# INTROGRESSION AND CHARACTERIZATION OF ALLOPLASMIC MALE STERILE LINES IN BRINJAL (*Solanum melongena* L.)

**Thesis**

**Submitted to the Punjab Agricultural University**
**in partial fulfillment of the requirements**
**for the degree of**

**MASTER OF SCIENCE**
**in**
**HORTICULTURE (Vegetable Science)**
**(Minor Subject: Plant Breeding and Genetics)**

**By**
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## CERTIFICATE I

This is to certify that the thesis entitled, **"Introgression and characterization of alloplasmic male sterile lines in brinjal (*Solanum melongena* L.)"** submitted for the degree of **M.Sc.** in the subject of **Horticulture (Vegetable Science)** (Minor subject: **Plant Breeding and Genetics**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Karmvir Singh Garcha (L-2014-A-156-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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**CERTIFICATE II**

This is to certify that the thesis entitled, **"Introgression and characterization of alloplasmic male sterile lines in brinjal (*Solanum melongena* L.)"** submitted by **Karmvir Singh Garcha** (Admn No. **L-2014-A-156-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Horticulture (Vegetable Science)** (Minor subject: **Plant Breeding and Genetics**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

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## ABSTRACT

A study was conducted to introgress and characterize male sterile lines carrying alloplasmic cytoplasm of *S. aethiopicum* into *S. melongena* at Punjab Agricultural University, Ludhiana. Three backcross generations *viz.* $BC_3$, $BC_4$ and $BC_5$ were evaluated along with 12 recurrent parents for twenty-three morphological traits in Factorial Randomized Complete Block Design during 2015-16. The analysis of variance indicated significant variability among all the genotypes for all the traits, however among generations petiole length, leaf blade width, calyx size, pedicel length and fruit length displayed non-significant differences. The vegetative growth of CMS-backcross progenies was vigorous than their fertile counterparts. Non-availability of viable pollen in shriveled anthers in all the backcross generations indicated stable expression in all genotypes. All CMS-lines *viz.* BR 104A, MR 319A, BL 219A, BL 201A, BL 214A, BL 12-4A, BL 216A, SR 5A, P 67A, SR 232A SR 93-213A and CB 99-231A in $BC_5$ generation were morphologically similar in flower traits with respective recurrent parent (B-line), except petal size, stamen size and days to 50% flowering. Significant differences among fruit traits except fruit length were found in A and B-lines. Genetic variability estimates revealed high genotypic co-efficient of variability, heritability and genetic advance for all the fruit characteristics in all the generations. The overall recovery of all the characteristics in $BC_5$ generation was 97.98%. The availability of male sterile lines in diverse genetic backgrounds would be useful in heterosis breeding of brinjal.



**Keywords:** *S. aethiopicum*, *S. melongena*, cytoplasmic male sterility, introgression, characterization


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**ਸਾਰ-ਅੰਸ਼**


ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਵਿੱਚ *S. melongena* ਵਿੱਚ *S. aethiopicum* ਦਾ ਐਲੋਪਲਾਸਮਿਕ ਸਾਇਟੋਪਲਾਜਮ ਰੱਖਦੀ ਖੱਸੀ ਲਾਈਨ ਨੂੰ ਤਿਆਰ ਅਤੇ ਚਿਤਰਾਂਕਨ ਕਰਨ ਲਈ ਇਕ ਅਧਿਐਨ ਕੀਤਾ ਗਿਆ। $BC_3$, $BC_4$ ਅਤੇ $BC_5$ ਵਰਗੀਆਂ ਪੀੜੀਆਂ ਦੇ ਤਿੰਨ ਬੈਕਕਰੋਸ ਨੂੰ 12 ਪੁਨਰਾਵਤੀ ਜੰਮਹੇਤੂ ਦੇ ਨਾਲ 23 ਆਕ੍ਰਿਤੀ ਸੰਬੰਧੀ ਗੁਣਾ ਲਈ ਫੈਕਟੋਰੀਅਲ ਰੈਂਡਡੋਮਾਇਜਡ ਕੰਪਲੀਟ ਬਲਾਕ ਡਿਜਾਇਨ ਵਿਚ ਜਾਂਚਿਆ ਅਤੇ ਪਰਖਿਆ ਗਿਆ। ਵੇਰੀਏਸ਼ਨ ਦਾ ਵਿਸ਼ਲੇਸ਼ਣ ਦਰਸਾਉਂਦਾ ਹੈ ਕਿ ਸਾਰੇ ਜੀਨੋਟਾਇਪ ਵਿਚ ਸਾਰੇ ਗੁਣਾ ਲਈ ਵਿਭਿੰਨਤਾ ਸੀ ਜਦਕਿ ਪੀੜ੍ਹੀਆਂ ਵਿਚ ਪੱਤੇ ਦੀ ਡੰਡੀ, ਪੱਤੇ ਦੀ ਚੌੜਾਈ, ਕੈਲਕਸ ਦਾ ਆਕਾਰ, ਫੁੱਲ ਦੀ ਡੰਡੀ ਅਤੇ ਫਲ ਦੀ ਲੰਬਾਈ ਵਿਚ ਫਰਕ ਅਰਥਪੂਰਨ ਨਹੀਂ ਸੀ। ਸਾਰੀਆਂ CMS-ਬੈਕਰਰੋਸ ਪੀੜ੍ਹੀਆਂ ਵਿਚ ਸਾਰੇ ਵਨਸਪਤੀਕ ਗੁਣ ਆਪਣੇ ਬਹੁਪ੍ਰਜਨਕ ਵਿਰੋਧੀਆਂ ਨਾਲੋਂ ਜਿਆਦੇ ਸਨ। ਸਾਰੀਆਂ ਬੈਕਕਰੋਸ ਪੀੜ੍ਹੀਆਂ ਸੁੰਗੜੇ ਹੋਏ ਪਰਾਗਕੋਸ਼ ਵਿੱਚ ਜੀਵਤ ਪਰਾਗ ਦੀ ਅਣਹੋਂਦ ਜੀਨੋਟਾਇਪ ਵਿਚ ਸਥਿਰਤਾ ਦਰਸਾਉਂਦੀ ਹੈ। BR 104A, MR 319A, BL 219A, BL 201A, BL 214A, BL 12-4A, BL 216A, SR-5A, P 67A, SR 232A, SR 93-213A ਅਤੇ CB 99-231A ਵਰਗੀਆਂ ਸਾਰੀਆਂ ਲਾਈਨਾਂ $BC_5$ ਪੀੜ੍ਹੀ ਵਿੱਚ ਫੁੱਲ ਸੰਬੰਧੀ ਗੁਣਾਂ ਵਿੱਚ ਆਕ੍ਰਿਤੀ ਸੰਬੰਧੀ ਗੁਣਾਂ ਲਈ ਇੱਕੋ ਜਿਹੀਆਂ ਸਨ, ਪਰ ਪੰਖੁਡੀ ਦਾ ਆਕਾਰ, ਪੰਕੇਸਰ ਆਕਾਰ ਅਤੇ 50% ਨਿਸਰਨ ਲਈ ਲੱਗਣ ਵਾਲੇ ਦਿਨਾਂ ਲਈ ਇਕੋ ਜਿਹੀਆਂ ਨਹੀਂ ਸਨ। ਫ਼ਲ ਦੀ ਲੰਬਾਈ ਨੂੰ ਛੱਡ ਕੇ ਬਾਕੀ ਸਾਰੇ ਫ਼ਲ ਸੰਬੰਧੀ ਗੁਣਾਂ ਲਈ ਏ ਅਤੇ ਬੀ ਲਾਈਨ ਵਿੱਚ ਅਰਥਪੂਰਨ ਫਰਕ ਸੀ। ਜਨੈਟਿਕ ਵਿਭਿੰਨਤਾ ਦਾ ਆਂਕਲਨ ਦਰਸਾਉਂਦਾ ਹੈ ਕਿ ਸਾਰੀਆਂ ਪੀੜ੍ਹੀਆਂ ਵਿੱਚ ਫ਼ਲ ਸੰਬੰਧੀ ਸਾਰੇ ਗੁਣਾਂ ਵੱਲ ਬਹੁਤ ਜ਼ਿਆਦਾ ਵਿਭਿੰਨਤਾ ਦਾ ਜੀਨੋਟਾਇਪਕ ਕੋ-ਏਫੀਸ਼ੈਂਟ, ਹੈਰੀਟੇਬਿਲਟੀ ਅਤੇ ਜੈਨੇਟਿਕ ਅਡਵਾਂਸ ਪਾਇਆ ਗਿਆ। $BC_5$ ਪੀੜ੍ਹੀ ਵਿੱਚ ਸਾਰੇ ਲੱਛਣਾਂ ਦੀ ਬਹਾਲੀ 97.98% ਸੀ। ਵਿਵਿਧ ਆਨੁਵਾਸ਼ਿਕੀ ਆਧਾਰ ਵਿਚ ਲਾਈਨਾਂ ਦੀ ਉਪਲੱਬਧਤਾ ਬੈਂਗਣ ਵਿਚ ਹੈਟਰੋਸਿਸ ਪ੍ਰਜਨਣ ਲਈ ਮੱਦਦਗਾਰ ਹੋ ਸਕਦੀ ਹੈ ।



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**CHAPTER-I**

# INTRODUCTION

Brinjal (*Solanum melongena* L.) is a diploid crop having 12 chromosomes (2n= 2x= 24). It is a non-tuberous species of family Solanaceae and also known with names of aubergine, eggplant or guinea squash in different parts of the world. Other cultivated species of *S. melongena* are *S. aethiopicum* L. and *S. macrocarpon* L. Cultivated brinjal and its wild relatives belong to subgenus Leptostenomum (Dunal), which includes more than 400 species distributed among 22 sections (Kantharajah and Golegaonkar 2004, Van Eck and Snyder 2006). Eggplant is of significant fiscal importance in many parts of Asia, Africa and other subtropical regions of the world (Sihachakr *et al* 1993). It is a good source of nutrients especially vitamins and minerals. Medicinal value of brinjal has been cited in *Sanskrit* literature also (Hinata 1986, Kalloo 1993). There are divergent views about the origin of brinjal and proposed to be originated in India (De Candolle 1984), China and India (Vavilov 1926, Vavilov 1951), South-west Asia (Rolfs 1919), Asia (Khan 1979) or African tropics (Lester and Hasan 1991). In India, it is cultivated over an area of 6.73 lakh hectares with 1.26 lakh tonnes production and 18.70 MT/ha productivity (Anonymous 2015). West Bengal covers highest area of 1.61 lakh hectares and produces 29.65 lakh tonnes of brinjal. Area under brinjal cultivation in Punjab is 3.9 thousand hectares with production of 82.8 thousand tonnes, whereas, productivity is 21.3 MT/ha (Anonymous 2015).

Brinjal bear hermaphrodite flowers, where heterostyled flowers are common in occurrence. Flowers have been classified into four types depending upon the style length *viz*. long styled, medium styled, pseudoshort styled and true short styled. Ovary sizes also vary in accordance with style length (Krishnamurthi and Subramaniam 1954). The percentage of flowers with long or medium styles is totally a varietal character, however flowers with long styled pistil are in majority (60%) followed by short (22%) and medium (15%) style flowers on a plant (Passam and Bolmatis 1997). Fruit setting with long styled flowers ranged between 70 to 86.7% and on medium styled between 12.5 to 55.6 %, whereas very rare fruit setting is there on short and pseudo short styled flowers (Rylski *et al* 1984). Brinjal is typically a self-pollinated crop, but cross-pollination can occur up to the extent of 29% with the help of the insects therefore designated as often cross pollinated crop. Anthesis in brinjal starts at 7:30 a.m. and continues until 11:30 a.m. however pollen viability decreases with increase in time. The pollen dehiscence is influenced mainly by the temperature along with light and humidity (Sidhu *et al* 1980). Pollinators found to have considerable effect on increasing the fruit set in the brinjal (Pal and Osvald 1967). The cultivated brinjal varieties exhibit great diversity in phenotypic, physiological and biochemical characteristics. The conventional breeding

approaches in brinjal with the exploitation of the genetic diversity present within the specie, have accomplished the feat of developing several improved varieties with regard to the fruit characteristics, resistance to insect-pests and diseases and yield (Kalloo 1993).

Heterosis, a biological phenomenon, expressed in $F_1$ generation is manifested with growth, earliness, uniformity, better quality, higher productivity and resistance to insect-pests and diseases. This tool has been exploited in brinjal also and number of commercial hybrids has been released in the country (Pal and Singh 1949, Mishra 1961, Sambandum 1962, Chadha and Sidhu 1982). Hybrid production involves crossing of female and male parents, wherein, control over pollination of female parent is pre-requisite for preventing self and ensuring cross-pollination. This control over pollination poses a great challenge in crops having hermaphrodite flowers. To overcome this problem several cultural, genetic and chemical measures like manual emasculation, genetic male sterility (GMS), cytoplasmic male sterility (CMS), cytoplasmic-genetic male sterility (CGMS), self-incompatibility, male-gametocides and biotechnological tools have been exploited. In India, seed production for hybrids in brinjal mainly relies upon manual emasculation and pollination, which is a time, labour and cost intensive. Therefore, reliable male sterility systems could become valuable tools for simplifying the procedure, reducing the time, labour, and cost of production for $F_1$ hybrid seeds as well as preventing contamination of selfed seed.

In brinjal, genetic male sterility (Jasmin 1954, Nuttall 1963, Chauhan 1984, Phatak and Jaworski 1989, Phatak *et al* 1991), cytoplasmic male sterility (Fang *et al* 1985, Saito *et al* 2009, Khan and Isshiki 2010, Khan and Isshiki 2011) and transgenic male sterility (Cao *et al* 2010, Toppino and Kooiker 2011) systems have been reported. Out of these, male sterility due to nuclear genes (GMS) has narrow practical utilization, because of its complex mode of inheritance and maintenance (Budar and Pelletier 2001). Besides, genetic male sterile lines reported instability in expression under varying environmental conditions (Hazra *et al* 2008). The acceptability of genetically engineered male sterility (transgenic male sterility) is still a mark of question due to non-acceptability by some anti GM organizations. Whereas, use and maintenance of CMS system have been preferred due to maternal inheritance and easy use in many field and vegetable crops (Hanson 1991, Schnable and Wise 1998, Kempken and Pring 1999).

Cytoplasmically inherited male sterility (CMS) is the result of specific nuclear/mitochondrial interactions. The association of nucleus and cytoplasm of different species often results in male sterility i.e. male sterility due to alloplasmic cytoplasm. The abnormal mitochondrial gene expressions causing sterility has been found in many cultivated plant species including maize (Levings 1990), petunia (Bino 1985) and sorghum (Pring *et al* 1995, Xu *et al* 1995). However, female fertility is mostly not affected by CMS, so the male sterile plants could produce seed, if viable pollen is provided. In brinjal also, cytoplasmic

male sterility due to interspecific crosses with wild species have been reported (Fang *et al* 1985, Isshiki and Kawajiri 2002, Saito *et al* 2009, Khan and Isshiki 2010, Khan and Isshiki 2011). The availability of this genetic mechanism can eliminate the need of emasculation and pollination for production hybrid seed. Although, CMS had been reported in several crop species, but scanty information is available in brinjal. Therefore, present study was focused to advance and characterize backcross generations of brinjal (*Solanum melongena* L.) carrying alloplasmic cytoplasm of *Solanum aethiopicum* with the following objectives:

- To transfer nuclear genome of brinjal having alloplasmic cytoplasm.
- To characterize alloplasmic male sterile lines for growth and yield traits of brinjal.

## CHAPTER-II

# REVIEW OF LITERATURE

Cytoplasmic male sterility is the result of specific nuclear and mitochondrial interactions. The association of cytoplasm and nucleus from different species often results in total or partial male sterility i.e. male sterility due to alloplasmic cytoplasm. The literature on effect of alloplasmic cytoplasm on male sterility in brinjal is very scanty. Further, very few reports are available on the effect of alien cytoplasm on morphological characters of brinjal. Therefore, relevant literature pertaining to the effect of alloplasmic cytoplasm on male sterility and different morphological characters has been reviewed under the following heads:

2.1 Mitochondrial genes causing cytoplasmic male sterility

2.2 Alloplasmic male sterility in *S. melongena*

2.3 Alloplasmic male sterility in other cultivated crops

2.4 Characterization of cytoplasmic male sterile lines

2.5 Characterization of backcross progenies

2.6 Genetic variability

### 2.1 Mitochondrial genes causing cytoplasmic male sterility:

Dewey *et al* (1987) reported that T-male sterile cytoplasm (cms-T) of maize is associated with mitochondrial DNA containing an open reading frame (ORF-13). Antibodies raised to a chemically synthesized oligopeptide corresponding to ORF-13 were used to establish the expression of a 13-kDa protein from this reading frame. This 13-kDa polypeptide was synthesized uniquely in CMS-T maize, whose presence was decreased with the availability of the nuclear restorer gene (*Rfl*), suggesting the role in cytoplasmic male sterility. Nivison and Hanson (1989) with the use of anti *urf* peptide antibodies recognized a protein with molecular mass of 20-kDa in both cytoplasmic male sterile and fertile lines. A 25 kDa protein was also recognized but only in cytoplasmic male sterile lines. An isolated mitochondria was found to be producing this protein. This 25-kDa protein was much lower in quantity in the fertile plants carrying nuclear fertility restorer gene (*Rf*) in the same background of cytoplasmic male sterile cytoplasm.

Horn *et al* (1991) found that mitochondrial DNA sequence in the vicinity of the *atpA* gene was difference between CMS-lines of sunflower *(Helianthus annuus)* and their fertile maintainers. A unique 16 kDa protein was reported in the mitochondrial translation products of CMS-lines carrying the alloplasmic *H. petiolaris* cytoplasm, but was not present in lines with normal cytoplasm i.e. cytoplasm of *H. annuus*. This 16 kDa protein was also observed in fertility restored lines, but not in *H. petiolaris* linking it with CMS in sunflower. A male sterility causing mitochondrial DNA sequence (*orf*239) was introduced from common bean into the tobacco nuclear genome by He *et al* (1996). Several transformants with this DNA

sequence depicted a semi-sterile or male sterile phenotype. Expression of the gene fusions in transformed male sterile anthers was confirmed using ELISA, RNA gel blotting and light and electron microscopic immuno-cytochemistry. Köhler *et al* (1991) established that the mitochondrial DNAs of male fertile and sterile lines of sunflower differed by an inversion of 11 kb and insertion of 5kb. The recombination events within an inverted repeat of 261 bp were reason for rearrangements. The cytoplasmic malesterile line CMS*Baso* showed additional three transcripts of the *atpA* locus of about 2500, 1200 and 250 nucleotides, which were not present in fertile line *Baso.* A new open reading frame *(orfH 522)* of 522 nucleotides was co-transcribed with the *atpA* gene as an additional larger transcript of about 2500 nucleotides in *CMSBaso.* Translation product of this orfH 522 might be reason of CMS in sunflower.

Bergman *et al* (2000) identified a novel reading frame, *orf 274*, located upstream of *atp1* in the mitochondrial genomes of both *Nicotiana tabacum* and *N. repanda*, along with the male sterile and fertility-restored plants. Co-transcripts of this open reading frame and *atp1* were detected by RT-PCR in all cultivars, but with northern hybridization, these transcripts were detectable only in male sterile plants. Measurement of ATP and ADP levels, however, revealed that the ATP/ADP ratio differed significantly in floral buds of male sterile plants than in fertile plants. Yoshimi *et al* (2013) studied alloplasmic brinjal lines having cytoplasms from wild Solanum species showing cytoplasmic male sterility along with the cultivated eggplant *S. melongena*. The molecular basis of cytoplasmic male sterility (CMS) in alloplasmic lines of brinjal was detected. Male sterile plants of both *viz.* anther indehiscent and non-pollen formation types of CMS showed novel transcription patterns of *atp*1. A different transcription pattern of *cox*2 was observed only in the anther indehiscent type. Based on these differences, the DNA sequences of about a 4 Kbp segment in the *atp*1 region was determined. The novel open reading frames (*orf*s) were found for each of the CMS types and the cultivated eggplant. The cytoplasm of *S. kurzii* inducing the anther indehiscent type CMS had *orf* 312, and those of *S. aethiopicum* and *S. grandifolium* of non-pollen production type CMS had *orf* 218. The correspondence between the transcription patterns of these *orf*s and phenotypic expression of male sterility strongly suggested that these *orf*s are causal genes for each type of CMS.

**2.2 Alloplasmic male sterility in *S. melongena***

A partial male sterility in $F_1$ plants of cross *S. indicum* × *S. melongena* with 48.9% stainable pollen was reported; however no fruit setting was observed upon selfing. Fertility was restored in amphidiploid (*S. indicum-melongena*) obtained with colchicine treatment (Rajasekaran 1970). Isshiki and Kawajiri (2002) developed male sterile brinjal plants by crossing *S. violaceum* with *S. melongena* and then repeatedly backcrossing with *S. melongena* until $BC_4$ generation. Cytoplasm of *S. violaceum* in all $BC_4$ generation plants was confirmed

with chloroplast and mitochondrial DNA analysis. Khan and Isshiki (2008) further developed anther indehiscent type of functional male sterile line of brinjal by using the cytoplasm of *S. virginianum*. An interspecific $F_1$ hybrid between *S. virginianum* and *S. melongena* was backcrossed to *S. melongena* 'Uttara' using 'Uttara' as a recurrent pollen parent to produce four backcross generations. It was observed that anther indehiscence was there in all the plants of backcross generations although the $F_1$ hybrid along with parents was dehiscent.The incompatibility between the cytoplasm of *S. kurzii* Brace & Prain and nuclear genes of *S. melongena* caused alloplasmic male sterility in brinjal. *S. kurzii* as cytoplasm donor and *S. melongena* as nucleus donor parent produced male sterile brinjal by continuous backcrossing (Khan and Isshiki 2009)

Saito *et al* (2009) used 'Taibyo VF', a Japanese rootstock cultivar, a *S. grandifolium*× *S. melongena* hybrid having difficulty in seed setting, but $F_1$ plants of 'Taibyo VF' × 'LS1934' (*S. melongena*) produced some seeds and $F_2$ plants segregated into male sterile and fertile plants. All plants in the backcross of sterile progeny with *S. melongena* were sterile, indicating cytoplasmic male sterility caused by the *S. grandifolium* cytoplasm. Khan and Isshiki (2010) developed a new male sterile line of brinjal (*S. melongena* L.) by backcrossing $F_1$ rootstock 'Assist' (*S. aethiopicum* Aculeatum L. Group × *S. melongena* 'DMP') to *S.melongena* 'Uttara' using 'Uttara' as a recurrent pollen parent up to $BC_4$ generation. They observed that all the plants in the $F_1$ 'Assist' were fertile, while male fertile and sterile plants were there in $BC_1$. Same segregation into male fertile and sterile was followed in $BC_2$, $BC_3$ and $BC_4$ backcross generations with segregation ratio fitting to 3:1 in each generation. However, $BC_3$ and $BC_4$ progenies obtained from male sterile progenies fixed to male sterility without segregation. The selfed progenies of the fertile $BC_2$ progeny segregated into male fertile and sterile plants with segregation ratio of 15:1. On the basis of these findings, they assumed that incompatibility between the cytoplasm of *S. aethiopicum* (Aculeatum) and the nucleus of *S. melongena* was leading to pollen non-formation type of CMS.

