.

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The RESEARCH REVIEW ARTICLES suggest new avenues of research, pointing out lacunae in research globally. Do not leave any point of future course of action.

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The page limit for a full length article is 3,500 words or 10-12 typed pages in double space, while for a Short Note, it is up to 1,500 words or 4-5 typed pages in double space.

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After receiving the article, it is scrutinized at the level of the Editor. If it fulfills the scientific requirement and is well-written and well-presented in the format of the Journal, it is sent to the subject-matter specialists/referees. The article is considered for publication on the basis of referee's comments.

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Soil science is becoming significant in regards to agriculture practices. The earth is divided into various forms of soils depending upon its structural & material content. One of the element catching the eyes world over in agriculture fraternity is silicon due to its involvement in almost all the life process of a plant. The earth soil has various silicon content & availability geographically, namely the areas can be called as Ultisols/spodosols – extensively weathered and somewhat silicon depleted soil, Oxisols – most highly weathered & most silicon depleted soil, Histosols – little mineral material & silicon deficient soil and Mollisols – less weathered and more silicon soil.

Silicon is the second most abundant element in the earth's crust. The average of the global soil consist of approximately 32 percent of silicon by weight. It is thus regarded as quasi essential for plants. Most of the plant species consist of up to 40 percent of silicon and is essential in various biochemical process.

Although a major portion of soil consist of various forms of silicon compounds, plant available silica (PAS) is mostly deficient. Practices like composting & recycling of organic waste material and its relevant ashes which are a good source of PAS are a diminishing factor in modern farming. It is therefore recommended that a silicon rich (PAS) soil amendment is necessary.

The key benefits of a good silicon amendment in the soil affects a plant life cycle in the following manner:

A good soil silica amendment can control both Biotic (plant disease and pest damage) and Biotic (salinity, drought, high temperature, chilling temperature, mineral nutrient uptake deficiency, UV radiation, heavy metal as well toxicity) stress conditions.

It can well maintain a relative water content (RWC) in a plant & helps in increasing chlorophyll content even during drought stress.

Post harvest upkeep quality of crops can be increased.

Silicon amendment increase active role in plant defence mechanism (plant response to various diseases).

Maintain homeostasis of plant cell for healthy growth.

High cationic exchange capacity.

Plants utilise silicon in the form of silica acid and are deposited in the epidermal cells of plants as silica gel in between cuticles and cell wall & between cell membrane & cell wall. This controls stress like drought by reducing evaporation and reduces pest attack as a physical barrier.

In view of above findings and research worldwide, silicon amendment has more significantly showed response over a better physiology and biochemistry of plants.

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