Khan and Isshiki (2011) developed a new cytoplasmic male sterile line of brinjal utilizing cytoplasm from the *Solanum anguivi.* The interspecific hybrid (*S. anguivi* eggplant Senryo 2 gou) was backcrossed repeatedly to brinjal variety 'Uttara' up to $BC_5$ generation to achieve cytoplasm substitution, which was confirmed by chloroplast and mitochondrial DNA analysis. They got male sterile plants, whose anthers were completely devoid of pollen in the backcross progenies. All the male-fertile seed parents segregated into male-fertile and sterile types with a 3:1 ratio, whereas those obtained from the male sterile one were all male sterile. The progeny from a male-fertile $BC_5$ plant segregated into 15:1 whose progeny upon selfing segregated into 3:1. These results indicated that two independent dominant fertility restorer (*Rf*) genes control pollen formation. Hasnunnahar *et al* (2012) continuously backcrossed 'Taibyo VF' (*S. grandifolium* × *S. melongena*) to recurrent pollen parent *S. melongena*, for

development a cytoplasmic substitution line of brinjal. All the plants in $BC_1$ were male fertile, while segregation into fertile and sterile types was there in $BC_2$. The $BC_3$, $BC_4$, $BC_5$ and $BC_6$ progenies, obtained from the male fertile seed parents, were also segregated into the above mentioned types, whereas backcross progenies from sterile plants were all male sterile without any segregation. Male fertile plants segregated into either 1:1 or 3:1 ratio upon backcrossing whereas, selfed progenies of the male fertile plants fitted well to either 3:1 or 15:1 ratios. The progenies obtained from crossing between male fertile and sterile backcross progenies were fitted to 3:1 ratio. On the basis of segregation patterns, they concluded that two independent dominant fertility restorer (*Rf*) genes control the pollen formation of the *S. melongena* with the cytoplasm of *S. grandifolium.*

Krommydas *et al* (2015) with the help of back cross method developed CMS-lines of three Hellenic eggplant cultivars (*viz.* 'Langada', 'Emi' and 'Tsakoniki') carrying cytoplasm of *S. violaceum*. Molecular analysis of cytoplasmic DNA (cp and mt DNA) in the CMS-lines proved the maternal inheritance of the cytoplasm.The cytoplasm of *S. violaceum* did not affect the female fertility but male fertile plants were obtained in the advanced backcross generations of CMS 'Tsakoniki'. The nuclear/cytoplasmic interactions effecting male fertility was confirmed with the presence of three male fertility phenotypes *viz.* male sterile, male fertile and potentially male fertile. The genetic analysis indicated that male fertility in the genetic background of *cv.* 'Tsakoniki' is controlled by one primary genetic locus and affected by a secondary modifying locus. Their results indicated that the expression of CMS as well as fertility restoration is affected by the genotype of the brinjal.

**2.3 Alloplasmic male sterility in other cultivated crops:**

Fan *et al* (1986) evaluated the $F_1$ progenies for male fertility from crosses involving 32 *Brassica napus* L. strains and alloplasmic male sterile plants with cytoplasms *viz. ogu*, *nap* and *pol*. All *B. napus* lines maintained the sterility in *ogu* cytoplasm while thirty strains fully restored the fertility of plants with the *nap* male sterility. The sterility in *nap* cytoplasm was partially maintained by cultivar *Bronowski,* whereas upon crossing with cultivar *Lergo* segregation *nap* male sterile plants segregated into for male fertile/sterile ones. For the *pol* male sterility all strains were maintainers or partial maintainers. Prakash and Chopra (1990) repeatedly backcrossed synthetic alloploid of cross *B. oxyrrhina* × *B. campestris* with *B. campestris* for substituting *B. campestris* nucleus genes in the cytoplasm of *B. oxyrrhina.* Alloplasmic plants, obtained in $BC_5$ generation, were all male sterile, but with mild chlorosis observed during initial development. Synthetic alloploid *B. oxyrrhina-campestris* was also crossed with *B. juncea* to transfer into *B. oxyrrhina* cytoplasm. In $BC_1$ and $BC_2$ progenies green and chlorotic plants were observed. However, with the help of selection in $BC_3$ normal green colour male sterile *B. juncea* plants were obtained in $BC_3$. Pollen abortion in both *B. campestris* and *B. juncea* was post-meiotic.

Dalamcio *et al* (1995) made crosses of 46 accessions of *Oryza perennis* and two strains of *O. rufipogon* as female parents were with two restorers *viz.* IR54, IR64 of WA cytosterility. Backcrosses of sterile hybrids with their respective recurrent parents were made. Of all the backcross progenies, one line with the nuclear background of IR64 and cytoplasm of *O. perennis* Acc 104823 was stable for male sterility. This CMS-line was designated as IR66707A. It was completely sterile under self-conditions. Crosses of IR66707A with already available 10 restorers of WA cytoplasm displayed almost complete (93-100%) sterility, depicting the different source of CMS system than existing WA sterility.

Malik *et al* (1999) developed two new CMS-lines in *B. juncea* using the bridge-cross hybrids (*Diplotaxis erucoides* × *B. campestris*) × *B. juncea* and (*Diplotaxis berthautii* × *B. campestris*) × *B. juncea*. Backcrosses with of *B. juncea* were made upto $BC_5$ generation. The CMS-line with cytoplasm of *D. erucoides* segregated into true breeding types of tall and short plants. Both were comparable to *B. juncea* in vegetative and floral characteristics and in cytology, except for a more number of secondary branches and smaller anthers with no pollen in the CMS-line. Female fertility of CMS-line was also comparable to the cultivar. The other CMS-line having *D. berthautii* cytoplasm was also lookalike to the cultivar in vegetative morphology and cytology. Four true breeding floral types *viz.* smaller and indehiscent anthers containing no pollen, all six stamens petaloid, one petaloid stamen and five stamens with no anther and apetalous flowers i.e. with no anthers in all six stamens were discovered. Islam *et al* (2000) assessed 148 exotic rice germplasm lines for pollen sterility at flowering stage. Sixteen genotypes showed hundred percent pollen sterility status, which was considered as completely male sterile lines (A-line). Other sixteen genotypes were displayed 80% and above pollen and spikelet fertility making them to be identified as completely fertile lines. For identification of maintainer lines, the identified 16 CMS-lines were open crossed with established known maintainer lines *viz.* GAN46B, IR 68888B, IR 58025B, IR 62829B and BRRI1B. Based on pollen fertility status of the $F_1$ lines, it was pointed out that 10 lines out of total 16 were maintained by line IR 58025B, eight by IR 62829B, three by IR 68888B and one by GAN46B and BRRI1B.

Deol *et al* (2003) developed CMS with the help of backcross method by substituting the nuclear genome of *B. rapa* into the cytoplasmic background of *Enarthrocarpus lyratus.* There were some abnormalities in alloplasmic male sterile plants such as pale green leaves, small flowers having narrow petals and undeveloped anthers. Female fertility was initially low but improved with the advancement of each backcross generation. Stable male sterility during the whole growing season was observed. All *B. rapa* accessions except for EC 339014, partially maintained the male sterility. Saxena *et al* (2005) developed a stable CMS system in pigeon pea (ICP 2039A) with inter-specific crossing of *Cajanus cajanifolius*, a wild relative with cultivar ICP 11501. A total of 150 crosses were made on *C. cajanifolius* plant using

fresh pollen from ICP 11501. The success rate was low and only nine pods were set that produced sixteen hybrid seeds, of which twelve germinated. The anthers of these plants were fully developed with light yellow in colour and contained small amounts of pollen. The acetocarmine test revealed partial pollen fertility in each plant and it ranged from 40 to 80%. All eight $BC_1F_1$, plants were completely male sterile with no trace of pollen grains. In $BC_2F_1$, out of five plants grown four were male sterile. In the further backcross generations ($BC_3F_1$–$BC_7F_1$), all plants were male sterile indicating cytoplasm as source of male sterility.

Rosamma and Vijaykunar (2005) assessed the maintainer/restorer ability of 34 lines/cultivars by crossing them with seven cytoplasmic-genic male sterile lines of rice (WA cytoplasm) and one line of *O. perennis* CMS source. Difference in fertility reactions upon crossing with WA cytoplasm was observed while stability in sterility in hybrids produced with crossing with *O. perennis* source was obtained. Effective restorers recognized for WA cytosterility were, IR 36, Mattatriveni, Annapoorna, Aiswarya and Kanchana. Jyothy acted as a stable maintainer i.e. produced completely sterile hybrids with all CMS-lines. Zhao and Gai (2006) made crosses between seventy cultivated and annual wild soybean accessions with three maintainers (N2899, N21249, and N23998) of CMS plant NJCMS1A for detecting potential new sources with male sterile cytoplasm. The results showed that in addition to already available CMS sources, crosses N21566 × N21249 and N23168 × N21249 had male sterile plants in their progenies. The male sterile plants derived from [(N21566 × N21249) $F_1$ × N21249] $BC_1F_1$ were back-crossed with the recurrent parent N21249 for five successive times, and a new CMS-line along with its maintainer line, i.e. NJCMS3A and NJCMS3B, respectively, were developed. Newly developed CMS-line had stable male sterility with no effect on female fertility. The male fertility of $F_1$ hybrids between the sterile line NJCMS3A and twenty male parents revealed that fertility was restored with seven accessions and other thirteen acted as maintainers of its male sterility.

Wan *et al* (2008) identified a new CMS system in *B. juncea*, and termed it as '*hau* CMS' (00-6-102A). With the help of interspecific hybridization this male sterility was further transferred to *B. napus.* All flowers on A-line were male sterile, and seeds harvested from them after crossing with the maintainer gave rise to totally sterile progeny. Thickened petal-like structures replaced the anthers in and pollen grains were not produced. Sharma *et al* (2011) used three CMS-lines of rice having WA cytosterility to cross with sixty genotypes for identifying the restorer/maintainer nature. Most of the genotypes gave variable fertility reactions on crossing with WA CMS-lines. Among the sixty genotypes, sixteen were restorers of all three CMS-lines. Three genotypes maintained complete sterility with all the three CMS-lines, which could be used in developing new male sterile lines. Vu *et al* (2011) developed alloplasmic lines in *Allium cepa*, with continuous backcrossing using *A. roylei* as cytoplasm donor. The chromosomes of a single $F_1$ plant between *A. roylei* and shallot were doubled, and

$BC_1$ as allotriploids was produced by backcrossing with shallot. Backcrossing of allotriploid with bulb onion was used for to produce $BC_2$ with 2n = 16, 17, 23 and 24. The pollen fertility was checked on $BC_2$ plants (16 chromosomes), and then backcrossing with bulb onion was again performed to evaluate seed-setting characteristics. The pollen fertility of $BC_2$ ranged from 0% to more than 10%. Pollen sterility was showed by a large number of plants. The results revealed that exploitation of *A. roylei* cytoplasm could be used for the development of hybrids with help from new CMS-lines in *Allium.*

Chamola *et al* (2013) crossed CMS-lines of *B. juncea* having mitochondrial genome of *Moricandia arvensis* and *B. napus* having *Erucastrum canariense*as mitochondrial genome, with cauliflower (*B. oleracea*) to transfer cytoplasmic male sterility. Embryo culture was required to recover these interspecific hybrids. Several back crosses were performed to transfer characters of *B. oleracea*. Recurrent parent phenotype recovery rate was faster in *B. napus* × *B. oleracea* than *B. juncea* × *B. oleracea*. $BC_3$ generation plants of cross *B. napus* × *B. oleracea* developed good compact curd and complete male sterility whereas those of cross *B. juncea* × *B. oleracea* were male sterile but still had genetic elements of *B. juncea.* Xian-Hua *et al* (2013) developed a novel CMS rice source, identified from Dongxiang wild rice (*O. rufipogon*). Dongxiang wild rice as female was crossed with Zhongzao 35, an indica inbred variety, as male and continuous backcrossing generations with Zhongzao 35 were produced. This sterile system was a of typical abortion type with less pollen compared with WA type cytoplasm. Sequential planting demonstrated complete and stable male sterility. Testcross experiment revealed that all the tested materials including maintainers and restorers of CMS-WA and Honglian type cytoplasm and other indica inbred varieties acted as maintainers with complete sterile progenies, suggesting that this novel CMS has totally different fertility restoration than CMS-WA and CMS-HL.

**2.4 Characterization of cytoplasmic male sterile lines:**

Isshiki and Yoshida (2002) found anther indehiscence was reason for sterility in the cytoplasmic male sterile line of brinjal carrying alloplasmic cytoplasm of *S. violaceum* Ort. Both number and stainability of pollen from male sterile plants were lower than nuclear donor i.e. *S. melongena*. Fruit set, number of seeds/fruit and seed germination of $BC_5$ male sterile plants was almost equal to those of *S. melongena* indicating that cytoplasm of *S. violaceum* had no negative effect on fertility of seed and other characteristics of alloplasmic brinjal. Pathania *et al* (2003) characterized cytoplasmic male sterile line of *B. juncea* carrying cytoplasm of *Diplotaxis catholica* derived through repeated back cross method. In CMS plants flowering was early by about 10 days, in addition CMS plants showed more growth and flowered for more time than fertile counterparts. The anthers were converted into petal like structures or tubular structures, flowers had smaller nectarines. Gynoecium exhibited a crooked style length and trilocular ovary. Seed fertility was also with sterility. RFLP analysis

specified that *mt*-genome of *D. catholica* and that of *B. juncea* were highly divergent. However, in Northern analysis, out of eight studied *mt* genes, an altered transcript pattern was recorded for only *atp*A whose transcript became shorter in restored plants, thereby making it associated with CMS.

Tao *et al* (2004) studied the effects of cytoplasm on agronomic traits in five *japonica* parents of *O. sativa* genotypes *viz.* Xinan 175, Reimei, Keqing No. 3, Todorokiwase, and Toride No. 1. These genotypes were used as females in crossing with 3 distinct *japonica* rice cultivars *viz.* 8-126, Lijiangxintuanheigu and Norinmochi No. 20. These nuclear genomes of these cultivars were substituted by seven backcrosses into the five cytoplasms using the original male as recurrent parent. Significant cytoplasm-nucleus interaction indicated that the cytoplasm and cytoplasm-nucleus interactions played important parts in yield along with low temperature tolerance and some other agronomic traits in *japonica* rice. Gireesh *et al* (2010) evaluated eighteen CMS-lines and their isogonic maintainers for agronomic and floral traits with standard check CMS-line IR 58025A. Generally A-lines took more number of days to 50 per cent flowering than the corresponding B-lines. All the CMS-lines were shorter than their corresponding maintainers. Number of panicles were more in B-line than in A-lines. More number of spikelets per panicle were observed in KCMS 17A, KCMS 16A and KCMS 21A. Maximum out crossing rate was noticed in KCMS 11A, KCMS 16A, CRMS 31A and KCMS 12A. Most of the CMS-lines showed 100 per cent pollen sterility and less than 3.4 per cent spikelet fertility. All the CMS-lines were non-scented in nature. Genotype KCMS 11A and IR 68888A were promising for panicle exertion, the CMS-lines IR 70369A, CRMS 32A, KCMS 17A, KCMS 12A, KCMS 22A and KCMS 25A had long stigma. The style length of RTN 10A and CRMS 32A were high, while KCMS 25A and CRMS 32A exhibited greater angle between stigma lobes. The maintainer lines KCMS 10B, IR 70369, RTN 10B, IR 68888B excelled in anther length, anther breadth and filament length. Two CMS-lines *viz.,* KCMS 11A, KCMS 16A and CRMS 31A were identified as promising ones as they showed high out crossing rate, low pollen fertility and low spikelet fertility and are suitable for hybrids development.

Dey *et al* (2011) developed three Ogura based improved CMS-lines of cauliflower (*Brassica oleracea* var. *botrytis* L.) *viz*. *Ogu*1A, *Ogu*2A and *Ogu*3A. Backcross method was followed for seven generations with snowball group used as recurrent parent. These newly developed CMS-lines were characterized with their respective maintainer (B) line for various floral traits like petal size, shape of style, morphological traits like curd size and yields and seed setting traits *viz.* total number of seeds per pod and seed yield per plant. All the three new CMS-lines were in line with maintainer lines for traits like days to curd maturity. The curd yield of *Ogu*1A and *Ogu*3A were comparable to their respective maintainer lines. However, alien *Ogura* cytoplasm significantly lowered the petal size, filament length, style

length and stamen length. Number of pods/ plant was lower in all three CMS-lines than their maintainer B-lines. Number of seeds/ pod and seed yield/plant in *Ogu*3A and its respective B-line was comparable but differed significantly in *Ogu*1A and *Ogu*2A after introgression of Ogura cytoplasm. Raghavendra and Hittalmani (2015) studied the behaviour of introgressed male sterility with respect to various morphological traits in rice two $BC_2F_1$ populations derived from IR70369A × MAS 99 and KCMS31A × MAS 99. The plants obtained were completely male sterile. High heritability (>60%), narrow differences between PCV and GCV were observed for most traits. However, traits like stigma and panicle exertion, number of tillers/plant and spikelets/ panicle exhibited moderate heritability with wider differences between PCV and GCV.

Thakur *et al* (2015) compared the CMS progenies of cauliflower with their respective fertile maintainer lines for various yield attributes. The CMS progenies were also evaluated for their sterility behaviour during the flowering regimes *viz*. 25-50%, 50-75% and 75-100%. The CMS progenies had the head shape index similar with their respective recurrent/ maintainer lines. Most of the CMS genotypes were comparable with their respective maintainer for traits i.e. days to harvest, heading (%), non-rapper leaves, marketable heads per plot and gross weight. But in case of characters like compactness, net weight of heads and marketable head yield (kg/plot), the differences between maintainer and sterile progenies up to extent of 50%.

**2.5 Back cross progeny characterization:**

Since the backcrossing approach had been proposed by Harlan and Pope (1922) it has become a widely used approach for improving diverse crop species. Backcross methods are widely used for improving the traits governed by one or few genes.

Robertson and Frey (1984) evaluated sixty populations of 20 oat lines each, representing reciprocal crosses of the $BC_0$, $BC_1$, and $BC_2$, of all possible matings among five *Avena sterilis* L. strains and two *A. sativa* L. cultivars, for different agronomic traits. On an average, cytoplasm of *A. sterilis* displayed late heading date by 1.1 days and increased grain yield up to 10%. Characters like yield of straw, unit straw weight, plant height and vegetative growth index were positively affected by *A. sterilis* cytoplasm in the $BC_0$, but these advantages decreased by $BC_1$ and disappeared by $BC_2$. *A. sterilis* cytoplasm did not affect harvest index in $BC_0$, but by $BC_2$ it had elevated this trait by 1.1%. The interaction the between level of backcrossing and cytoplasmic effect for morphological traits resulted mainly because of interactions between *A. sterilis* cytoplasms and *A. sativa* nuclear genes or because of particular combinations of *A. sterilis* and *A. sativa* nuclear genes. Amoah (1988) used recurrent backcrosses to introgress nuclear genes from S. *tuberosum* L. ssp. *tuberosum* into the cytoplasm of Andean potatoes *S. tuberosum* ssp. *andigena* (Juz. and Buk.) and *S. phureja* (Juz. and Buk.). Reciprocal backcrosses were made in each generation to test the cytoplasmic

substitution effect on yield. Crosses direction had no constant effect on yield components substitution lines of ssp. *andigena*. In *S. phureja* significant differences for reciprocal progenies were there. The differences mostly resulted in the higher yield, when ssp. *tuberosum* was the pistillate parent, but in some progenies lower yields were also observed. The reciprocals difference was mainly due to chromosomally encoded gene action, resulting from maternal and/or paternal effects.

Atienza *et al* (2007) investigate the effect of *Hordeum chilense* cytoplasm on agronomic traits in common wheat (AABBDD). For this, the nuclear genome of bread wheat was transferred into the cytoplasm of *H. chilense* by repeated backcrossing to produce. Each alloplasmic line was compared with its respective euplasmic control. Almost all the traits were affected by the interaction between *H. chilense* cytoplasm and common wheat genome. Alloplasmic lines had delayed anthesis besides lower plant height and yield. Aegilops cytoplasm substitution is also known for causing the similar effects. Melo *et al* (2015) evaluated that $BC_1$ plants showed greater dissimilarity to their recurrent parent, but showed high genetic similarity with the non-recurrent *P. sublanceolata*.

**2.6 Genetic variability**

Mohanty (2002) evaluated 15 genotypes of brinjal for six quantitative characters and found that phenotypic coefficients were greater than corresponding genetic coefficients of variation for all the traits. High heritability, moderate to high genetic gain and high GCV were reported for fruit weight, fruit number/plant and branches on a plant. High heritability along with low GCV and genetic gain was there in case of plant height, days to first harvest and yield. Dhaka and Soni (2012) studied 20 genotypes of brinjal for genetic variability and found high PCV and GCV for average fruit weight and yield per plant, while low values were observed for days to first flowering and first picking. The high heritability and genetic advance as percent of mean was observed for number of fruits/ plant and average fruit weight. Prabhu *et al* (2009) observed high heritability and low genetic advance in the $BC_3F_1$ and $BC_3F_2$ progenies of EP 45 × *S. viarum* for mean fruit weight. Moderate heritability with low genetic advance for fruit weight was observed in the $BC_3F_1$ and $BC_3F_2$ progenies of CO 2 × *S. viarum* and the same were low for marketable yield in EP 65 × *S. viarum* and MDU 1 × *S. viarum.* High heritability with either high or moderate or high GA was observed in $BC_3F_1$ and $BC_3F_2$ generations of EP 45 × *S. viarum* and CO 2 × *S. viarum* for shoot borer infestation. High estimates of PCV and GCV for calyx length, number of fruits/ plant, little leaf incidence, total phenols, length and yield of fruit/ plant were there (Kumar *et al* 2013)

Lokesh *et al* (2013) did evaluation for 14 characters in 60 brinjal germplasm lines. High PCV and GCV values along with high GA(%) were found for traits *viz.* plant height, spread, number of branches/ plant, fruit diameter, weight and yield per plant. Mili *et al* (2014) studied genetic variability in 36 different genotypes of brinjal and observed high GCV and

heritability for single fruit weight, diameter, pulp seed ratio, fruits/ plant. High heritability estimates for fruit length, width and weight per fruit in were observed in $F_1$ generation by Singh *et al* (2014). Madhavi *et al* (2015) conducted investigation with 21 diverse brinjal lines and observed high PCV and GCV, heritability and GA for all of fruit characteristics. Patel *et al* (2015) did assessment of 35 brinjal genotypes for 21 characters and revealed higher PCV than GCV for all traits. Maximum PCV and GCV was registered for fruit length: diameter ratio while high estimate of heritability and genetic advance was found for most of the yield and its contributing characters.

**CHAPTER - III**

# MATERIALS AND METHODS

The present investigation entitled, "Introgression and characterization of alloplasmic male sterile lines in brinjal" was carried out during year 2014-15 and 2015-16 at Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana (Punjab). The details of the materials used and the techniques adopted during the course of investigation have been presented in this chapter.

## 3.1 Experimental site and climate

The experiment was conducted at Vegetable Research-Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana which is 30˚ 54' N latitude and 75˚ 48' E longitude at a mean height of 247 meters above sea level. The mean maximum and minimum temperature show considerable fluctuations during the summer, while minimum temperature falls below freezing point accompanied by frosty spells during winter. The average rainfall is about 500-700 mm, most of which is normally received from July-September.

## 3.2 Experimental details

### 3.2.1 Experimental plant material

Twelve $BC_3$, $BC_4$ and $BC_5$ progenies carrying alloplasmic cytoplasm of *Solanum aethiopicum* viz., BR 104, MR 319, BL 219, BL 201, BL 214, BL 12-4, BL 216, SR 5, P 67, SR 232, SR 93-213 and CB 99-231 along with their respective recurrent parents were used as experimental material.

### Methodology

A cross between *Solanum aethiopicum* × *Solanum melongena var.* Punjab Barsati was made during 2007 resulted into male sterile $F_1$. It was subsequently backcrossed with round (BR 104, MR 319), long (BL 219, BL 201, BL 214, BL 12-4, BL 216) and small-round fruited (SR 5, P 67, SR 232, SR 93-213 and CB 99-231) genotypes of brinjal. The backcross progenies having alloplamic cytoplasm of *Solanum aethiopicum* were at $BC_3$ stage during 2015. The crosses to produce seeds for $BC_4$ generation were attempted during July-August of 2015. The flower buds of male sterile as well as of recurrent parent going to open in next morning were covered with white parchment bags in the evening one day prior to pollination. In the next morning, the bags from the freshly opened male sterile and fertile flowers were removed and male sterile flowers were pollinated with the pollen of freshly opened flowers of respective recurrent parents. After pollination, each pollinated flower was tagged and again covered with help of cotton. Likewise, the crosses were made in all the twelve lines and simultaneously each male fertile parent (recurrent parent) was selfed by pollinating the bagged flowers with pollen from anthers of same flower or from other flower of same plant.

The $BC_4$ seeds of different lines and selfed seeds of recurrent parents were collected during October 2015. The $BC_3$ and $BC_4$ seeds were stored under low temperature conditions at 4 $^0$C.

Nursery to produce $BC_4$ generation of CMS-lines was sown in mid Nov 2015 along with the recurrent parent and transplanted in mid Feb 2016. Again crosses to produce seeds of $BC_5$ generation were attempted with respective recurrent parent. The seed of $BC_5$ generation was collected in May and sown along with $BC_3$, $BC_4$ and recurrent parent seed in the nursery. Seven plants of each set *viz.* BR 104A × BR 104B , MR 319A × MR 319B, BL 219A × BL 219B, BL 201A × BL 201B, BL 214A × BL-214B, BL 12-4A × BL 12-4, BL 216A × BL 216B, SR 5A × SR 5B, P 67A × P 67B, SR 232A × SR 232B, SR 93-213 × SR 93-213B and CB 99-231A × CB 99-231B and of each generation *viz.* $BC_3$, $BC_4$, $BC_5$ were transplanted in mid July 2016 at a distance of 60 cm between rows and 45 cm between plants. The experiment was laid out in a Factorial Randomized Complete Block Design (FRBD) with three replications. Five plants from each treatment were selected to record the observations. All package of practices recommended by the Punjab Agricultural University to raise a healthy crop of brinjal were followed (Anonymous 2015).

To ensure optimum fruit setting on male sterile lines, each flower was pollinated everyday with pollen collected by the Pollen-Collector. This process was continued for eight weeks period of flowering.

**3.3 Observations:**

Following observations were recorded during the course of experimentation:

**3.3.1 Plant height (cm)**

The plant height was recorded on five plants after last picking in November and mean values were calculated.

**3.3.2 Plant spread (cm)**

The plant spread was measured in criss-cross direction on five plants after last picking in November and mean values were calculated.

**3.3.3 Petiole length (cm)**

The petiole length of 5$^{th}$ leaf from top at full foliage stage of five plants was measured and mean values were calculated.

**3.3.4 Petiole colour**

The petiole colour of 5$^{th}$ leaf from top at full foliage stage of five plants was recorded with standard colour chart of the Royal Horticultural Society.

**3.3.5 Leaf blade length (cm)**

The leaf blade length of 5$^{th}$ leaf from top at full foliage stage of five plants was measured and mean values were calculated.

**3.3.6 Leaf blade width (cm)**

The leaf blade width of 5$^{th}$ leaf from top at full foliage stage of five plants was

measured and mean values were calculated.

**3.3.7 Leaf blade colour**

The leaf blade colour of 5$^{th}$ leaf from top at full foliage stage of five plants was recorded with standard colour chart of Royal Horticultural Society.

**3.3.8 Days to 50% flowering**

The days to 50% flowering was taken as number of days taken from sowing to anthesis of the first flower on three out of five plants.

**3.3.9 Calyx colour**

The calyx colour of five randomly selected flowers at full bloom stage of five plants was recorded with standard colour chart of Royal Horticultural Society.

**3.3.10 Calyx size (mm)**

The calyx size of five randomly selected flowers at full bloom stage of five plants was measured and mean values were calculated.

**3.3.11 Corolla colour**

The corolla colour of five randomly selected flowers at full bloom stage of five plants was recorded with standard colour chart of the Royal Horticultural Society.

**3.3.12 Petal size (mm)**

The aggregate size of all petals from five randomly selected flowers at full bloom stage of five plants was measured and mean values were calculated.

**3.3.13 Stamen size (mm)**

The stamen size including anther and filament of five randomly selected flowers at full bloom stage of five plants was measured and mean values were calculated.

**3.3.14 Pistil size (mm)**

The pistil size including stigma, style and ovary of five randomly selected flowers at full bloom stage of five plants was measured and mean values were calculated.

**3.3.15 Male fertility status (sterile/fertile)**

Male fertility was recorded on the basis of presence or absence of viable pollen in the anthers and observed under the microscope.

**3.3.16 Pedicel length (cm)**

The pedicel size of five randomly selected flowers at full bloom stage of five plants was measured and mean values were calculated.

**3.3.17 Fruit length (cm)**

The length of five randomly selected fruits at 3$^{rd}$ harvest from each treatment was measured from the middle of the fruit with Vernier's Caliper and mean values were calculated.

**3.3.18 Fruit girth (cm)**

The girth of five randomly selected fruits at 3$^{rd}$ harvest from each treatment was

measured from the middle of the fruit with Vernier's Caliper and mean values were calculated.

**3.3.19 Fruit length: girth ratio**

The recorded length and girth was used to calculate fruit length: girth ratio.

**3.3.20 Fruit colour**

The fruit colour of five randomly selected fruits at third harvest was recorded with standard colour chart of Royal Horticultural Society.

**3.3.21 Number of fruits/ plant**

The number of fruits per plant was computed by summing up the total number of fruits harvested in six picking of five plants of each treatment and calculating the average.

**3.3.22 Average fruit weight (g)**

The average fruit weight was calculated at third harvest by averaging weight of five randomly selected fruits.

**3.3.23 Fruit yield/ plant (kg/ plant)**

The total yield of five randomly selected plants was averaged to calculate per plant yield.

**3.4 Statistical Analysis:**

The data were compiled, tabulated and subjected to the statistical analysis as follows:

1. Analysis of variance for design of the experiment
2. Restoration of recurrent parent in backcross generations
3. Heritability and genetic advance analysis

**3.4.1 Analysis of variance for design of the experiment**:

Statistical analysis was done according to the CPCS-1 PACKAGE of Punjab Agricultural University, Ludhiana, using Factorial Randomized Complete Block Design. For conducting analysis of variance mean values of five plants from each generation of each genotype in three replications were used of twelve brinjal genotypes. The analysis of variance for Randomized Complete Block Design was based on following model:

$$Y_{ijk} = \mu + \delta_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + e_{ijk}$$

(i=1, 2, -------- r)

(j=1, 2, -------- a)

(k=1, 2, ------- b)

Where,

$Y_{ijk}$ = $i^{th}$ phenotypic performance of $j^{th}$ genotype in $k^{th}$ replication

$\mu$ = General mean effect

$\delta_i$ = $i^{th}$ replication effect

$\alpha_j$ = Effect of $j^{th}$ genotype

$\beta_k$ = Effect of $k^{th}$ generation

$(\alpha\beta)_{jk}$ = Effect of interaction due to $j^{th}$ genotype and $k^{th}$ generation

$e_{ijk}$ = Experimental error

**Analysis of variance**

| Source of variation | Degree of freedom | S.S | M.S.S | F value |
|---|---|---|---|---|
| **Replications** | r-1 | SSR | MSR = SSR/r-1 | MSR/MSE |
| **Genotypes** | a-1 | SSA | MSA = SSA/a-1 | MSA/MSE |
| **Generations** | b-1 | SSB | MSB = SSB/b-1 | MSB/MSE |
| **Genotype × Generation** | (a-1) (b-1) | SS A×B | MS A×B = SS A×B/ (a-1) (b-1) | MS A×B/MSE |
| **Error** | (r-1) (ab-1) | SSE | MSE = SSE/(r-1) (ab-1) | |
| **Total** | rab-1 | TSS | | |

*from a new table of 'ab' observations made by adding r observations in each cell

Where

$SST1 = \Sigma Y_{jk}^2/r - C.F$

C.F = Correction factor = $G^2/N$

TSS = Total Sum of Squares = $\Sigma_{ijk}^2$- C.F.

SSR = Replication Sum of Squares = $\Sigma r^2_i/ab$- C.F

SSA = Genotypes Sum of Squares = $\Sigma a^2_j/rb$- C.F.

SSB = Generations Sum of Squares = $\Sigma b^2_k/ar$- C.F.

SS A×B = Genotype × Generation interaction Sum of Squares = SST1-SSA-SSB

SSE = Error Sum of Squares = TSS-SSR-SSA-SSSB-SS A×B

MSR = Mean Sum of Squares due to replications

MSA = Mean Sum of squares due to genotypes

MSB = Mean Sum of squares due to generations

MS A×B = Mean Sum of squares due to Genotype × Generation interaction

MSE =` Mean Sum of Squares due to error

Calculated F values were compared with table values at error degrees of freedom at 5% level of significance.

Critical Difference (CD) values

$CD_A = SE\ t_{\text{error df, }\alpha} \frac{\sqrt{2MSE}}{\sqrt{rb}}$

$CD_B = SE\ t_{\text{error df, }\alpha} \frac{\sqrt{2MSE}}{\sqrt{ar}}$

$CD_{A\times B} = SE\ t_{\text{error df, }\alpha} \frac{\sqrt{2MSE}}{\sqrt{r}}$

### 3.4.2 Restoration of recurrent parent in backcross generations

Recipient parents shift towards recurrent parents with each backcross was estimated by

comparing trends in different generations, wherein, recurrent parent for each trait was set equal to 100% and all other generation means were calculated using following formula:

$$Restoration\ of\ character\ (\%) = \frac{Observed\ value}{Recurrent\ parent\ value} \times 100$$

The data was compared with expected theoretical rate of 93.75 in $BC_3$, 96.875 in $BC_4$ and 98.7544 in $BC_5$ generation (Fehr 1987) and the proportionate restoration of observed value over theoretical values were calculated.

### 3.4.3 Heritability and genetic advance analysis

**Analysis of variance for the design of the experiment**

For conducting analysis of variance mean values of five plants from each generation of each genotype among three replications were used for twelve brinjal genotypes. The analysis of variance for Randomized Complete Block Design was based on following model:

**Analysis of variance**

$Y_{ij} = m + g_i + b_j + e_{ij}$

Where,

$Y_{ij}$ = phenotypic value of the $i^{th}$ genotype of a generation grown in the $j^{th}$ replication

m = general population mean

$g_i$= effect of the $i^{th}$ genotype, where i = 1....g

$b_j$ = effect of the $j^{th}$ replication, where j = 1....r

$e_{ij}$= environmental effect

Analysis of variance based on the above model led to the following components of variance.

**Analysis of variance**

| Source of variation | Df | SS | MSS | | F value |
|---|---|---|---|---|---|
| | | | Observed | Expected | |
| Replications | r-1 | $Sr = \Sigma r^2/g - (\Sigma x)^2/N$ | $M_r = S_r / r-1$ | $\sigma_e + g\sigma_r$ | $M_r / M_e$ |
| Genotypes | g-1 | $Sg = \Sigma g^2/r - (\Sigma x)^2/N$ | $M_g = S_g / g-1$ | $\sigma_e + r\sigma_g$ | $M_g / M_e$ |
| Error | (r-1)(g-1) | $S_e = S_t - S_r - S_g$ | $Me = S_e / (r-1)(g-1)$ | $\sigma_e$ | |
| Total | gr-1 | $S_t$ | | | |

Where,

r = number of replications

g = number of genotypes

N = total number of observations

$S_r$ = replication sum of squares

$S_g$ = genotype sum of squares

$S_e$= error sum of squares

$S_t$ = total sum of squares

$\sigma_r$ = replication variance

$\sigma_g$ = genotypic variance

$\sigma_e$ = error variance

The genotypic variance was tested against error variance by 'F' test for (g-1) and (r-1) (g-1) degrees of freedom. Similarly the replication variance could be tested against error variance for (r-1) and (r-1) (g-1) degrees of freedom.

The standard error of difference between the genotypic means is based on r replications. It was estimated as follows:

$$SD(d) = \pm\sqrt{\frac{2M_e}{r}}$$

Critical Difference (CD) = SE (d) × $t_{(r-1)(g-1)}$ at 5% level of significance.

**3.4.3.1 Parameters for calculating heritability and genetic advance**

**a) Genotypic variance (VG)** = $M_g$-$M_e$/r

Where $M_g$ is genotypic mean square, $M_e$ is error and r is number of replications taken for particular character.

**b) Phenotypic variance (VP)** = $M_g$/r

Where $M_g$ is genotypic mean square and r is number of replications taken for particular character.

**c) Phenotypic coefficient of variance (PCV)** = Square root of phenotypic variance (VP) × 100 / Grand mean

**d) Genotypic coefficient of variance (GCV)** = Square root of genotypic variance (VG) × 100 / Grand mean

**e) Heritability percentage in broad sense ($H^2$)** = Genotypic variance (VG) / Phenotypic variance (VP) × 100

**f) Genetic advance (GA)** = Square root of phenotypic variance (VP) × $H^2$ × k

Where k is differential selection and for 5% selection k = 2.06

**g) Genetic advance percentage of mean** = Genetic advance × 100 / Grand mean

**3.4.3.2 Categories of the coefficients of variation:**

Coefficients of variation were categorized according to Sivsubramanian and Menon (1973) as follows:

| **Percent of variability** | **Category** |
|---|---|
| 0-10% | Lower |
| 11-20% | Medium |
| > 20% | High |

Categories of heritability values in accordance with Robinson *et al* (1949):

| **Per cent of heritability** | **Category** |
|---|---|
| 0-30% | Low |
| 31-60% | Medium |
| > 60% | High |

Further, the percent genetic advance over mean was classified as below:

| **GA as per cent of mean** | **Category** |
|---|---|
| <10% | Low |
| 10-20% | Medium |
| > 20% | Higher |

**CHAPTER-IV**

## RESULTS AND DISCUSSION

The results pertaining to the present investigation "Introgression and characterization of alloplasmic male sterile lines in brinjal (*Solanum melongena* L.)" have been discussed as under in different sub headings:

### 4.1 ANOVA for experimental design

The results pertaining to the analysis of variance for experimental design are given in Table 4.1. The mean sum of square values for genotypes were significant for all the characters *viz.*, plant height, plant spread, petiole length, leaf blade length, leaf blade width, days to 50% flowering, calyx size, petal size, stamen size, pistil size, pedicel length, fruit length, fruit girth, number of fruits/ plant, fruit weight and yield/ plant. This indicated significant differences among genotypes for all the characters studied. Mean sum of square values for different generations (recurrent parents and CMS backcross generations) were also significant

**Table 4.1: ANOVA for experimental design**

| Source | Replications | Genotypes | Generations | Genotype × generations | Error |
|---|---|---|---|---|---|
| **df** | 2 | 11 | 3 | 33 | 94 |
| **Plant height** | 950.22 | 842.6* | 612.32* | 15.37 | 31.78 |
| **Plant Spread** | 289.02 | 614.77* | 212.73* | 13.48 | 30.43 |
| **Petiole length** | 0.59 | 3.49* | 0.48 | 0.32 | 0.12 |
| **Leaf blade length** | 0.88 | 13.05* | 6.28* | 0.12 | 0.56 |
| **Leaf blade width** | 1.33 | 17.42* | 1.09 | 0.6 | 0.52 |
| **Days to 50% flowering** | 16.35 | 283.29* | 965.91* | 46.88* | 13.67 |
| **Calyx Size** | 8.94 | 27.14* | 0.48 | 0.16 | 1.06 |
| **Petal Size** | 2.44 | 38.33* | 9.86* | 0.96 | 2.16 |
| **Stamen size** | 0.67 | 6.85* | 93.05* | 0.79* | 0.38 |
| **Pistil size** | 0.66 | 13.27* | 1.92* | 0.38 | 0.53 |
| **Pedicel length** | 0.35 | 34.52* | 0.3 | 0.18 | 0.74 |
| **Fruit length** | 0.2 | 86.75* | 0.22 | 0.19 | 0.35 |
| **Fruit girth** | 0.15 | 15.34* | 1.61* | 0.12 | 0.88 |
| **Fruit number/ plant** | 1.38 | 1699.72* | 259.56* | 6.61 | 11.56 |
| **Fruit weight** | 218.24 | 25402.8* | 132.6* | 4.51 | 27.19 |
| **Fruit yield/plant** | 0.12 | 2.24* | 1.24* | 0.3 | 0.64 |

*Significant at 5% level

in plant height, plant spread, leaf blade length, days to 50% flowering, petal size, stamen

size, pistil size, fruit girth, number of fruits/ plant, fruit weight and yield/ plant revealing significant differences among generations for these characters. However, petiole length, leaf blade width, calyx size, pedicel length and fruit length displayed non-significant differences among the generations, revealing early recovery of these characters in comparison with respective recurrent parents. Genotype × generation interaction was significant only in days to 50% flowering and stamen size, which might be due cytoplasmic male sterility effect as CMS plants were late in flowering and had shriveled anthers with no pollen.

**4.2 Characterization of CMS-lines**

**4.2.1 Plant height**

The comparison of different backcross generations of CMS-lines with recurrent parent for plant height (cm) is presented in Table 4.2. Overall mean of $BC_3$ (71.40), $BC_4$ (68.92) and $BC_5$ (66.59) generations of recipient CMS-lines (A-line) was significantly different from the recurrent parents (B-line). Among different backcross generations, $BC_3$ had maximum plant height, but was at par with $BC_4$ and significantly different than $BC_5$ and recurrent parents. A significant difference was observed for plant height among genotypes, wherein, maximum plant height was recorded in SR 93-213 (84.85), which was significantly

**Table 4.2: Comparison of different backcross generations of CMS-lines with recurrent parents for plant height (cm) in brinjal**

| Genotype | B-line | Generations of A-lines* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 64.36 | 66.70 | 67.82 | 69.75 | **67.16** |
| **MR 319 (B or A)** | 64.99 | 68.01 | 69.57 | 70.22 | **68.20** |
| **SR 5 (B or A)** | 62.24 | 68.78 | 70.30 | 73.67 | **68.75** |
| **P 67 (B or A)** | 63.29 | 71.55 | 73.74 | 78.60 | **71.79** |
| **CB 99-231 (B or A)** | 66.28 | 73.95 | 77.78 | 80.89 | **74.72** |
| **BL 219 (B or A)** | 44.87 | 55.80 | 56.15 | 58.03 | **53.71** |
| **BL 214 (B or A)** | 64.60 | 69.12 | 71.93 | 74.67 | **70.08** |
| **BL 201 (B or A)** | 65.44 | 68.04 | 69.06 | 69.83 | **68.09** |
| **BL 12-4 (B or A)** | 63.08 | 62.85 | 63.11 | 63.51 | **63.14** |
| **BL 216 (B or A)** | 51.94 | 56.43 | 59.96 | 62.60 | **57.73** |
| **SR 232 (B or A)** | 52.00 | 56.81 | 59.70 | 61.94 | **57.61** |
| **SR 93-213 (B or A)** | 77.31 | 81.06 | 87.96 | 93.07 | **84.85** |
| ***Mean*** | **61.70 -** | **66.59 (+7.93)**** | **68.92 (+11.7)**** | **71.40 (+15.72)**** | |
| **CD (P=0.05)** | **Genotype = 4.57** | **Generations = 2.64** | **Genotype × Generations = NS** | | |

* Cytoplasmic Male sterile (CMS) lines

** Percent deviation from recurrent parent mean

taller than all other genotypes. The minimum height was recorded in BL 219 (53.71) with non-significant differences from SR 232 (57.61) and BL 216 (57.73). However, interaction of backcross generations with the genotypes displayed non-significant results.

Over the recurrent parent, increase in plant height of recipient CMS $BC_5$, $BC_4$ and $BC_3$ generations was 7.93, 11.70 and 15.72%, respectively. The increase may be attributed to less number of fruit set and yield on male sterile plants than that of their fertile counterpart. Similar results were obtained by Pathania *et al* (2003) in CMS-lines of cabbage, which continued to grow for longer duration than their maintainer lines and attained more height. Rao *et al* (1994) also reported taller plants in $BC_4$ generation of *D. siifolia* based CMS system in *B. juncea.* However, lower plant height of CMS rice lines as compared to their maintainers was reported by Gireesh *et al* (2010). Singh and Srivastava (2006) reported no difference in plant height of $BC_3$ generation CMS plants with their maintainers in *Trachystoma* and *Moricandia* based CMS-lines of *B. juncea.*

**4.2.2 Plant Spread**

The data on the plant spread (cm) recorded after the last picking is presented in Table 4.3. A perusal of data indicated numerically decrease in plant spread with advancement in

**Table 4.3: Comparison of different backcross generations of CMS-lines with recurrent parent for plant spread (cm) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 85.72 | 86.07 | 85.49 | 87.47 | **87.47** |
| **MR 319 (B or A)** | 63.91 | 64.45 | 63.79 | 62.72 | **62.72** |
| **SR 5 (B or A)** | 62.61 | 64.33 | 69.01 | 67.60 | **67.60** |
| **P 67 (B or A)** | 69.72 | 75.23 | 75.29 | 76.86 | **76.86** |
| **CB 99-231 (B or A)** | 73.47 | 77.33 | 79.28 | 80.86 | **80.86** |
| **BL 219 (B or A)** | 60.39 | 64.09 | 67.63 | 68.06 | **68.06** |
| **BL 214 (B or A)** | 67.44 | 71.67 | 71.90 | 73.71 | **73.71** |
| **BL 201 (B or A)** | 74.47 | 75.15 | 75.57 | 76.45 | **76.45** |
| **BL 12-4 (B or A)** | 80.12 | 81.28 | 81.05 | 81.90 | **81.90** |
| **BL 216 (B or A)** | 64.06 | 66.08 | 67.12 | 70.06 | **70.06** |
| **SR 232 (B or A)** | 66.39 | 77.11 | 72.94 | 76.53 | **76.53** |
| **SR 93-213 (B or A)** | 72.00 | 79.67 | 83.67 | 86.30 | **86.30** |
| ***Mean*** | **70.03 -** | **73.54 (+5.01)**** | **74.40 (+6.24)**** | **75.71 (+8.11)**** | |
| **CD (P=0.05)** | **Genotype = 4.48** | **Generations = 2.58** | | **Genotype × Generations = NS** | |

*Cytoplamic Male sterile Lines

**Percent deviation from maintainer lines mean

each CMS backcross generation. Overall plant spread mean of recurrent parents (70.03) was minimum and differed significantly from overall means of all recipient CMS backcross generations. However, statistically there was no difference between all three backcross generations i.e. $BC_3$ (75.71), $BC_4$ (74.40) and $BC_5$ (73.54). Among genotypes, differences for plant spread were significant with maximum value in BR 104 (87.47) followed by SR 93-213 (86.30) and minimum in SR 319 (62.72). Non-significant results for interaction between genotypes and generations were observed.

Over recurrent parent, plant spread of CMS $BC_5$, $BC_4$ and $BC_3$ generations positively deviated by 5.01, 6.24, 8.11%, respectively. The increase may be attributed to less number of fruit set and yield on male sterile plants than that of fertile counterparts. The results may be verified from the facts that increase in fruit setting and yield resulted in lower plant height and spread of brinjal. Such observations on plant growth of cabbage and *B. juncea* have been given by Pathania *et al* (2003) and Singh and Sirivastava (2006).

**4.2.3 Petiole length**

The comparison of different backcross CMS generations (A-lines) with their recurrent parents for petiole length (cm) is presented in Table 4.4. The results revealed non-

**Table 4.4: Comparison of different backcross generations of CMS-lines with recurrent parent for petiole length (cm) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 3.45 | 3.48 | 3.43 | 3.43 | **3.45** |
| **MR 319 (B or A)** | 3.76 | 3.25 | 3.20 | 3.42 | **3.41** |
| **SR 5 (B or A)** | 3.31 | 3.37 | 3.25 | 3.43 | **3.34** |
| **P 67 (B or A)** | 4.68 | 4.74 | 4.53 | 4.64 | **4.65** |
| **CB 99-231 (B or A)** | 2.88 | 2.78 | 2.81 | 2.82 | **2.82** |
| **BL 219 (B or A)** | 3.96 | 3.73 | 3.79 | 3.76 | **3.81** |
| **BL 214 (B or A)** | 4.33 | 4.36 | 4.18 | 4.18 | **4.26** |
| **BL 201 (B or A)** | 3.35 | 3.54 | 3.63 | 3.57 | **3.52** |
| **BL 12-4 (B or A)** | 4.14 | 4.16 | 4.07 | 4.08 | **4.11** |
| **BL 216 (B or A)** | 4.24 | 4.32 | 4.31 | 4.22 | **4.27** |
| **SR 232 (B or A)** | 3.94 | 3.82 | 3.97 | 3.83 | **3.89** |
| **SR 93-213 (B or A)** | 4.50 | 4.55 | 4.40 | 4.37 | **4.45** |
| ***Mean*** | **3.88 -** | **3.84 (-1.03)**** | **3.80 (-2.06)**** | **3.81 (-1.80)**** | |
| **CD (P=0.05)** | **Genotype = 0.28** | **Generations = NS** | | **Genotype × Generations = NS** | |

*Cytoplasmic Male sterile

** Percent deviation from recurrent parent mean

significant differences for mean petiole length of different backcross generations and recurrent parents. However, numerically maximum petiole length was found in recurrent parent (3.88) followed by $BC_5$ (3.84), $BC_4$ (3.80) and $BC_3$ (3.81). There were significant differences among genotypes for petiole length, wherein, maximum length was recorded in P 67 (4.65) followed by SR 93-213 (4.45) and BL-216 (4.27) and minimum in CB 99-231 (2.82). Overall, there was reduction in percent of petiole length in $BC_5$ (-1.03), $BC_4$ (-2.06) and $BC_3$ (-1.8) generations of A-lines than the fertile B-lines. The interaction between genotype and generations also manifested non-significant results. The results are in concordance with of Wan *et al* (2014) who reported no difference in petiole length in $BC_8$ generation of *hau* based CMS-line 0912A and maintainer line 0912B in leaf mustard.

**4.2.4 Petiole Colour**

Table 4.5 reveals the petiole colour of different backcross generations of CMS-lines along with their respective recurrent parents. There was no variation in colour of different backcross generations of recipient CMS-lines from their recurrent parents, hence can be assumed as a highly heritable trait in brinjal. There was green petiole colour in eight genotypes viz. MR 319, SR 5, BL 219, BL 214, BL 201, BL 12-4, SR 232, SR 93-213, grayed purple in three *viz.* BR 104, P 67, CB 99 - 231 and purple in one *viz.* BL 216. Further among

**Table 4.5: Comparison of different backcross generations of CMS-lines with recurrent parent for petiole colour in brinjal**

| Genotypes | B-line | Generations of A-lines | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Grayed Purple (139A) | Grayed Purple (139A) | Grayed Purple (139A) | Grayed Purple (139A) |
| **MR 319 (B or A)** | Green (143A) | Green (143A) | Green (143A) | Green (143A) |
| **SR 5 (B or A)** | Green (143B) | Green (143B) | Green (143B) | Green (143B) |
| **P 67 (B or A)** | Grayed Purple (N 186A) | Grayed Purple (N 186A) | Grayed Purple (N 186A) | Grayed Purple (N 186A) |
| **CB 99-231 (B or A)** | Grayed Purple (139A) | Grayed Purple (139A) | Grayed Purple (139A) | Grayed Purple (139A) |
| **BL 219 (B or A)** | Green (143A) | Green (143A) | Green (143A) | Green (143A) |
| **BL 214 (B or A)** | Green (143A | Green (143A | Green (143A | Green (143A |
| **BL 201 (B or A)** | Green (143B) | Green (143B) | Green (143B) | Green (143B) |
| **BL 12-4 (B or A)** | Green (143A) | Green (143A) | Green (143A) | Green (143A) |
| **BL 216 (B or A)** | Purple (79A) | Purple (79A) | Purple (79A) | Purple (79A) |
| **SR 232 (B or A)** | Green (143A) | Green (143A) | Green (143A) | Green (143A) |
| **SR 93-213 (B or A)** | Green (143A) | Green (143A) | Green (143A) | Green (143A) |

* Cytoplasmic Male sterile

**Value in parenthesis represents shades of colour as per The Royal Horticultural Society Colour Charts

green petioles, a slight variation for intensity of colour (143A and 143B) distinguished with the charts of The Royal Horticultural Society was observed. Similarly, in grayed-purple colour two shades *viz.* 139A and N-186A was noticed.

### 4.2.5 Leaf blade length

The data pertaining to the leaf blade length (cm) of various recipient generations along with their recurrent parents is presented in Table 4.6. The results revealed that leaf blade length decreases with advancement of backcross generation. The overall mean of leaf blade length of recurrent parents (13.98) differed significantly from all three backcross generations. The maximum leaf blade length was observed in $BC_3$ (14.90), but was at par with $BC_4$ (14.79) and $BC_5$ (14.70). The leaf blade length of different genotypes differed significantly with maximum value in BR 104 (15.83) and minimum in BL 12-4 (12.64). Whereas, genotypes BR 104, SR 93-213, BL 214, P 67 and BL 216 did not show any differences among themselves. The interaction between genotype and generations for this trait was non-significant.

**Table 4.6: Comparison of different backcross generations of CMS-lines with recurrent parent for leaf blade length (cm) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 15.12 | 15.79 | 16.03 | 16.38 | **15.83** |
| **MR 319 (B or A)** | 13.61 | 13.60 | 13.65 | 13.70 | **13.64** |
| **SR 5 (B or A)** | 13.40 | 14.47 | 14.57 | 14.61 | **14.26** |
| **P 67 (B or A)** | 14.97 | 15.61 | 15.63 | 15.69 | **15.48** |
| **CB 99-231 (B or A)** | 14.53 | 15.23 | 15.37 | 15.42 | **15.14** |
| **BL 219 (B or A)** | 13.27 | 14.50 | 14.68 | 15.08 | **14.38** |
| **BL 214 (B or A)** | 15.00 | 15.64 | 15.73 | 15.78 | **15.54** |
| **BL 201 (B or A)** | 13.01 | 13.57 | 13.84 | 13.83 | **13.56** |
| **BL 12-4 (B or A)** | 12.19 | 12.74 | 12.84 | 12.77 | **12.64** |
| **BL 216 (B or A)** | 14.46 | 15.60 | 15.54 | 15.51 | **15.28** |
| **SR 232 (B or A)** | 12.97 | 13.90 | 13.76 | 14.08 | **13.67** |
| **SR 93-213 (B or A)** | 15.20 | 15.73 | 15.87 | 15.93 | **15.68** |
| ***Mean*** | **13.98 -** | **14.70 (5.15)**** | **14.79 (5.79)**** | **14.90 (6.58)**** | |
| **CD (P=0.05)** | **Genotype = 0.61** | **Generations = 0.35** | **Genotype × Generations = NS** | | |

*Cytoplasmic Male sterile lines
** Percent deviation from recurrent parent mean

The leaf blade length of $BC_3$, $BC_4$ and $BC_5$ generations deviated positively by 6.58, 5.79 and 5.15%, than the recurrent parent, respectively. The excessive leaf blade length could be due to more vegetative growth and less fruits per plant on CMS-lines. Such findings have also been reported in mustard (Wan *et al* 2014), rice (Guo *et al* 2009) and wheat (Atiezna *et al* 2007).

### 4.2.6 Leaf blade width

The leaf blade width (cm) of different generations of A-lines along with respective B-lines of twelve genotypes is given in Table 4.7. However, leaf blade width of $BC_3$ generation (9.50) was highest, but was at par with other backcross generations *viz*. $BC_4$ (9.37) and $BC_5$ (9.32) and recurrent parent (9.08) averages. Among all twelve genotypes, maximum leaf blade width was observed in SR 93-213 (11.36) and minimum in CB 99-231 (7.83). The interaction between genotypes and generations showed non-significant differences for this trait.

The backcross generations *viz.* $BC_3$, $BC_4$ and $BC_5$ demonstrated 4.63, 3.19 and 2.64 percent more width then the recurrent parent, respectively. The excessive leaf growth could be due to more vegetative growth and less fruits setting per plant on CMS-lines. More vegetative growth has also been reported in mustard (Wan *et al* 2014), rice (Guo *et al* 2009) and wheat (Atiezna *et al* 2007).

**Table 4.7: Comparison of different backcross generations of CMS-lines with recurrent parent for leaf blade width (cm) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 10.13 | 10.35 | 10.57 | 10.64 | **10.42** |
| **MR 319 (B or A)** | 9.39 | 9.40 | 9.20 | 9.23 | **9.30** |
| **SR 5 (B or A)** | 9.02 | 9.18 | 9.08 | 9.13 | **9.10** |
| **P 67 (B or A)** | 10.57 | 10.79 | 11.13 | 11.37 | **10.96** |
| **CB 99-231 (B or A)** | 7.60 | 7.81 | 7.87 | 8.03 | **7.83** |
| **BL 219 (B or A)** | 8.15 | 8.45 | 8.59 | 8.69 | **8.47** |
| **BL 214 (B or A)** | 8.69 | 8.88 | 8.93 | 9.15 | **8.91** |
| **BL 201 (B or A)** | 7.82 | 8.14 | 8.31 | 8.32 | **8.15** |
| **BL 12-4 (B or A)** | 7.56 | 7.98 | 7.99 | 7.90 | **7.86** |
| **BL 216 (B or A)** | 10.11 | 10.57 | 10.41 | 10.50 | **10.40** |
| **SR 232 (B or A)** | 8.62 | 9.03 | 9.13 | 9.41 | **9.05** |
| **SR 93-213 (B or A)** | 11.30 | 11.33 | 11.22 | 11.58 | **11.36** |
| ***Mean*** | **9.08 -** | **9.32 (+2.64)**** | **9.37 (+3.19)**** | **9.50 (+4.63)**** | |
| **CD (P=0.05)** | **Genotype = 0.58** | **Generations = NS** | **Genotype × Generations = NS** | | |

*Cytoplasmic Male sterile lines
** Percent deviation from recurrent parent mean

### 4.2.7 Leaf blade colour

Leaf blade colour observed from various generations including CMS backcross generations and recurrent parents of twelve genotypes is given in Table 4.8. There was no variation in leaf blade colour of different A and B-lines. All genotypes have green (137 A, B

and C) leaf blade, except P 67 of violet blue (N 92) as per colour charts of The Royal Horticultual Society. There was no variation in colour of different backcross generations of recipient CMS-lines from their recurrent parents, hence can be considered as a highly heritable trait in brinjal. Prakash and Chopra (1990) also observed that synthetic alloploid *B. oxyrrhina-campestris* obtained through continuous backcrossing of *B. oxyrrhina* with *B. campestris* demonstrated normal green colour.

**Table 4.8: Comparison of different backcross generations of CMS-lines with recurrent parent for leaf blade colour in brinjal**

| Genotypes | B-line | Generations of A-line* | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Green (137B) | Green (137B) | Green (137B) | Green (137B) |
| **MR 319 (B or A)** | Green (137A) | Green (137A) | Green (137A) | Green (137A) |
| **SR 5 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **P 67 (B or A)** | Violet Blue (N 92) | Violet Blue (N 92) | Violet Blue (N 92) | Violet Blue (N 92) |
| **CB 99-231 (B or A)** | Green (137A) | Green (137A) | Green (137A) | Green (137A) |
| **BL 219 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **BL 214 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **BL 201 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **BL 12-4 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **BL 216 (B or A)** | Green (137C) | Green (137C) | Green (137C) | Green (137C) |
| **SR 232 (B or A)** | Green (137 C) | Green (137 C) | Green (137 C) | Green (137 C) |
| **SR 93-213 (B or A)** | Green (137 C) | Green (137 C) | Green (137 C) | Green (137 C) |

*Cytoplasmic Male sterile lines

** Value in parenthesis represents shades of colour as per The Royal Horticultural Society Colour Charts

**4.2.8 Days to 50% flowering**

Days to 50% flowering is an important parameter for determining the earliness of crops. Data presented in Table 4.9 demonstrated that days to 50% flowering differ significantly among backcross generations and recurrent parents. It was observed that all backcross generations differed significantly from recurrent parents (46.50), but were at par among themselves. Genotypes also differed significantly for days to 50% flowering, wherein, minimum number of days were taken by SR 5 (46.00) and maximum by BL 214 (61.33). Interaction between genotypes and backcross generations was also significant. The comparison of respective $BC_5$ generation and recurrent parents of MR 319, CB 99-231, BL 219, SR 232 and SR 93-213 showed non-significant differences among themselves, however, BR 104, SR 5, P 67, BL 214, BL 201, BL 12-4 and BL 216 differ significantly for days to

flowering. Overall, $BC_5$, $BC_4$ and $BC_3$ generations of A-line took 18.2, 21.33 and 25.36 percent more days to flowering, respectively. Our results are in concordance with the results of Kirti *et al* (1995) who reported that $BC_5$ generation CMS-line of *B. juncea* developed from somatic hybrid of *Trachystoma ballii and B. juncea* took 5-7 more days to flowering. Rao *et al* (1994) also reported delayed flowering in $BC_4$ generation of alloplasmic CMS-lineof *B. juncea* derived from cross of *D. siifolia* × *B. juneea* var. Pusa Bold. Majority of CMS A-lines of wheat flowered late than their respective B-lines (Tomar and Anbalagan 2004). Delayed flowerig in male sterile plants of GMS lines of muskmelon, chilli and pigeonpea have also been observed (Dhatt and Singh 1999 and Dhatt and Gill 2000).

**Table 4.9: Comparison of different backcross generations of CMS-lines with recurrent parent for days to 50% flowering in brinjal**

| Genotype | B-line | Generations of A-lines* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 42.67 | 53.33 | 50.67 | 57.0 | **50.92** |
| **MR 319 (B or A)** | 49.0 | 51.67 | 48.0 | 52.67 | **50.33** |
| **SR 5 (B or A)** | 39.67 | 47.0 | 48.0 | 49.33 | **46.00** |
| **P 67 (B or A)** | 47.33 | 55.67 | 62.0 | 65.0 | **57.50** |
| **CB 99-231 (B or A)** | 45.67 | 49.0 | 51.0 | 52.33 | **49.50** |
| **BL 219 (B or A)** | 47.67 | 49.33 | 53.67 | 56.33 | **51.75** |
| **BL 214 (B or A)** | 47.0 | 66.33 | 67.33 | 64.67 | **61.33** |
| **BL 201 (B or A)** | 42.33 | 64.67 | 70.33 | 69.33 | **61.67** |
| **BL 12-4 (B or A)** | 40.67 | 53.0 | 55.0 | 56.0 | **51.17** |
| **BL 216 (B or A)** | 48.33 | 55.33 | 50.67 | 56.0 | **52.58** |
| **SR 232 (B or A)** | 50.67 | 53.33 | 57.67 | 55.33 | **54.25** |
| **SR 93-213 (B or A)** | 53.67 | 57.0 | 58.67 | 61.33 | **57.67** |
| ***Mean*** | **46.22 -** | **54.64 (+18.20)**** | **56.08 (+21.33)**** | **57.94 (+25.36)**** | |
| **CD (P=0.05)** | **Genotype = 3.0** | **Generations = 1.73** | | **Genotype × Generations = 6.0** | |

*Cytoplasmic Male sterile lines
** Percent deviation from recurrent parent mean

**4.2.9 Calyx colour**

Colour of calyx of different genotypes and their different populations is presented in Table 4.10. There was no divergence in calyx colour of all backcross generations from the respective recurrent parent. However three colours *viz.* violet, green and purple with variable intensity were observed in different genotypes as per the colour charts of The Royal Horticultural Society. Calyx of genotype MR 319, SR 5, BL 219, BL 201, BL 12-4, SR 232, and SR 93-213 were green; P 67, CB 99-231, BL 214 and BL 216 purple; BR 104 violet

coloured. Presence of same calyx colour in all generation revealed the fact of being highly heritable character.

**Table 4.10: Comparison of different backcross generations of CMS-lines with recurrent parent for days to calyx colour in brinjal**

| Genotypes | B-line | Generations of A-line | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Violet Blue (N 92 A) | Violet Blue (N 92 A) | Violet Blue (N 92 A) | Violet Blue (N 92 A) |
| **MR 319 (B or A)** | Green (138B) | Green (138B) | Green (138B) | Green (138B) |
| **SR 5 (B or A)** | Green (137B) | Green (137B) | Green (137B) | Green (137B) |
| **P 67 (B or A)** | Purple (79C) | Purple (79C) | Purple (79C) | Purple (79C) |
| **CB 99-231 (B or A)** | Purple (279A) | Purple (279A) | Purple (279A) | Purple (279A) |
| **BL 219 (B or A)** | Green (138B) | Green (138B) | Green (138B) | Green (138B) |
| **BL 214 (B or A)** | Purple (79C) | Purple (79C) | Purple (79C) | Purple (79C) |
| **BL 201 (B or A)** | Green (138A) | Green (138A) | Green (138A) | Green (138A) |
| **BL 12-4 (B or A)** | Green (138B) | Green (138B) | Green (138B) | Green (138B) |
| **BL 216 (B or A)** | Purple (279A) | Purple (279A) | Purple (279A) | Purple (279A) |
| **SR 232 (B or A)** | Green (138B) | Green (138B) | Green (138B) | Green (138B) |
| **SR 93-213 (B or A)** | Green (138B) | Green (138B) | Green (138B) | Green (138B) |

*Cytoplasmic Male sterile lines

** Value in parenthesis represents shades of colour as per The Royal Horticultural Society Colour Charts

### 4.2.10 Calyx size

The data presented in the Table 4.11 depicts the calyx size (mm) of different backcross generations of male sterile A-lines along with the maintainer B-lines of different genotypes. Non-significant differences were found among different generations of A-line and recurrent parents (B-line). In general, calyx size of fertile recurrent parents (16.21) was more than the sterile recipient parent. Genotypic difference for calyx size was significant with maximum value of SR 5 (18.17) followed by SR 93-213 (18.06) and MR 319 (17.68) and, was smallest in BL 214 (13.89). The interaction between genotypes and backcross generations was non-significant. The percentage deviation of calyx size from recurrent parent was -1.36, -1.48 and -0.66 of $BC_3$, $BC_4$ and $BC_5$ generations, respectively. The results were contrasting to Yang *et al* (2005) who reported smaller sepal length and width in $BC_1$, $BC_2$ and $BC_3$ generations of CMS leaf mustard as compared to their maintainer.

**Table 4.11: Comparison of different backcross generations of CMS-lines with recurrent parent for calyx size (mm) in brinjal**

| Genotypes | B-line | Generations of A-lines* | | | |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | *Mean* |
| **BR 104 (B or A)** | 17.07 | 16.48 | 16.12 | 16.16 | **16.46** |
| **MR 319 (B or A)** | 17.59 | 17.32 | 17.94 | 17.86 | **17.68** |
| **SR 5 (B or A)** | 18.13 | 18.39 | 18.08 | 18.07 | **18.17** |
| **P 67 (B or A)** | 14.78 | 14.68 | 14.37 | 14.63 | **14.62** |
| **CB 99-231 (B or A)** | 16.97 | 16.43 | 15.89 | 16.17 | **16.37** |
| **BL 219 (B or A)** | 15.57 | 15.77 | 15.37 | 15.64 | **15.59** |
| **BL 214 (B or A)** | 14.04 | 13.95 | 13.82 | 13.76 | **13.89** |
| **BL 201 (B or A)** | 15.09 | 14.77 | 14.53 | 14.41 | **14.70** |
| **BL 12-4 (B or A)** | 14.32 | 14.08 | 14.12 | 14.05 | **14.14** |
| **BL 216 (B or A)** | 17.04 | 17.34 | 17.03 | 16.92 | **17.08** |
| **SR232 (B or A)** | 15.85 | 16.04 | 16.35 | 16.17 | **16.10** |
| **SR 93-213 (B or A)** | 18.09 | 18.16 | 17.98 | 17.99 | **18.06** |
| ***Mean*** | **16.21 -** | **16.12 (-0.66)**** | **15.97 (-1.48)**** | **15.99 (-1.36)**** | |
| **CD (P=0.05)** | **Genotype = 0.84** | **Generations = NS** | | **Genotype × Generations = NS** | |

*Cytoplasmic Male sterile

** Percent deviation from recurrent parent mean

### 4.2.11 Corolla colour

Corolla colour representing petals of different generations of recipient male sterile and recurrent parents is given in Table 4.12. There were no differences in colour of all backcross generations from the respective recurrent parent. Corolla colour of all genotypes was violet with variable intensity as per charts of The Royal Horticultural Society. As P 67 showed violet colour with N 92 A score, SR 5 with N 87 A, CB 99-231, BL 214 and BL 12-4

**Table 4.12: Comparison of different backcross generations of CMS-lines with recurrent parent for corolla colour in brinjal**

| Genotypes | B-Line | Generations of A-line | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) |
| **MR 319 (B or A)** | Violet (N87C) | Violet (N87C) | Violet (N87C) | Violet (N87C) |
| **SR 5 (B or A)** | Violet (N 87A) | Violet (N 87A) | Violet (N 87A) | Violet (N 87A) |
| **P 67 (B or A)** | Violet (N 92A) | Violet (N 92A) | Violet (N 92A) | Violet (N 92A) |
| **CB 99-231 (B or A)** | Violet (N 87B) | Violet (N 87B) | Violet (N 87B) | Violet (N 87B) |
| **BL 219 (B or A)** | Violet (N87C) | Violet (N87C) | Violet (N87C) | Violet (N87C) |
| **BL 214 (B or A)** | Violet (N 87B) | Violet (N 87B) | Violet (N 87B) | Violet (N 87B) |
| **BL 201 (B or A)** | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) |
| **BL 12-4 (B or A)** | Violet (N87B) | Violet (N87B) | Violet (N87B) | Violet (N87B) |
| **BL 216 (B or A)** | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) | Violet (N 87C) |
| **SR 232 (B or A)** | Violet (N87C) | Violet (N87C) | Violet (N87C) | Violet (N87C) |
| **SR 93-213 (B or A)** | Violet (N87C) | Violet (N87C) | Violet (N87C) | Violet (N87C) |

*Cytoplasmic Male sterile lines

**Value in parenthesis represents shades of colour as per The Royal Horticultural Society Colour Charts

with N 87B and rest with the 87C. Similar results for petal colour were observed in different CMS-lines and their maintainers by Dey *et al* (2011) in cauliflower. Nothnagel *et al* (2016) also reported similar petal colour to recurrent parent in CMS $BC_3$ and $BC_5$ generations of *Eruca sativa.*

### 4.2.12 Petal size

The petal size (mm) of all backcross generations showed significant differences (Table 4.13) and the largest was of recurrent parents (31.84), followed by $BC_5$ (31.35), $BC_4$ (30.92) and $BC_3$ (30.63) generations. The genotypes also showed significant differences for petal size. Among all, BL 12-4 (35.97) was largest by having significantly better value than the others. However, smallest size was recorded in SR 232 (29.32) followed by BL 214 (29.85), BR 104 (29.52) and BL 201 (29.45). The interactions of genotypes and backcross generations were found non-significant.

It was observed that petal size of all male sterile backcross generations was smaller than the normal recurrent parent or in other words size improved with each successive backcross. Compared with normal recurrent parent, it was smaller by -3.79, -3.89 and -1.54% in $BC_3$, $BC_4$ and $BC_5$ generations. Significant difference in petal size of $BC_7$ generation of CMS plants with their maintainers in cauliflower was reported by Dey *et al* (2011). CMS developed by crossing *Diplotaxis berthauti* with *B. juncea* also gave smaller petal size than *B. juncea* (Malik *et al* 1999). Yang *et al* (2005) also reported smaller petal length and width than maintainer line in $BC_1$, $BC_2$ and $BC_3$ generation of CMS-line of leaf mustard.

**Table 4.13: Comparison of different backcross generations of CMS-lines with recurrent parent for petal size (mm) in brinjal**

| Genotype | B-line | Generations of A-lines* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 30.54 | 29.21 | 29.20 | 29.13 | **29.52** |
| **MR 319 (B or A)** | 32.09 | 31.88 | 31.54 | 31.91 | **31.86** |
| **SR 5 (B or A)** | 32.31 | 32.13 | 30.81 | 30.07 | **31.33** |
| **P 67 (B or A)** | 31.43 | 30.84 | 31.36 | 29.15 | **30.70** |
| **CB 99-231 (B or A)** | 32.17 | 31.31 | 31.24 | 31.43 | **31.54** |
| **BL 219 (B or A)** | 32.22 | 31.82 | 30.81 | 31.16 | **31.50** |
| **BL 214 (B or A)** | 30.96 | 30.61 | 29.87 | 27.95 | **29.85** |
| **BL 201 (B or A)** | 29.42 | 29.48 | 29.33 | 29.58 | **29.45** |
| **BL 12-4 (B or A)** | 37.17 | 35.62 | 35.16 | 35.92 | **35.97** |
| **BL 216 (B or A)** | 31.95 | 32.49 | 30.80 | 30.98 | **31.56** |
| **SR232 (B or A)** | 29.76 | 29.11 | 29.43 | 28.99 | **29.32** |
| **SR 93-213 (B or A)** | 32.01 | 31.66 | 31.49 | 31.33 | **31.62** |
| ***Mean*** | **31.84 -** | **31.35 (-1.54)**** | **30.92 (-2.89)**** | **30.63 (-3.79)**** | |
| **CD (P=0.05)** | **Genotype = 1.19** | **Generations = 0.69** | **Genotype × Generations = NS** | | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean

### 4.2.13 Stamen size

The comparison of different backcross generations of CMS-lines with recurrent parent for stamen size (mm) is given in Table 4.14. Average stamen length of recurrent parent (11.29) was significantly higher over $BC_3$ (8.09), $BC_4$ (8.06) and $BC_5$ (8.09) generations. Among genotypes, significant differences were observed with maximum length in BL 12-4 (10.20) followed by BL 216 (10.17). The minimum size was recorded in MR-319 (8.06) with non-significant differences in BR 104 (8.07), BL 214 (8.14), SR 232 (8.30) and BL 219 (8.46).

**Table 4.14: Comparison of different backcross generations of CMS-lines with recurrent parent for stamen size (mm) in brinjal**

| Genotype | B-line | Generations of A-lines* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 10.78 | 7.25 | 7.20 | 7.07 | **8.07** |
| **MR 319 (B or A)** | 10.06 | 7.38 | 7.33 | 7.47 | **8.06** |
| **SR 5 (B or A)** | 10.97 | 7.83 | 8.10 | 8.03 | **8.73** |
| **P 67 (B or A)** | 11.90 | 8.12 | 7.81 | 7.65 | **8.87** |
| **CB 99-231 (B or A)** | 11.15 | 8.27 | 8.73 | 8.55 | **9.17** |
| **BL 219 (B or A)** | 10.19 | 7.98 | 7.76 | 7.93 | **8.46** |
| **BL 214 (B or A)** | 10.16 | 7.52 | 7.42 | 7.45 | **8.14** |
| **BL 201 (B or A)** | 11.30 | 8.01 | 8.27 | 8.05 | **8.91** |
| **BL 12-4 (B or A)** | 13.59 | 9.19 | 8.99 | 9.03 | **10.20** |
| **BL 216 (B or A)** | 11.56 | 9.91 | 9.59 | 9.63 | **10.17** |
| **SR 232 (B or A)** | 10.38 | 7.40 | 7.42 | 7.99 | **8.30** |
| **SR 93-213 (B or A)** | 13.47 | 8.19 | 8.09 | 8.23 | **9.50** |
| ***Mean*** | **11.29 -** | **8.09 (-28.44)**** | **8.06 (-28.61)**** | **8.09 (-28.44)**** | |
| **CD (P=0.05) Genotype = 0.50** | | **Generations = 0.27** | | **Genotype × Generations = 0.99** | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean

The interaction of different back cross generations with recurrent parents also differed significantly. The stamen size of male sterile flowers of all backcross generations was significantly smaller than the respective fertile recurrent parent (Figure 1, 2, 3 and 4). The reduction in size of stamens was more than 28% in all backcross generations. Development of very small sized and shrivelled anthers have been reported in male sterile lines due to introgression of *S. aethiopicum* cytoplasm into *S. melongena* (Khan and Issihiki 2010). Dey *et al* (2011) also reported smaller anther size in $BC_7$ generation of CMS-lines in cauliflower. Singh and Sirivastava (2006) reported variable stamen length in different CMS systems of *B. juncea*. Stamens were longer than style in *oxyrrhina,* equal in *moricandia* and shorter in *ogura, siifolia, tournefortii* and *trachystoma* male sterile lines. Significantly smaller anther

and filament sizes of CMS-lines than the maintainers were reported in wheat by Tomar and Anbalagan (2004).


**Figure1: Flowers of different generation: A=Recurrent parent, B=CMS $BC_5$ generation, C=CMS $BC_4$ generation, D=CMS $BC_3$ generation**

**Figure 2: Flower of recurrent parent of BR-104B**

**Figure 3: Flower of CMS $BC_5$ of BR-104A**

### 4.2.14 Pistil size

Data on the pistil size (mm) of different generations is presented in Table 4.15. Wherein, average pistil length of recurrent parent (16.03) was significantly better than all male sterile backcross generations, but, pistil size of $BC_3$, $BC_4$ and $BC_5$ generations was statistically at par among themselves. The genotypic differences for pistil size were significant and longest pistil was recorded in BL 12-4 (17.87), which was significantly better than all the genotypes. The shortest pistil was measured in P 67 (14.32) with no difference than BL 201 (14.82). The average reduction in pistil length of CMS-lines in $BC_5$ generation was -1.68% than the recurrent parents. The interaction effects of genotype × generation were non-significant, which indicated that alloplasmic cytoplasm did not effect the growth of female organ of the flower (Figure 4). Khan and Isshiki (2010) observed no effect on female

fertility of *S. aethiopicum* based cytoplasmic male sterility in different backcross generations. However, in cauliflower smaller style length in $BC_7$ generation of CMS-lines than the maintainers was observed by Dey *et al* (2011). Malik *et al* (1999) also reported smaller pistil size in CMS-line of *B. juncea* as compared to its maintainer lines. Significantly smaller ovary and style length in CMS-lines than their maintainers was reported by Tomar and Anbalagan (2004) in wheat. Non-significant differences in style length of alloplasmic CMS-lines with their maintainers in *B. juncea* were reported by Singh and Sirivastava (2006).

**Table 4.15: Comparison of different backcross generations of CMS-lines with recurrent parent for pistil size (mm) in brinjal**

| Genotype | B-line | Generations of A-lines* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 15.47 | 15.07 | 14.52 | 14.62 | **14.92** |
| **MR 319 (B or A)** | 15.70 | 15.26 | 14.17 | 15.28 | **15.10** |
| **SR 5 (B or A)** | 15.59 | 15.13 | 15.05 | 14.72 | **15.12** |
| **P 67 (B or A)** | 15.10 | 14.20 | 14.21 | 13.77 | **14.32** |
| **CB 99-231 (B or A)** | 16.08 | 16.17 | 16.71 | 15.80 | **16.19** |
| **BL 219 (B or A)** | 16.31 | 16.86 | 16.15 | 15.70 | **16.25** |
| **BL 214 (B or A)** | 15.25 | 15.00 | 15.40 | 14.62 | **15.07** |
| **BL 201 (B or A)** | 14.97 | 14.81 | 14.48 | 15.03 | **14.82** |
| **BL 12-4 (B or A)** | 18.43 | 17.59 | 17.73 | 17.71 | **17.87** |
| **BL 216 (B or A)** | 17.32 | 17.19 | 16.41 | 16.83 | **16.94** |
| **SR232 (B or A)** | 15.56 | 15.20 | 15.53 | 15.35 | **15.41** |
| **SR 93-213 (B or A)** | 16.61 | 16.71 | 16.32 | 17.05 | **16.67** |
| ***Mean*** | **16.03 -** | **15.76 (-1.68)**** | **15.56 (-2.93)**** | **15.54 (-3.06)**** | |
| **CD (P=0.05)** | **Genotype = 0.59** | **Generations = 0.34** | | **Genotype × Generations = NS** | |

*Cytoplasmic Male sterile lines
**Percent deviation from recurrent parent mean

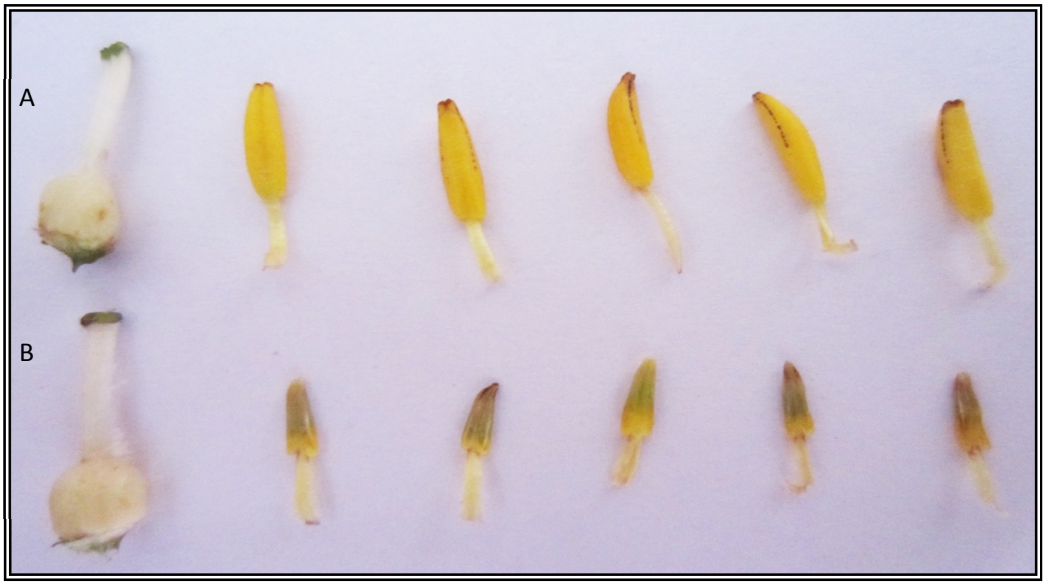



**Figure 4: A: Pistil and stamens of male fertile recurrent line; B: Pistil and deformed stamens of male sterile line**

### 4.2.15 Male fertility status

The male fertility status of different male sterile lines of $BC_3$, $BC_4$ and $BC_5$ generations along with their respective recurrent parentis given in Table 4.16. The examination of anthers under microscope revealed no viable pollen grains in all backcross generations of CMS-lines and thus designated as male sterile, whereas, viable pollen was observed in all the recurrent parents. The pollen non formation type of cytoplasmic male sterility was due to transfer of alloplasmic cytoplasm (*S. aethiopicum*) into *S. melongena* (Khan and Isshiki 2010). Rao *et al* (1994) also reported no pollen formation in alloplasmic CMS in *B. juncea.*

**Table 4.16: Comparison of different backcross generations of CMS-lines with recurrent parent for male fertility status in brinjal**

| Genotype | B-line | Generations of A-line* | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **MR 319 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **SR 5 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **P 67 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **CB 99-231 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **BL 219 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **BL 214 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **BL 201 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **BL 12-4 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **BL 216 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **SR 232 (B or A)** | Fertile | Sterile | Sterile | Sterile |
| **SR 93-213 (B or A)** | Fertile | Sterile | Sterile | Sterile |

*Cytoplasmic Male sterile lines

### 4.2.16 Pedicel length

The comparison of different backcross generations of CMS-lines with recurrent parent for pedicel length (mm) of brinjal is presented in Table 4.17. Mean pedicel length (mm) of different backcross CMS-lines along with their recurrent parents did not showed any substantial differences. However, genotypes differed considerably for pedicel length with a range of 15.18 (BR 104) to 21.41 mm (BL 201). The genotype × generation interaction manifested non-significant results for this trait. Our results were in concordance with Rao *et al* (1994) who observed smaller pedicel length in $BC_4$ generation of alloplasmic male sterile line derived through crossing of *B. juncea* and *Diplotaxis siifolia.* These differences in pedicel length could be due to genotypes and species involved in crossing.

**Table 4.17: Comparison of different backcross generations of CMS-lines with recurrent parent for pedicel length (mm) in brinjal**

| Genotypes | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 15.33 | 14.95 | 15.63 | 14.79 | **15.18** |
| **MR 319 (B or A)** | 16.63 | 16.18 | 16.85 | 16.36 | **16.51** |
| **SR 5 (B or A)** | 19.20 | 18.83 | 19.26 | 18.85 | **19.03** |
| **P 67 (B or A)** | 16.88 | 16.91 | 17.00 | 16.78 | **16.89** |
| **CB 99-231 (B or A)** | 21.58 | 21.67 | 21.62 | 20.77 | **21.41** |
| **BL 219 (B or A)** | 16.73 | 16.66 | 17.13 | 17.04 | **16.89** |
| **BL 214 (B or A)** | 18.24 | 18.00 | 17.82 | 17.95 | **18.00** |
| **BL 201 (B or A)** | 17.41 | 17.32 | 17.22 | 17.67 | **17.41** |
| **BL 12-4 (B or A)** | 19.26 | 19.30 | 19.34 | 19.32 | **19.30** |
| **BL 216 (B or A)** | 19.74 | 19.84 | 20.12 | 20.34 | **20.01** |
| **SR 232 (B or A)** | 17.81 | 17.78 | 17.31 | 17.55 | **17.61** |
| **SR 93-213 (B or A)** | 18.50 | 18.23 | 18.25 | 17.99 | **18.24** |
| ***Mean*** | **18.11 -** | **17.97 (-0.77)**** | **18.13 (+0.1)**** | **17.95 (-0.89)**** | |
| **CD (P=0.05)** | **Genotype = 0.70** | **Generations = NS** | **Genotype × Generations = NS** | | |

*Cytoplasmic Male sterile lines
** Percent deviation from recurrent parent mean

### 4.2.17 Fruit length

The fruit length (cm) of different recipient backcross generations of CMS-lines along with their respective maintainers is depicted in Table 4.18. Statistically there was no difference between recipient $BC_3$, $BC_4$ and $BC_5$ generations and their recurrent parents mean (Figure 5). However, significant differences were there between genotypes with maximum value in BL 12-4 (13.72) followed by BL 216 (12.23) and BL 201 (10.74). The minimum fruit-length was recorded in small fruited SR 5 (5.54) and SR 232 (5.84). The interaction between genotypes and generations was non-significant. Overall differences of recurrent parent with recipient $BC_5$, $BC_4$ and $BC_3$ was -0.35, -0.58 and -0.47 percent, respectively. Dey *et al* (2011) also observed no difference in CMS-lines and their maintainers in curd length of cauliflower. The ear length of $BC_4$ generation and its recurrent parent was similar in maize also (Chen *et al* 2010).

**Table 4.18: Comparison of different backcross generations of CMS-lines with recurrent parent for fruit length (cm) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 7.69 | 7.67 | 7.66 | 7.64 | **7.66** |
| **MR 319 (B or A)** | 6.61 | 6.59 | 6.60 | 6.58 | **6.60** |
| **SR 5 (B or A)** | 5.35 | 5.57 | 5.52 | 5.72 | **5.54** |
| **P 67 (B or A)** | 7.12 | 7.20 | 7.00 | 7.06 | **7.09** |
| **CB 99-231 (B or A)** | 6.36 | 6.33 | 6.24 | 6.40 | **6.33** |
| **BL 219 (B or A)** | 10.16 | 10.27 | 10.35 | 10.66 | **10.36** |
| **BL 214 (B or A)** | 8.91 | 9.45 | 9.33 | 9.49 | **9.30** |
| **BL 201 (B or A)** | 10.79 | 10.70 | 10.75 | 10.70 | **10.74** |
| **BL 12-4 (B or A)** | 14.09 | 13.84 | 13.19 | 13.76 | **13.72** |
| **BL 216 (B or A)** | 12.13 | 12.29 | 12.48 | 12.01 | **12.23** |
| **SR232 (B or A)** | 6.49 | 5.84 | 5.88 | 5.13 | **5.84** |
| **SR 93-213 (B or A)** | 7.05 | 6.59 | 7.07 | 7.08 | **6.95** |
| ***Mean*** | **8.56 -** | **8.53 (-0.35)**** | **8.51 (-0.58)**** | **8.52 (-0.47)**** | |
| **CD (P=0.05)** | **Genotype = 0.48** | **Generations = NS** | | **Genotype × Generations = NS** | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean

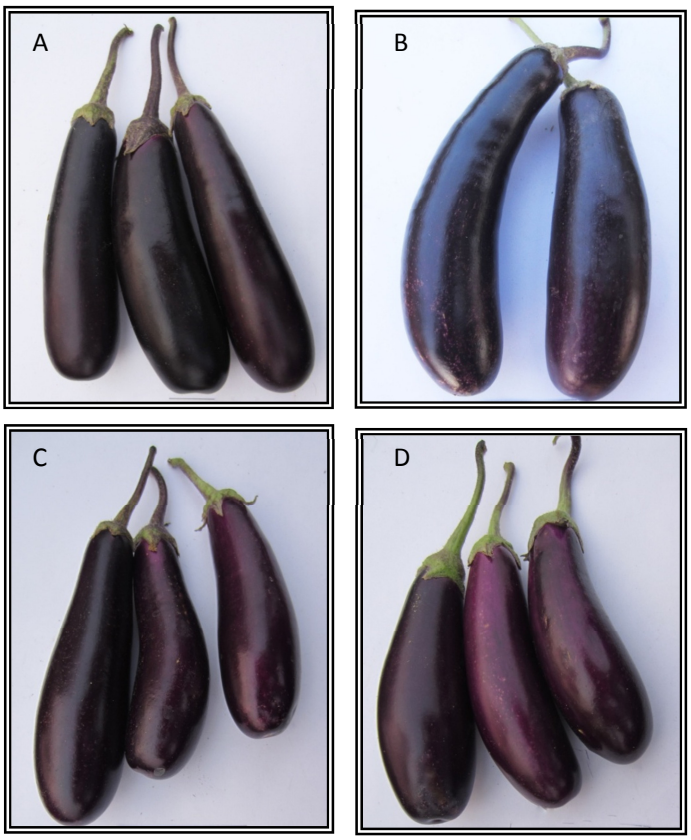



**Figure 5: Fruits of Recurrent parent (A), $BC_5$ (B), $BC_4$ (C) and $BC_3$ (D) generations of BL 216**

### 4.2.18 Fruit girth

The data presented in Table 4.19 reveals the comparison of fruit girth (cm) of different CMS backcross generations ($BC_3$, $BC_4$ and $BC_5$) and their respective recurrent parents. There was significant difference for fruit girth in all the generations. The maximum overall mean of recurrent parents (4.97) was significantly more than all the three generations. However, backcross generations *viz.* $BC_5$ (4.64), $BC_4$ (4.55) and $BC_3$ (4.51) did not differ among themselves (Figure 6). Genotypes for girth of the fruit vary significantly with maximum value in BR 104 (7.44) followed by MR 319 (5.68) and minimum in BL 12-4 (3.15) followed by BL 219 (3.66). The interaction of genotypes with different generations manifested non-significant results. The fruit girth of $BC_5$, $BC_4$ and $BC_3$ generation was -6.64, -8.45 and -9.26% less than the recurrent parent. The results were contrasting to Dey *et al* (2011), where curd width in CMS-lines of cauliflower was more than their maintainers. Nothnagel *et al* (2016) reported no difference in silique length of $BC_5$ alloplasmic CMS-line of *E. sativa* with its maintainer. Rao *et al* (1994) also reported no difference in $BC_4$ generation of alloplasmic CMS-line of *B. juncea* with maintainer cultivar Pusa Bold. Chen *et al* (2010) also reported small diameter of ear in $BC_4$ generation than its recurrent parent in maize.

**Table 4.19: Comparison of different backcross generations of CMS-lines with recurrent parent for fruit girth (cm) in brinjal**

| Genotypes | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 7.57 | 7.58 | 7.38 | 7.25 | **7.44** |
| **MR 319 (B or A)** | 5.99 | 5.47 | 5.97 | 5.30 | **5.68** |
| **SR 5 (B or A)** | 5.06 | 4.49 | 4.21 | 4.40 | **4.54** |
| **P 67 (B or A)** | 5.23 | 4.83 | 4.60 | 4.64 | **4.82** |
| **CB 99-231 (B or A)** | 5.27 | 4.60 | 4.46 | 4.53 | **4.72** |
| **BL 219 (B or A)** | 3.86 | 3.69 | 3.53 | 3.56 | **3.66** |
| **BL 214 (B or A)** | 4.84 | 4.32 | 4.33 | 4.00 | **4.37** |
| **BL 201 (B or A)** | 5.11 | 4.21 | 3.95 | 4.03 | **4.33** |
| **BL 12-4 (B or A)** | 3.23 | 3.14 | 3.12 | 3.12 | **3.15** |
| **BL 216 (B or A)** | 5.66 | 5.42 | 5.40 | 5.39 | **5.47** |
| **SR232 (B or A)** | 4.04 | 3.95 | 3.77 | 3.97 | **3.93** |
| **SR 93-213 (B or A)** | 3.82 | 3.95 | 3.88 | 3.93 | **3.89** |
| ***Mean*** | **4.97 -** | **4.64 (-6.64)**** | **4.55 (-8.45)**** | **4.51 (-9.26)**** | |
| **CD (P=0.05) Genotype = 0.24 Generations = 0.14 Genotype × Generations = NS** | | | | | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean


**Figure 6: Fruits of recurrent parent (A), $BC_5$ (B), $BC_4$(C) and $BC_3$ (D) generations of SR 5**

### 4.2.19 Fruit length: girth ratio

Fruit length: girth ratio determines the shape and important from consumers point of view. Ratio close to one indicates round and more than one elongated shape of the fruit. In present study, fruit length: girth ratio ranged from 1.85 in recurrent parents mean to 2.02 in $BC_3$ generation (Table 4.20). However, among genotypes a range from 1.03 (BR 104) to 4.35 (BL 12-4) revealed great variability in all the genotypes for shape of the fruit (Table 4.20). The data depicts that BR-104 A&B (1.03), MR-319 A&B (1.16) and SR-5 A&B (1.23) were round in shape; P-67 A&B (1.47) and CB-99-231 A&B (1.35) and SR-232 A&B (1.48) oblong and rest of the genotypes were long in shape.

### 4.2.20 Fruit colour

Fruit colour is an important horticultural trait for consumer preference as purple colour is more favoured in Punjab markets. There was no difference in colour between $BC_3$, $BC_4$ and $BC_5$ CMS generations and their respective recurrent parents. However, variability was recorded for fruit colour among all the genotypes, wherein five genotypes viz. P 67, BL 214, BL 201, BL 216 and SR 232 displayed purple (N 79A) fruit colour as per colour chart of The Royal Horticultural Society, whereas four viz. MR 319, BL 219, BL 12-4, and SR 93-213

depicted garyed purple colour (187 A, B) and three viz. BR 104, SR 5 and CB 99-231 violet blue (N 92A) (Table 4.21).

**Table 4.20: Comparison of different backcross generations of CMS-lines with recurrent parent for fruit length: girth ratio in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 1.02 | 1.01 | 1.04 | 1.05 | **1.03** |
| **MR 319 (B or A)** | 1.10 | 1.21 | 1.10 | 1.24 | **1.16** |
| **SR 5 (B or A)** | 1.06 | 1.24 | 1.31 | 1.30 | **1.23** |
| **P 67 (B or A)** | 1.36 | 1.49 | 1.52 | 1.52 | **1.47** |
| **CB 99-231 (B or A)** | 1.21 | 1.38 | 1.40 | 1.41 | **1.35** |
| **BL 219 (B or A)** | 2.10 | 2.38 | 2.39 | 2.66 | **2.38** |
| **BL 214 (B or A)** | 2.31 | 2.56 | 2.64 | 2.66 | **2.54** |
| **BL 201 (B or A)** | 2.11 | 2.54 | 2.72 | 2.65 | **2.51** |
| **BL 12-4 (B or A)** | 4.36 | 4.41 | 4.23 | 4.41 | **4.35** |
| **BL 216 (B or A)** | 2.14 | 2.27 | 2.31 | 2.23 | **2.24** |
| **SR 232 (B or A)** | 1.61 | 1.48 | 1.56 | 1.29 | **1.48** |
| **SR 93-213 (B or A)** | 1.84 | 1.67 | 1.82 | 1.80 | **1.79** |
| ***Mean*** | **1.85** | **1.97** | **2.00** | **2.02** | |

*Cytoplasmic Male sterile lines

**Table 4.21: Comparison of different backcross generations of CMS-lines with recurrent parent for fruit colour in brinjal**

| Genotypes | B-line | Generations of A-line | | |
|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ |
| **BR 104 (B or A)** | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) |
| **MR 319 (B or A)** | Grayed Purple (187A) | Grayed Purple (187A) | Grayed Purple (187A) | Grayed Purple (187A) |
| **SR 5 (B or A)** | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) |
| **P 67 (B or A)** | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) |
| **CB 99-231 (B or A)** | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) | Violet Blue (N 92A) |
| **BL 219 (B or A)** | Grayed Purple (N 186B) | Grayed Purple (N 186B) | Grayed Purple (N 186B) | Grayed Purple (N 186B) |
| **BL 214 (B or A)** | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) |
| **BL 201 (B or A)** | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) |
| **BL 12-4 (B or A)** | Grayed Purple (N 186B) | Grayed Purple (N 186B) | Grayed Purple (N 186B) | Grayed Purple (N 186B) |
| **BL 216 (B or A)** | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) |
| **SR 232 (B or A)** | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) | Purple (N 79A) |
| **SR 93-213 (B or A)** | Grayed Purple (187A) | Grayed Purple (187A) | Grayed Purple (187A) | Grayed Purple (187A) |

*Cytoplasmic Male sterile line

** Value in parenthesis represents shades of colour as per The Royal Horticultural Society Colour Charts

### 4.2.21 Number of fruits/ plant

The data presented in Table 4.22 represents the number of fruits/ plant in different backcross generations of CMS-lines and their recurrent parents. The number of fruits/ plant differed significantly among generations and was maximum in recurrent parents (34.37) than all the three recipient backcross generations (Figure 7). However, all three generations did not show substantial differences among themselves. Number of fruits/ plant differed significantly among genotypes. The maximum number was counted in SR 5 (52.42) and SR 93-213 (50.52), which was significantly better than the other genotypes. The interaction of different CMS backcross generations with recurrent parents was non-significant. The reason for lesser number fruits on male sterile lines could be the hand pollination, as 100% fruit setting could not be expected with this approach. In spite of all efforts of artificial pollination 13.68% less fruit setting was observed in $BC_5$ generation of all the male sterile lines than the recurrent parent. Delay in flowering of CMS-lines also lead towards late fruit setting in our study. The results were in line with Dey *et al* (2011) who observed significant differences in number of pods/ plant in CMS-lines with their maintainers in cauliflower. Prohens *et al* (2012) also reported reduced number of flowers and less fruit setting in backcross generations produced by crossing of of *S. melongena* with *S. aethiopicum.* About 80% fruit set in $BC_4$ was reported

**Table 4.22: Comparison of different backcross generations of CMS-lines with recurrent parent for number of fruits/ plant in brinjal**

| Genotypes | B-line | Generations of A-line | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 10.96 | 8.73 | 8.37 | 7.51 | **8.89** |
| **MR 319 (B or A)** | 13.55 | 11.44 | 11.32 | 10.29 | **11.65** |
| **SR 5 (B or A)** | 52.82 | 44.87 | 45.81 | 44.67 | **47.04** |
| **P 67 (B or A)** | 43.26 | 38.50 | 38.21 | 34.55 | **38.63** |
| **CB 99-231 (B or A)** | 28.94 | 25.41 | 27.71 | 26.06 | **27.03** |
| **BL 219 (B or A)** | 34.61 | 28.66 | 25.77 | 26.99 | **29.00** |
| **BL 214 (B or A)** | 30.78 | 25.83 | 22.41 | 24.96 | **25.99** |
| **BL 201 (B or A)** | 33.00 | 27.48 | 29.36 | 25.02 | **28.71** |
| **BL 12-4 (B or A)** | 41.48 | 38.33 | 35.55 | 35.71 | **37.77** |
| **BL 216 (B or A)** | 32.05 | 28.60 | 30.48 | 26.87 | **29.50** |
| **SR 232 (B or A)** | 44.40 | 40.98 | 37.32 | 36.35 | **39.76** |
| **SR 93-213 (B or A)** | 50.16 | 44.46 | 42.63 | 44.03 | **45.32** |
| ***Mean*** | **34.67 -** | **30.27 (-12.68)**** | **29.58 (-14.67)**** | **28.58 (-17.55)**** | |
| **CD (P=0.05) Genotype = 2.76 Generations = 1.60 Genotype × Generations = NS** | | | | | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean

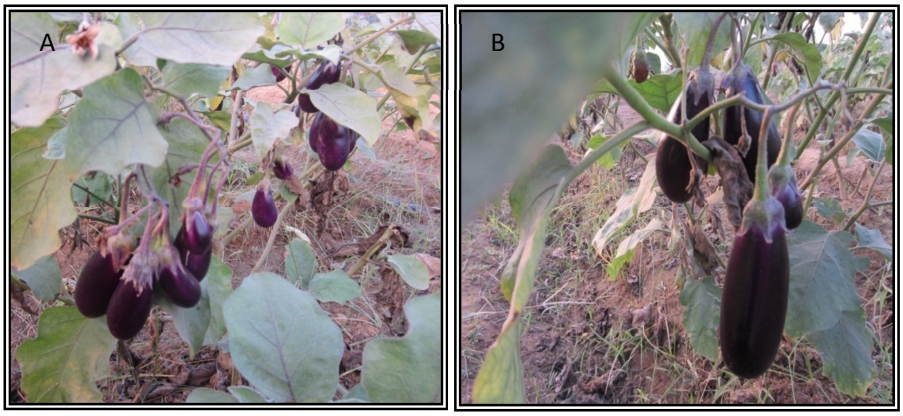



**Figure 7: Fruit setting on fertile recurrent parent (A) and $BC_5$ generation of CMS plant (B)**

in male sterile *S. aethiopicum* × *S. melongena* cross with artificial pollination by Khan and Isshiki (2010). Krommydas *et al* (2015) observed gradual increase in successful backcrosses up to $BC_5$ generation in cross of *S. violaceum* × *S. melongena* when *S. melongena* was used as recurrent parent.

### 4.2.22 Fruit weight

The comparison of different backcross generations of CMS-lines with recurrent parent for fruit weight (g) was found significant and presented in Table 4.23. Mean fruit

**Table 4.23: Comparison of different backcross generations of CMS-lines with recurrent parent for fruit weight (g) in brinjal**

| Genotype | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 186.88 | 183.76 | 182.21 | 178.79 | **182.91** |
| **MR 319 (B or A)** | 142.76 | 138.50 | 136.02 | 132.70 | **137.50** |
| **SR 5 (B or A)** | 36.81 | 33.26 | 33.88 | 32.03 | **34.00** |
| **P 67 (B or A)** | 56.35 | 55.12 | 54.29 | 51.94 | **54.43** |
| **CB 99-231 (B or A)** | 38.40 | 35.38 | 35.05 | 33.12 | **35.49** |
| **BL 219 (B or A)** | 64.96 | 62.91 | 62.16 | 60.92 | **62.74** |
| **BL 214 (B or A)** | 52.79 | 51.63 | 53.13 | 49.98 | **51.88** |
| **BL 201 (B or A)** | 76.03 | 74.97 | 73.94 | 74.40 | **74.83** |
| **BL 12-4 (B or A)** | 65.76 | 63.77 | 60.87 | 59.57 | **62.49** |
| **BL 216 (B or A)** | 78.66 | 77.66 | 75.75 | 73.95 | **76.51** |
| **SR232 (B or A)** | 35.85 | 34.31 | 33.91 | 34.15 | **34.56** |
| **SR 93-213 (B or A)** | 32.22 | 31.49 | 31.28 | 30.50 | **31.37** |
| ***Mean*** | **72.29 -** | **70.23 (-2.85)**** | **69.37 (-4.03)**** | **67.67 (-6.39)**** | |
| **CD (P=0.05)** | **Genotype = 4.23** | **Generations = 2.44** | **Genotype × Generations = NS** | | |

*Cytoplasmic Male sterile lines

** Percent deviation from recurrent parent mean

weight of recurrent parents (72.29) was maximum having non-significant differences with $BC_5$ (70.23) and significant with $BC_4$ (69.37) and $BC_3$ (67.67) generations. Genotypes also exhibited significant differences for fruit weightiness, wherein, BR 104 (182.91) recorded maximum fruit weight followed by MR 319 (137.50) and BL 216 (76.51). Minimum weight was observed in SR 93-213 (31.37) with non-significant differences in SR 5 (34.0), SR 232 (34.56) and CB 99-231 (35.49). Compared with recurrent parent improvement in fruit weight from $BC_3$ to $BC_5$ was from -6.39 to -2.85%, respectively. The interaction of different back cross generations with the recurrent parents did not manifest any difference. In cauliflower, curds with uniform weight were recorded in male sterile and maintainer lines (Dey *et al* 2011).

### 4.2.23 Fruit yield/ plant

Comparison for fruit yield/ plant (kg) of twelve genotypes in CMS-lines and their respective maintainers is presented in Table 4.24. Significant differences were observed among backcross generations and their recurrent parents. Highest mean yield recorded in recurrent parents (2.04) was significantly better than $BC_3$ (1.61), $BC_4$ (1.69) and $BC_5$ (1.74) generations. Yield of genotypes also displayed substantial differences. Highest yield was observed in BL 12-4 (2.41) with non-significant differences from BL 201(2.30) and BL 216 (2.25) and lowest in CB 99-231 (0.98). Compared with recurrent parent improvement in fruit yield/plant was from -20.79 to -14.79% from $BC_3$ to $BC_5$ generation. Variable responses in yield/ plot in various CMS-lines and their fertile counterparts were reported in cabbage by Thakur *et al* (2015).

**Table 4.24: Comparison of different backcross generations of CMS-lines with recurrent parent for yield /plant (kg) in brinjal**

| Genotypes | B-line | Generations of A-line* | | | *Mean* |
|---|---|---|---|---|---|
| | Recurrent parent | $BC_5$ | $BC_4$ | $BC_3$ | |
| **BR 104 (B or A)** | 2.07 | 1.67 | 1.61 | 1.41 | **1.69** |
| **MR 319 (B or A)** | 1.99 | 1.65 | 1.55 | 1.45 | **1.66** |
| **SR 5 (B or A)** | 1.95 | 1.53 | 1.56 | 1.50 | **1.63** |
| **P 67 (B or A)** | 2.30 | 2.12 | 2.08 | 1.87 | **2.09** |
| **CB 99-231 (B or A)** | 1.15 | 0.91 | 0.97 | 0.90 | **0.98** |
| **BL 219 (B or A)** | 2.26 | 1.85 | 1.74 | 1.73 | **1.90** |
| **BL 214 (B or A)** | 1.70 | 1.35 | 1.28 | 1.27 | **1.40** |
| **BL 201 (B or A)** | 2.54 | 2.08 | 2.14 | 2.43 | **2.30** |
| **BL 12-4 (B or A)** | 2.72 | 2.57 | 2.28 | 2.08 | **2.41** |
| **BL 216 (B or A)** | 2.50 | 2.25 | 2.17 | 2.10 | **2.25** |
| **SR 232 (B or A)** | 1.61 | 1.43 | 1.38 | 1.21 | **1.41** |
| **SR 93-213 (B or A)** | 1.65 | 1.42 | 1.47 | 1.41 | **1.49** |
| ***Mean*** | **2.04 -** | **1.74 (-14.79)**** | **1.69 (-17.17)**** | **1.61 (-20.79)**** | |
| **CD (P=0.05) Genotype = 0.21** | | **Generations = 0.12** | | **Genotype × Generations = NS** | |

*Cytoplasmic Male sterile line

** Percent deviation from recurrent parent mean

Similarly, for seed yield in cauliflower significant differences in two $BC_7$ generation of CMS-lines and their respective maintainers were observed by Dey *et al* (2011).

### 4.3 Restoration of recurrent parent in backcross generations

#### 4.3.1 Restoration for fruit traits of recurrent parent

The percent restoration of different fruit characteristics (fruit length, fruit girth, number of fruits/ plant, fruit weight and fruit yield/ plant) is compared in Figure 8. Among various fruit characteristics the highest refurbishment of recurrent parents in $BC_5$ CMS generation was revealed by fruit length (99.65%) followed by fruit weight (97.15), fruit girth (93.36), number of fruits/ plant (87.32) and fruit yield/ plant (85.21). Overall the highest mean restoration for all the fruit characters was presented in $BC_5$ CMS generations (92.54) followed by $BC_4$ (91.02) and $BC_3$ (89.11) generations (Figure 8). However, the proportionate revival of the recurrent parent over the theoretical values (PR/TV) 93.75 ($BC_3$), 96.87 ($BC_4$) and 98.44 ($BC_5$) was 95.05 in $BC_3$, 93.96 in $BC_4$ and 93.72% in $BC_5$. The expected recovery rate of nuclear genome with each backcross is $1-(1/2)^{t+1}$ (Babu *et al* 2004). But variability in the advancement of characters of recurrent parent with backcross generations was confirmed by Fehr (1987). Bayles *et al* (2005) also observed only 96% recovery (recurrent parent) of the theoretical rate in $BC_4$ generation for various traits in cotton. Varshney *et al* (2014) reported three *Fusarium* wilt resistant lines in $BC_3F_4$ generation with 92.7-95.2% recurrent parent genome in chickpea.

#### 4.3.2 Restoration for flower characteristics of recurrent parent

In case of flower characteristics the percent restoration of the recurrent parent traits *viz.* calyx size, petal size, pistil size and pedicel length is presented in Figure 9. Among various flower characters maximum similarity to recurrent parent was reported in calyx size (99.44), followed by pedicel length (99.23), petal size (98.46), pistil size (98.32). The high revival was supported by non-significant results in $BC_5$ generation with recurrent parent. The stamen size was showing least similarity to recurrent parents (71.66) with the reason being shrivelled anthers with no pollen (Khan and Issihiki 2010). Overall restoration of flower traits (except stamen size) in $BC_3$, $BC_4$ and $BC_5$ generations of CMS A-line was 97.73, 98.2 and 98.86% of the recurrent parent, respectively (Figure 9). However, the recovery percentage did not follow the rule of 50% advancement towards recurrent parent with each generation as per suggested by Allard (1960). The average percentage recovery was 104.24% of the required 93.75% in $BC_3$, 101.37% of the 96.875% in $BC_4$ and 100.43% of the required 98.4375% in $BC_5$. The results suggest that flower traits recovery was very fast as average percentage (except stamen size) over the recurrent parent was more than expected even in $BC_3$. High homozygosity of 95.6±3.3% against the expected 87.5% was reported in $BC_3$ generation by Ahmadikhah *et al* 2015. Chen *et al* (2010) reported that after four backcross generations a new line would look alike to the recurrent parent.

**Figure 8: Restoration of different fruit traits in $BC_3$, $BC_4$, $BC_5$ generation along with recurrent parent**

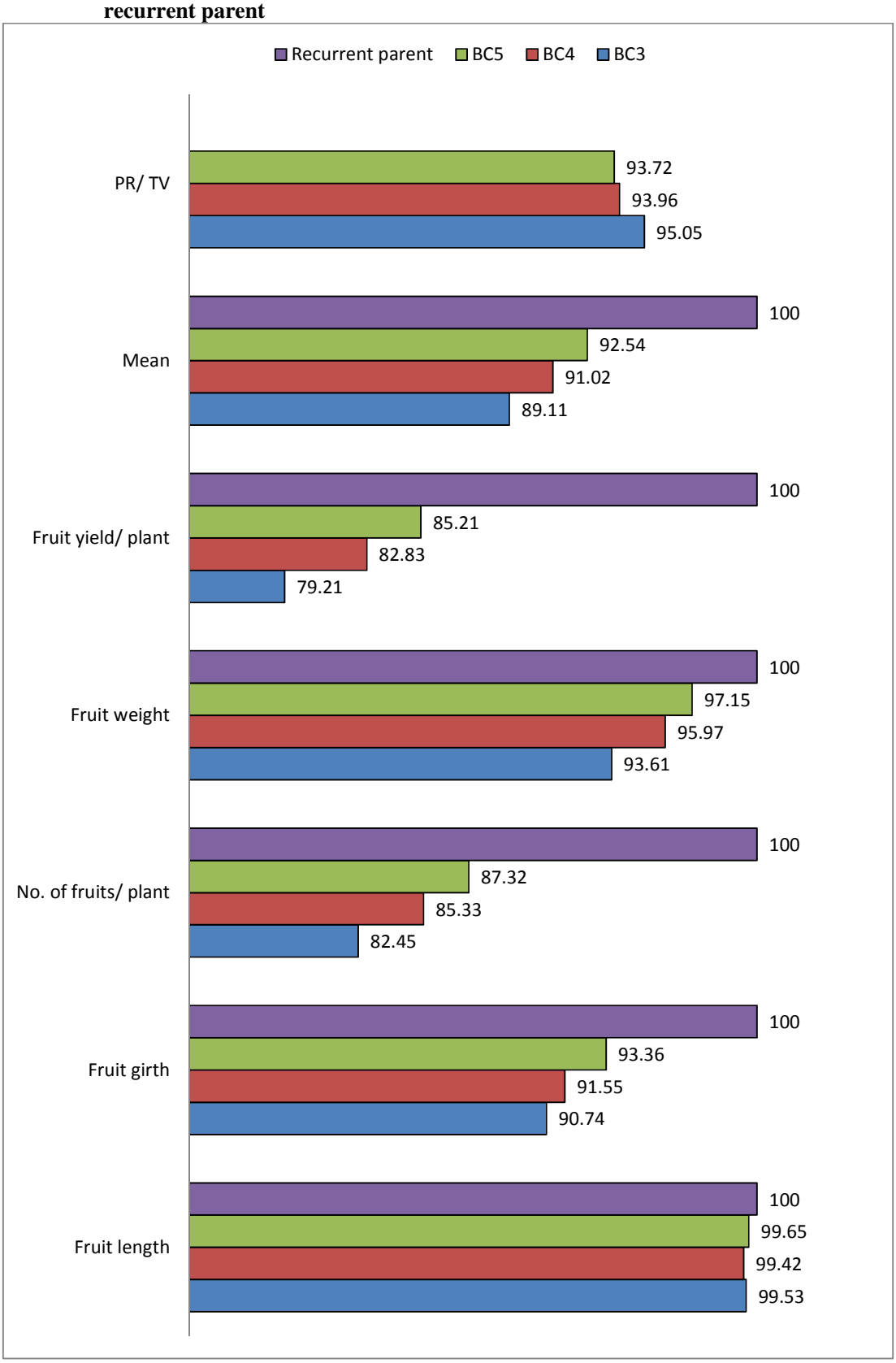


**Figure 9: Restoration of different flower characteristics in $BC_3$, $BC_4$, $BC_5$ generation along with recurrent parent**

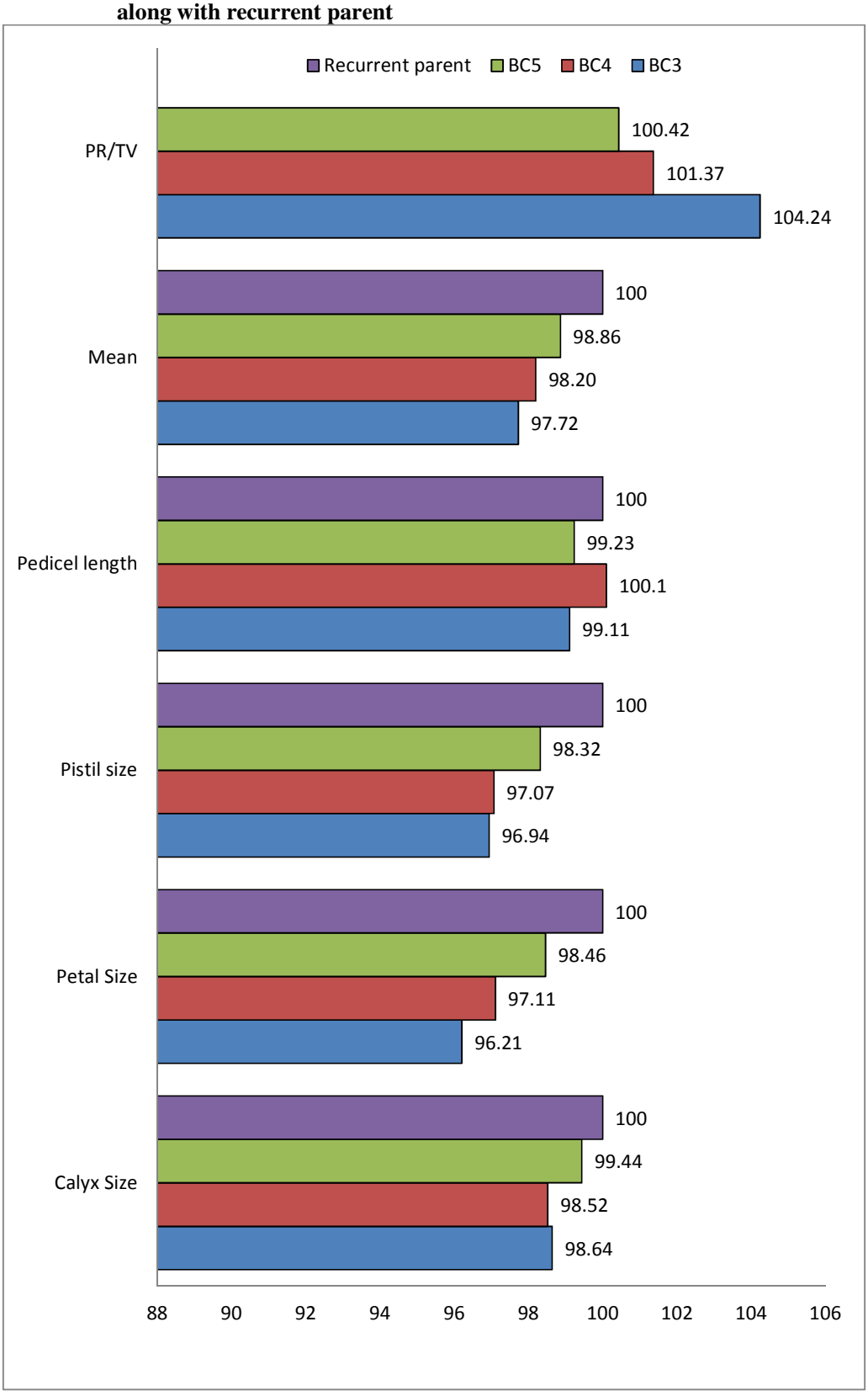
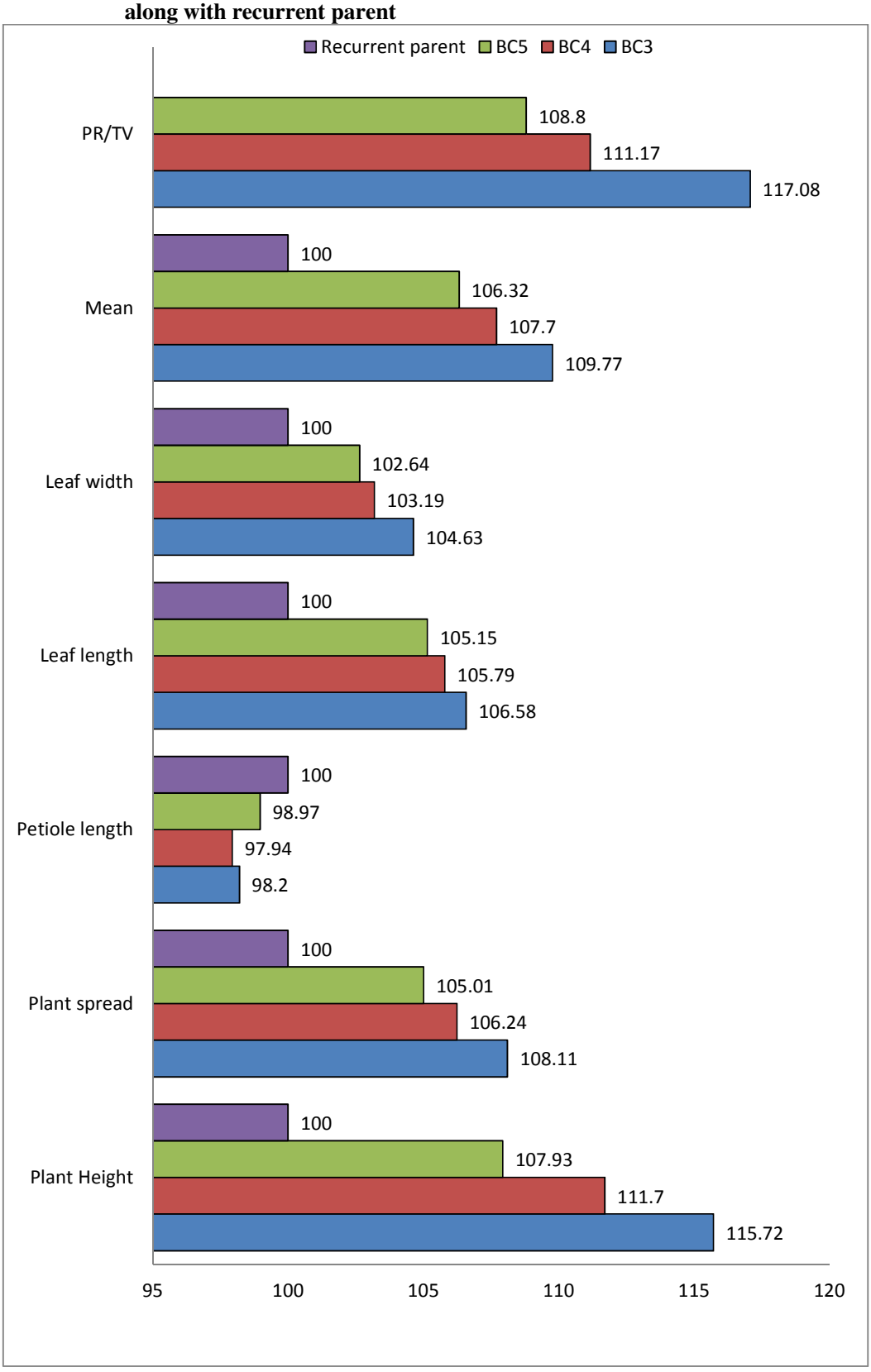


**Figure 10: Restoration of different vegetative traits in $BC_3$, $BC_4$ and $BC_5$ generation along with recurrent parent**



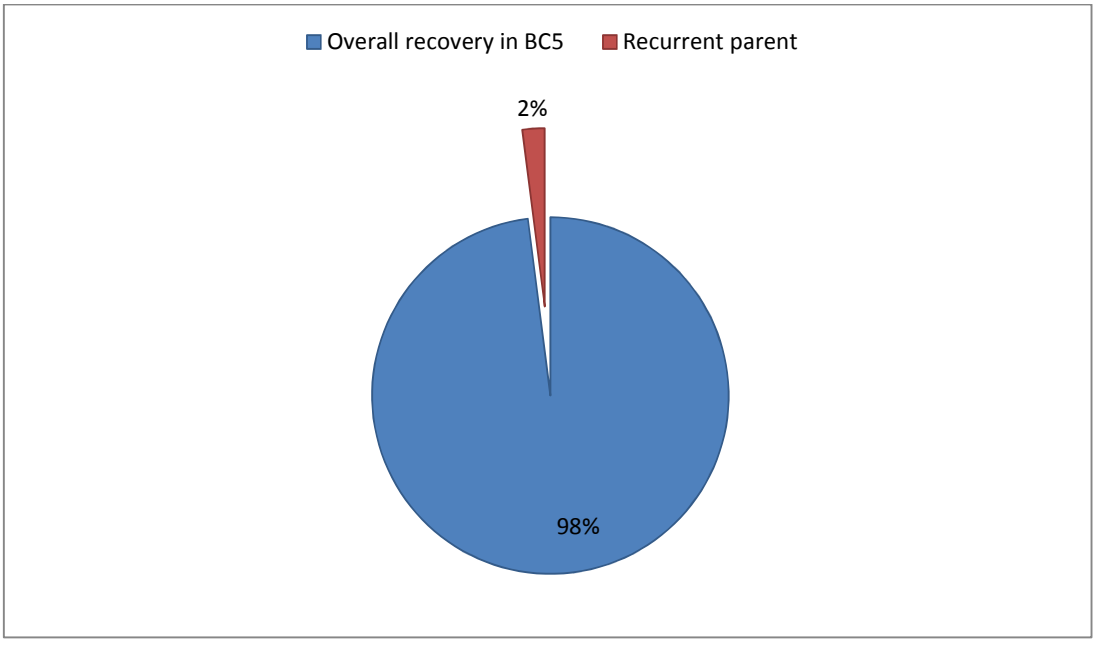
### 4.3.3 Restoration os recurrent parent for vegetative traits

Restoration of recurrent parent characteristics for vegetative characters *viz.* plant height, plant spread, petiole length, leaf blade length, leaf blade width and days to 50% flowering is depicted in figure 10. The restoration rate of vegetative characters was very fast with characters *viz.* Days to 50% flowering (118.2), plant height (107.93), leaf blade length (105.15), plant spread (105.01) and leaf blade width (102.64) depicting more than 100% revival. But excessive vegetative growth was mainly because of lesser fruit set, less yield and CMS-lines being late in flowering. Thakur *et al* (2015) also reported that CMS-lines of cauliflower displayed higher vegetative growth. Because of excessive vegetative growth the average refurbishment of vegetative characters was also high *viz.* 109.77, 107.70 and 106.32% of the recurrent parent in $BC_3$, $BC_4$ and $BC_5$ (Figure 10). Also, the percentage recovery over theoretical value was also high than expected i.e. 117.08 of the required 93.75% in $BC_3$, 111.17% of the required 96.875% in $BC_4$ and 108.0% of the required 98.4735 in $BC_5$ generation of CMS A-line displaying deviation in recovery of different fruit characteristics in different backcross generations. Dey *et al* (2014) also reported excessive vegetative growth of CMS brassica.

### 4.3.4 Overall similarity percentage in $BC_5$

In the $BC_5$ generation the characters were similar to recurrent parent to the extent of 97.98%. The expected recovery for $BC_5$ is 98.48%. Therefore the overall restoration percentage of recurrent parent traits was almost in line with the expected percentage although variations were there for various characters.

**Figure 11: Overall similarity percentage to recurrent parent in $BC_5$:**



### 4.4 Genetic variability

#### 4.4.1 Genetic variability in recurrent parents

The analysis of variance for sixteen quantitative characters of twelve genotypes (recurrent parents) is offered in Table 4.25. The results demonstrated that the genotypes were significantly different for all the traits, depicting the existence of vast amount of variability for various growth and yield attributes. In present investigation, variability between recurrent parents was important for the development of diverse CMS-lines in different background of their respective maintainers. The variability available for the sixteen characters under study in twelve genotypes was investigated using PCV, GCV, $H^2$ and genetic advance (Table 4.26).

**Table 4.25 Analysis of variance for recurrent parents**

| Source | Replications | Genotypes | Error |
|---|---|---|---|
| **df** | **2** | **11** | **22** |
| **Plant height** | 485.11 | 213.33* | 29.87 |
| **Plant spread** | 117.68 | 170.41* | 32.74 |
| **Petiole length** | 0.09 | 0.88* | 0.11 |
| **Leaf blade length** | 0.28 | 3.13* | 0.57 |
| **Leaf blade width** | 0.69 | 4.55* | 0.38 |
| **Calyx size** | 0.39 | 6.35* | 1.29 |
| **Petal size** | 0.95 | 11.39* | 2.21 |
| **Stamen size** | 2.09 | 4.28* | 0.65 |
| **Pistil size** | 1.24 | 3.07* | 0.47 |
| **Days to 50% flowering** | 21.19 | 52.69* | 12.1 |
| **Pedicel length** | 1.24 | 8.52* | 0.81 |
| **Fruit length** | 0.55 | 21.64* | 0.32 |
| **Fruit girth** | 0.14 | 4.07* | 0.09 |
| **Fruit weight** | 51.78 | 6606.29* | 40.77 |
| **Number of fruits/ plant** | 9.69 | 504.54* | 22.49 |
| **Yield/ plant** | 0.03 | 0.63* | 0.09 |

*Significant at 5% level

Table 4.26 reveals that phenotypic (PCV) and genotypic (GCV) co-efficient of variation marked substantial differences within all the studied attributes except fruit length and girth. The PCV was higher than the GCV for all of the traits, signifying that environmental factors were affecting the expression of these characters. Higher values of PCV and GCV have been obtained for fruit weight (65.31 and 64.71), number of fruits/ plant (39.04 and 36.56), fruit length (31.82 and 31.13), fruit girth (23.89 and 23.15) and yield/ plant (25.6 and 20.67). High magnitude of coefficients of variations indicates the presence of high degree of variability between the recurrent parents offering better possibility for further improvement. Moderate levels of PCV along with GCV were obtained for petiole length (15.7

and 13.05), leaf blade width (14.65 and 13) and plant height (15.46 and 12.67). However, low values for GCV have been obtained for all other characters indicating narrow range of variation between genotypes due to substantial effect of environment, thus providing very narrow scope for selection. This indicates that high genetic variation with negligible effect of environment predominantly responsible for the different expression of these attributes and effective selection may possibly be made on the basis of phenotype. These results were in accordance with Kushwah and Bandhyopadhya (2005) who displayed high PCV and GCV for several yield related traits in brinjal.

**Table 4.26 Estimates of components of variance, broad sense heritability, GA and GA as percent of the mean for 16 characters in twelve recurrent parents of CMS brinjal**

| Character | General Mean | Variances | | Coefficient of variability | | $H^2$ (%) | GA | % GA |
|---|---|---|---|---|---|---|---|---|
| | | VG | VP | GCV | PCV | | | |
| **Plant height** | 61.7 | 61.15 | 91.02 | 12.67 | 15.46 | 67.18 | 13.2 | 21.4 |
| **Plant spread** | 70.03 | 45.89 | 78.63 | 9.67 | 12.66 | 58.36 | 10.66 | 15.22 |
| **Petiole length** | 3.88 | 0.26 | 0.37 | 13.05 | 15.7 | 69.09 | 0.87 | 22.34 |
| **Leaf blade length** | 13.98 | 0.85 | 1.42 | 6.61 | 8.53 | 60.11 | 1.48 | 10.56 |
| **Leaf blade width** | 9.08 | 1.39 | 1.77 | 13 | 14.65 | 78.71 | 2.16 | 23.75 |
| **Calyx size** | 16.21 | 1.69 | 2.98 | 8.02 | 10.64 | 56.79 | 2.02 | 12.45 |
| **Petal size** | 31.84 | 3.06 | 5.27 | 5.49 | 7.21 | 58.01 | 2.74 | 8.62 |
| **Stamen size** | 11.29 | 1.21 | 1.86 | 9.73 | 12.08 | 64.9 | 1.82 | 16.15 |
| **Pistil size** | 16.04 | 0.87 | 1.34 | 5.82 | 7.21 | 65.12 | 1.55 | 9.67 |
| **Days to 50% flowering** | 46.22 | 13.53 | 25.63 | 7.96 | 10.95 | 52.78 | 5.5 | 11.91 |
| **Pedicel length** | 18.11 | 2.57 | 3.38 | 8.85 | 10.16 | 75.93 | 2.88 | 15.89 |
| **Fruit length** | 8.56 | 7.11 | 7.43 | 31.13 | 31.82 | 95.71 | 5.37 | 62.74 |
| **Fruit girth** | 4.98 | 1.33 | 1.42 | 23.15 | 23.89 | 93.98 | 2.3 | 46.24 |
| **Fruit weight** | 72.29 | 2188.5 | 2229.28 | 64.71 | 65.31 | 98.17 | 95.48 | 132.09 |
| **Number of fruits/ plant** | 34.67 | 160.68 | 183.17 | 36.56 | 39.04 | 87.72 | 24.46 | 70.55 |
| **Yield/ plant** | 2.04 | 0.18 | 0.27 | 20.67 | 25.6 | 65.19 | 0.7 | 34.37 |

High heritability estimates were obtained for traits *viz.* fruit weight (98.17), fruit length (95.71), fruit girth (93.98), number of fruits/ plant (87.72), leaf blade width (78.71), pedicel length (75.93), petiole length (69.09), plant height (67.18), pistil size (65.12), yield/ plant (65.19), stamen size (64.9) and leaf blade length (60.11). These high estimates suggested that phenotypic performance could lead to more effective selection. However, only heritability values may not provide clear forecast of the breeding value. Heritability along

with %GA is more effective and provides reliable results in predicting the outcome of selection (Johnson *et al* 1955). In the present study, high heritability along with high GA% was observed for fruit weight (98.17 and 132.09), fruit length (95.71 and 62.74), fruit girth (93.98 and 46.24), number of set fruits plant (87.72 and 70.55), leaf blade width (78.71 and 23.75), petiole length (69.09 and 22.34), plant height (67.18 and 21.4) and yield/ plant (65.19 and 34.37). Similar results of high heritability and percent genetic advance were reported by Bora and Shadeque (1993) and Sharma and Swaroop (2000).

**4.2.2 Genetic variability in CMS $BC_3$ generation:**

Analysis of variance for $BC_3$ CMS generation is presented in Table 4.27. The significant mean square values for all the characters under investigation revealed the occurrence of variability between different $BC_3$ CMS generations of twelve recurrent parents. It emphasized that the CMS character was being introgressed into different backgrounds of their respective maintainer parents. These could be further selected and used in succeeding backcrosses with their respective recurrent parents for improvement.

**Table 4.27 Analysis of variance for various morphological traits in CMS $BC_3$ generation**

| | Replications | Genotypes | Error |
|---|---|---|---|
| **df** | 2 | 11 | 22 |
| **Plant height** | 250.41 | 284.12* | 35.54 |
| **Plant spread** | 133.32 | 176.39* | 20.99 |
| **Petiole length** | 0.13 | 0.78* | 0.15 |
| **Leaf blade length** | 0.04 | 3.61* | 0.76 |
| **Leaf blade width** | 1.46 | 4.7* | 0.63 |
| **Calyx size** | 1.82 | 7.09* | 0.81 |
| **Petal size** | 0.93 | 12.77* | 1.84 |
| **Stamen size** | 0.52 | 1.52* | 0.24 |
| **Pistil size** | 0.07 | 3.98* | 0.82 |
| **Days to 50% flowering** | 17.53 | 105.69* | 17.56 |
| **Pedicel length** | 0.83 | 8.54* | 1.46 |
| **Fruit length** | 0.07 | 22.5* | 0.34 |
| **Fruit girth** | 0.06 | 3.52* | 0.09 |
| **Fruit weight** | 56.66 | 6082.41* | 33.16 |
| **Number of fruits/ plant** | 0.39 | 398.25* | 8.06 |
| **Yield/ plant** | 0.05 | 0.58* | 0.08 |

*Significant at 5% level

The data displayed in Table 4.28 reveals out the differences between magnitude of PCV and GCV for all the characters studied in $BC_3$ generation of CMS-lines. PCV of all the traits depicted higher values than GCV that representing the environmental effect on the expression on these characters. However the difference between GCV and PCV of average

fruit weight was too negligible that revealing limited consequence of environment on its phenotype. Highest PCV was exhibited for fruit weight (66.9) followed by number of fruits/ plant (41.12), fruit length (32.63), yield/ plant (30.89). High GCV was exhibited for fruit weight (66.36) followed by number of fruits/ plant (39.9), fruit length (31.91), yield/ plant (25.34) and fruit girth (23.7), which implies that phenotypic variability is reliable is measure of genotypic variability for these characters. Petal size (7.64 and 6.23), leaf blade length (8.77 and 6.54) and pistil size (8.81 and 6.6) displayed comparatively low PCV and GCV. High genotypic and phenotypic variances have also been reported by Patel *et al* (2004), Kushwah and Bandhyopadhya (2005) and Singh and Kumar (2005).

**Table 4.28: Estimates of components of variance, broad sense heritability, GA and GA as percent of the mean for 16 characters $BC_3$ generation CMS plants**

| Character | Mean | Variances | | Coefficient of variation | | $H^2$ (%) | GA | GA% |
|---|---|---|---|---|---|---|---|---|
| | | VG | VP | GCV | PCV | | | |
| **Plant height** | 71.4 | 82.86 | 118.40 | 12.75 | 15.24 | 69.98 | 15.69 | 21.97 |
| **Plant spread** | 75.71 | 51.80 | 72.79 | 9.51 | 11.27 | 71.16 | 12.51 | 16.52 |
| **Petiole length** | 3.81 | 0.21 | 0.36 | 12.08 | 15.73 | 59.02 | 0.73 | 19.12 |
| **Leaf blade length** | 14.9 | 0.95 | 1.71 | 6.54 | 8.77 | 55.5 | 1.49 | 10.03 |
| **Leaf blade width** | 9.5 | 1.36 | 1.99 | 12.26 | 14.84 | 68.18 | 1.98 | 20.85 |
| **Calyx size** | 15.99 | 2.09 | 2.90 | 9.05 | 10.66 | 72.04 | 2.53 | 15.82 |
| **Petal size** | 30.63 | 3.64 | 5.48 | 6.23 | 7.64 | 66.51 | 3.21 | 10.47 |
| **Stamen size** | 8.09 | 0.43 | 0.67 | 8.08 | 10.07 | 64.36 | 1.08 | 13.36 |
| **Pistil size** | 15.54 | 1.05 | 1.87 | 6.6 | 8.81 | 56.13 | 1.58 | 10.18 |
| **Days to 50% flowering** | 57.94 | 29.38 | 46.94 | 9.35 | 11.82 | 62.59 | 8.83 | 15.24 |
| **Pedicel length** | 17.95 | 2.36 | 3.82 | 8.56 | 10.89 | 61.78 | 2.49 | 13.85 |
| **Fruit length** | 8.52 | 7.39 | 7.73 | 31.91 | 32.63 | 95.63 | 5.48 | 64.28 |
| **Fruit girth** | 4.51 | 1.14 | 1.23 | 23.7 | 24.6 | 92.81 | 2.12 | 47.03 |
| **Fruit weight** | 67.67 | 2016.42 | 2049.58 | 66.36 | 66.9 | 98.38 | 91.75 | 135.58 |
| **Number of fruits/ plant** | 28.58 | 130.06 | 138.12 | 39.9 | 41.12 | 94.16 | 22.8 | 79.76 |
| **Yield/ plant** | 1.61 | 0.17 | 0.25 | 25.34 | 30.89 | 67.29 | 0.69 | 42.82 |

The heritability estimates were high (>60%) for characters *viz.* fruit weight (98.38) pursued by fruit length (95.63), number of fruits per plant (94.16) and fruit girth (92.81), calyx size (72.04), plant spread (71.16), plant height (69.98), leaf blade width (68.18), yield/ plant (67.29), petal size (66.51), stamen size (64.36), days to fifty percent flowering (62.59) and pedicel length (61.78), whereas, the moderate estimates of heritability were found in leaf

blade length (55.5), pistil size (56.13) and petiole length (59.02). GA (%) was also found highest in fruit weight (135.58) followed by number of fruits/ plant (79.76), fruit length (64.28), fruit girth (47.03), yield/ plant (42.82) and leaf blade width (20.85). In all other characters moderate GA (%) was found. High heritability and GA (%) in fruit weight, fruit length, no. of fruits/ plant, fruit girth and yield/plant signify that the phenotype was the true expression of genotype for these characters and selection could be made only on the basis of phenotype. High heritability estimates with high GA was also reported by Chadha and Paul (1984), Gautam and Srinivas (1992). These results were substantiated with findings of Johnson *et al* (1955).

### 4.4.3 Genetic variability in CMS $BC_4$ generation

Analysis of variance for $BC_4$ CMS generation is presented in Table 4.29. The mean square values for all the characters under investigation revealed significant variability between different $BC_4$ CMS generations of respective recurrent parents. It highlighted the introgression of CMS character into different backcross populations of their respective recurrent parents. The selection could be made from these backcross populations for succeeding backcrosses with their respective recurrent parents for the improvement.

**Table 4.29: Analysis of variance for various morphological traits in CMS $BC_4$ generation**

| Source | Replication | Genotypes | Error |
|---|---|---|---|
| **df** | 2 | 11* | 22 |
| **Plant height** | 65.97 | 228.82* | 36.66 |
| **Plant spread** | 31.08 | 144* | 32.41 |
| **Petiole length** | 0.01 | 0.87* | 0.1 |
| **Leaf blade length** | 0.19 | 3.36* | 0.57 |
| **Leaf blade width** | 0.54 | 4.17* | 0.34 |
| **Calyx size** | 4.54 | 7.29* | 1.01 |
| **Petal size** | 2.69 | 7.6* | 2.34 |
| **Stamen size** | 0.06 | 1.62* | 0.27 |
| **Pistil size** | 1.18 | 3.81* | 0.55 |
| **Days to 50% flowering** | 6.25 | 162.55* | 11.28 |
| **Pedicel length** | 0.34 | 8.36* | 0.42 |
| **Fruit length** | 0.22 | 20.86* | 0.46 |
| **Fruit girth** | 0.11 | 4.2* | 0.11 |
| **Fruit weight** | 60.63 | 6272.45* | 18.83 |
| **Number of fruits/ plant** | 4.89 | 397.51* | 7.62 |
| **Yield/ plant** | 0.04 | 0.49* | 0.04 |

*Significant at 5% level

Table 4.30 reveals that characters *viz.* fruit weight, number of fruits/ plant, fruit length and yield/ plant showed comparatively high genotypic and phenotypic coefficients of variation. GCV of all the characters varied between 4.28 and 65.81, while PCV ranged between 6.55 and 66.11 (Table 4.30). The highest GCV was observed in fruit weight (65.81) followed by number of fruits/ plant (38.54), fruit length (30.66), fruit girth (25.66) and yield/ plant (23.02) which could easily be selected in single environment. Differences between GCV and PCV were present in almost all the characters indicating that there was environmental factors were influencing their phenotypic expression. The selection in $BC_4$ CMS population according to respective recurrent parent could be practised based on traits with high GCV. Results were in line with Arivalagan *et al* (2013) who also observed higher PCV than GCV in most of cases indicating environmental effect along with genotypic effect in variation.

**Table 4.30: Estimates of components of variance, broad sense heritability, GA and GA as percent of the mean for 16 characters $BC_4$ generation CMS plants**

| Character | Mean | Variances | | Coefficient of variation | | $H^2$ (%) | GA | %GA |
|---|---|---|---|---|---|---|---|---|
| | | VG | VP | GCV | PCV | | | |
| **Plant height** | 68.92 | 64.05 | 100.71 | 11.61 | 14.56 | 63.6 | 13.15 | 19.08 |
| **Plant spread** | 74.4 | 37.20 | 69.61 | 8.2 | 11.21 | 53.44 | 9.18 | 12.34 |
| **Petiole length** | 3.8 | 0.26 | 0.36 | 13.36 | 15.8 | 71.56 | 0.88 | 23.28 |
| **Leaf blade length** | 14.79 | 0.93 | 1.50 | 6.52 | 8.29 | 61.83 | 1.56 | 10.56 |
| **Leaf blade width** | 9.37 | 1.28 | 1.62 | 12.05 | 13.58 | 78.83 | 2.07 | 22.05 |
| **Calyx size** | 15.97 | 2.09 | 3.10 | 9.06 | 11.04 | 67.4 | 2.45 | 15.32 |
| **Petal size** | 30.92 | 1.75 | 4.09 | 4.28 | 6.55 | 42.77 | 1.78 | 5.77 |
| **Stamen size** | 8.06 | 0.45 | 0.72 | 8.33 | 10.5 | 62.92 | 1.1 | 13.61 |
| **Pistil size** | 15.56 | 1.09 | 1.64 | 6.71 | 8.22 | 66.53 | 1.75 | 11.27 |
| **Days to 50% flowering** | 56.08 | 50.42 | 61.70 | 12.66 | 14.01 | 81.72 | 13.22 | 23.58 |
| **Pedicel length** | 18.13 | 2.65 | 3.07 | 8.97 | 9.66 | 86.32 | 3.11 | 17.17 |
| **Fruit length** | 8.51 | 6.80 | 7.26 | 30.66 | 31.68 | 93.65 | 5.2 | 61.12 |
| **Fruit girth** | 4.55 | 1.36 | 1.47 | 25.66 | 26.63 | 92.82 | 2.32 | 50.92 |
| **Fruit weight** | 69.37 | 2084.54 | 2103.37 | 65.81 | 66.11 | 99.1 | 93.63 | 134.97 |
| **Number of fruits/ plant** | 29.58 | 129.96 | 137.58 | 38.54 | 39.65 | 94.46 | 22.83 | 77.16 |
| **Yield/ plant** | 1.69 | 0.15 | 0.19 | 23.02 | 26.04 | 78.13 | 0.71 | 41.91 |

Heritability in broad sense includes both fixable (additive) and non-fixable (dominant and epistatic) variances and also provides a good indication about the repeatability of the traits. The estimates of heritability for different characters ranged between 42.77 and 99.1%. Most of characters *viz.* fruit weight (99.1), number of fruits/ plant (94.46), fruit length (93.65) and fruit girth (92.82), pedicel length (86.32), days to fifty percent flowering (81.72), leaf blade width (78.83), yield/ plant (78.13), petiole length (71.56), calyx size (67.4), pistil size

(66.53), plant height (63.6), stamen size (62.92) and leaf blade length (61.83) displayed high heritability percentage. Although, the presence of high heritability indicated the effectiveness of selection on the basis of phenotypic performance of different backcross populations, but did not show any sign for the genetic progress with selection of the best individuals. Genetic advance gave percent progress which was found the highest in fruit weight (134.97) followed by number of fruits per plant (77.16), fruit length (61.12) and fruit girth (50.92), yield/ plant (41.91), days to fifty percent flowering (23.58), petiole length (23.28) and leaf blade width (22.05). High heritability and GA (%) for different traits indicated high proportion of genotypic variance in phenotypic expression for these traits in $BC_4$ populations. Therefore, reliable selection for these traits may possibly be made for succeeding backcross with respective recurrent parents. The results of high heritability and genetic advance in different traits among backcross populations were similar as reported by Prabhu and Natarajan (2007) and Senapati *et al* (2009).

**4.4.4 Genetic variability in CMS $BC_5$ generation:**

Analysis of variance for $BC_5$ CMS generation is presented in Table 4.31. The mean square values for all the characters under investigation unveiled significant differences between respective $BC_5$ CMS populations of different recurrent parents. It highlighted the transfer of CMS character into different backcross populations of their respective recurrent parents. The selection from these backcross populations for succeeding backcrosses with their respective recurrent parents could be made for the further improvement

Different genetic variables among different CMS $BC_5$ backcross generations raised from different recurrent parents are displayed in Table 4.32. GCV values of different characters ranged between 4.9 and 65.94%. GCV values were high for characters *viz.* fruit weight (65.94), number fruits/ plant (38.59), fruit length (31.75), yield/ plant (25.45) and fruit girth (24.43). High GCV values in brinjal were also reported by Sabolu *et al* (2014) and Singh *et al* (2014). However, the lower GCV values were revealed by petal size (4.9), pistil size (6.69), leaf blade length (6.77), calyx size (8.65), plant spread (8.89), stamen size (9), pedicel length (9.85) and days to fifty percent flowering (9.95). PCV values ranged between 6.93 to 66.27%. All the traits demonstrated higher PCV than GCV indicating environmental effect on the expression of these traits. The characters with high GCV i.e. fruit weight, number of fruits/ plant, fruit length, yield/ plant, fruit girth can be selected on the basis of phenotypes expressed in $BC_5$ generation. The selection of characters with low GCV *viz.* petal size, pistil size, leaf blade length, calyx size, plant spread, stamen size, pedicel length and days to fifty percent flowering on the basis of their phenotypic expression will not be reliable. Peter and Singh (1974) also reported variability in different characters for GCV and PCV values.

**Table 4.31: Analysis of variance for various morphological traits in CMS $BC_5$ generation**

| Source | Replications | Genotypes | Error |
|---|---|---|---|
| **df** | **2** | **11** | **22** |
| **Plant height** | 251.63 | 162.44* | 24.31 |
| **Plant spread** | 90.13 | 164.9* | 36.29 |
| **Petiole length** | 0.04 | 1.05* | 0.14 |
| **Leaf blade length** | 1.35 | 3.31* | 0.34 |
| **Leaf blade width** | 0.07 | 4.18* | 0.72 |
| **Calyx size** | 5.91 | 6.91* | 1.08 |
| **Petal size** | 3.11 | 9.44* | 2.36 |
| **Stamen size** | 0.39 | 1.82* | 0.23 |
| **Pistil size** | 0.39 | 3.55* | 0.22 |
| **Days to 50% flowering** | 6.19 | 103* | 14.32 |
| **Pedicel length** | 0.41 | 9.65* | 0.25 |
| **Fruit length** | 0.03 | 22.31* | 0.32 |
| **Fruit girth** | 0.1 | 3.92* | 0.07 |
| **Fruit weight** | 66.2 | 6455.19* | 21.85 |
| **Number of fruits/ plant** | 0.89 | 419.25* | 9.89 |
| **Yield/ plant** | 0.13 | 0.63* | 0.05 |

*Significant at 5% level

**Table 4.32: Estimates of components of variance, broad sense heritability, GA and GA as percent of the mean for 16 characters $BC_5$ generation CMS plants**

| Character | Mean | Variances | | Coefficient of variation | | $H^2$ (%) | GA | % GA |
|---|---|---|---|---|---|---|---|---|
| | | VG | VP | GCV | PCV | | | |
| **Plant height** | 66.76 | 46.04 | 70.35 | 10.16 | 12.56 | 65.44 | 11.31 | 16.94 |
| **Plant spread** | 73.54 | 42.70 | 78.99 | 8.89 | 12.09 | 54.06 | 9.9 | 13.46 |
| **Petiole length** | 3.84 | 0.30 | 0.44 | 14.32 | 17.39 | 67.77 | 0.93 | 24.28 |
| **Leaf blade length** | 14.7 | 0.99 | 1.33 | 6.77 | 7.84 | 74.43 | 1.77 | 12.02 |
| **Leaf blade width** | 9.33 | 1.15 | 1.87 | 11.51 | 14.69 | 61.43 | 1.73 | 18.59 |
| **Calyx size** | 16.12 | 1.94 | 3.02 | 8.65 | 10.79 | 64.23 | 2.3 | 14.28 |
| **Petal size** | 31.35 | 2.36 | 4.72 | 4.9 | 6.93 | 50.05 | 2.24 | 7.14 |
| **Stamen size** | 8.09 | 0.53 | 0.76 | 9 | 10.77 | 69.82 | 1.25 | 15.49 |
| **Pistil size** | 15.77 | 1.11 | 1.33 | 6.69 | 7.32 | 83.5 | 1.98 | 12.59 |
| **Days to 50% flowering** | 54.64 | 29.56 | 43.88 | 9.95 | 12.12 | 67.37 | 9.19 | 16.83 |
| **Pedicel length** | 17.97 | 3.13 | 3.38 | 9.85 | 10.23 | 92.73 | 3.51 | 19.54 |
| **Fruit length** | 8.53 | 7.33 | 7.65 | 31.75 | 32.44 | 95.76 | 5.46 | 63.99 |
| **Fruit girth** | 4.64 | 1.28 | 1.35 | 24.43 | 25.13 | 94.56 | 2.27 | 48.94 |
| **Fruit weight** | 70.23 | 2144.45 | 2166.30 | 65.94 | 66.27 | 98.99 | 94.91 | 135.14 |
| **Number of fruits/ plant** | 30.27 | 136.45 | 146.34 | 38.59 | 39.96 | 93.24 | 23.24 | 76.75 |
| **Yield/ plant** | 1.74 | 0.19 | 0.24 | 25.45 | 28.32 | 80.77 | 0.82 | 47.12 |

Heritability estimates (above 60%) along with GA (%) (above 20%) are useful in predicting percent gain during selection. In this study, fruit weight (98.99 and 135.14), fruit length (95.76 and 63.99), fruit girth (94.56 and 48.94), number of fruits/ plant (93.24 and 76.75), yield/ plant (80.77 and 47.12) and petiole length (67.77 and 24.28) depicted high heritability and GA (%), respectively. Chung-won *et al* (2003) also reported high heritability and GA in many attributes of brinjal. The high estimates of heritability along with medium estimates of genetic advance was observed for plant height, calyx size, stamen size, pistil size, days to fifty percent flowering, pedicel length, leaf blade length and leaf blade width rendering them unsuitable for improvement through selection. Low genetic advance along with high heritability is observed if character is controlled by non-additive gene action (Panse 1957). The percent genetic advance for some traits *viz.* stamen size and days to 50% flowering was also affected due to male sterility.

## CHAPTER V

## SUMMARY

Brinjal is an important vegetable crop and large number of varieties and hybrids has been released worldwide. Heterosis in brinjal is exploited through hand emasculation and pollination techniques, requiring a lot of resources and skill. Some reports indicates that wide hybridization generate male sterility in *Solanum* species. Therefore, attempts were made to cross *S. aethipicum and S. melongena,* which induced alloplasmic male sterility. Hence, present study on 'Introgression and characterization of alloplasmic male sterile lines in brinjal (*Solanum melongena* L.)' was carried out during 2015-16 in at Department of Vegetable Science, Punjab Agricultural University, Ludhiana with the following objectives:

- To transfer nuclear genome of brinjal having alloplasmic cytoplasm.
- To characterize alloplasmic male sterile lines for growth and yield traits of brinjal

The experimental material comprised of twelve recurrent parents *viz*., BR 104, MR 319, BL 219, BL 201, BL 214, BL 12-4, BL 216, SR 5, P 67, SR 232, SR 93-213 and CB 99-231 along with their $BC_3$, $BC_4$ and $BC_5$ male sterile progenies carrying alloplasmic cytoplasm of *S. aethiopicum.* The $BC_4$ and $BC_5$ generations of twelve cytoplasmic male sterile (CMS) lines were produced in two seasons from $BC_3$ and $BC_4$ in 2015-16, respectively. All the three male sterile backcross generations along with their fertile recurrent parents were evaluated for various morphological traits in 2016-17 using Factorial Randomized Complete Block Design along with three replications. Observations were recorded for plant height (cm), plant spread (cm), petiole length (cm), petiole colour, leaf blade length (cm), leaf blade width (cm), leaf blade colour, days to 50% flowering, calyx colour, calyx size (mm), corolla colour, petal size (mm), stamen size (mm), pistil size (mm), male fertility status (sterile/fertile), pedicel length (mm), fruit length (cm), fruit girth (cm), fruit length: girth ratio, fruit colour, number of fruits/plant, average fruit weight (g), fruit yield/plant (kg). Analysis of variance revealed that genotypes were statistically different for all the characters, while generations were at par for characters like petiole length, leaf blade width, calyx size, pedicel length and fruit length. All the CMS backcross progenies were stable in their expression for male sterility caused by *S. aethiopicum* cytoplasm having no viable pollen grains in the shriveled anthers.

The vegetative characters like plant height i.e. 71.40, 68.92, 66.59, plant spread i.e. 75.71, 74.40, 73.54 and leaf blade length i.e. 14.90, 14.79, 14.70 were significantly better in $BC_3$, $BC_4$ and $BC_5$ generations than their recurrent parents i.e. 61.70, 70.03, 13.98, respectively. However, plant height, plant spread and leaf blade length decreased with the advancement of backcross generations succeeding towards revival of recurrent characters. The overall petiole length (3.88) and leaf blade width (9.08) of recurrent parents was statistically at par with all three CMS backcross generations. Among genotypes SR 93-213

(84.85) was significantly tallest, whereas BR 104 (87.47) followed by SR 93-213 (86.30) were high in plant spread over different generations. The maximum value of leaf blade length was observed in genotype BR 104 (15.83) followed by SR 93-213 (15.68), while of leaf blade width was in SR 93-213 (11.36) followed by BR 104 (10.42). Petiole length was maximum in SR 93-213 (4.45) followed by BL 214 (4.26).On the basis of The Royal Horticultural Society Colour Charts leaf blade colour and petiole colour did not present any difference between backcross generations and their respective recurrent parents, however purple pigmented and green coloured were able to be differentiated at genotypic level.

In general, most of the flowering characters such as calyx size (16.12), petal size (31.35), pistil size (15.76) and pedicel length (17.97) of $BC_5$ CMS generation were at par with recurrent parent values, except days to 50% flowering (54.64) and stamens (11.29) of male sterile lines recipient parents. Among genotypes, SR 5 had overall best value for days to 50% flowering (46.0) and calyx size (18.17); BL 12-4 for petal size (35.97), stamen size (10.20) and pistil size (17.87); CB 99-231 (21.41) for pedicel length. Calyx and corolla colour on the basis of The Royal Horticultural Society Colour Charts did not present any difference between generations. Three calyx colours *viz.* green, purple and violet blue were observed in different genotypes, while only shade differences for violet colour were observed among the genotypes for corolla colour.

Among yield related characteristics, mean performance for fruit girth, number of fruits/ plant, fruit weight and fruit yield/ plant were significantly better in recurrent parent i.e. 4.97, 34.67, 72.29, 2.04 than $BC_3$ i.e. 4.51, 28.58, 67.67, 1.61, $BC_4$ i.e. 4.55, 29.58, 69.37, 1.69 and $BC_5$ i.e. 4.64, 30.27, 70.23, 1.74 generations, respectively. However, comparable differences were observed for fruit length among recurrent parent mean (8.56) and all three CMS backcross generations viz. $BC_5$ (8.53), $BC_4$ (8.51) and $BC_3$ (8.52). Genotypes differed for yield traits with maximum fruit length in BL 12-4 (13.72); fruit girth (7.44) and weight (182.91) in BR 104; number of fruits/ plant in SR 5 (47.04) and fruit yield/ plant in BL 12-4 (2.41). Based on The Royal Horticultural Society Colour Charts, fruit colour did not depict any difference between generations. Grayed purple, purple and violet blue colours of the fruits were observed in different genotypes over the generations, but shade differences were observed only among the genotypes carrying grayed purple fruit colour. Fruit length: girth ratio was higher in backcross generations, but was nearing towards recurrent parent with advancement of each generation.

High level of genetic coefficient of variation for fruit weight, fruit length, fruit girth, number of fruits/plant, yield/ plant in $BC_3$, $BC_4$, $BC_5$ and their maintainer-lines indicated vast variability among different genotypes. High heritability along with high percent GA also observed in all fruit characteristics, which indicated effectiveness of selection for improving superior breeding lines. Recovery percent of fruit trait in $BC_5$ was 92.54%, while it was

98.86% in floral traits (except stamen size). Vegetative characteristics including days to 50% flowering gave more growth i.e. 106.32% of the recurrent parents (100%). Overall, in all the traits CMS generations were becoming closer to recurrent parents with of 97.98 % of recurrent parent similarity in $BC_5$.

Therefore, it can be concluded that all alloplasmic male sterile backcross generations were stable in male sterility and were vigorous in vegetative growth. Most of the flowering traits in $BC_5$ generation were comparable to recurrent parent except petal size, stamen size and days to 50% flowering. Fruit characteristics like girth, weight, numbers and yield were lower than recurrent parent, however, high heritability along with high genetic advance are the good indicators for their improvement. After $BC_5$ generation of backcrossing all the traits showed 97.98% similarity to the recurrent parent. Thus male sterility induced from cross of *S. aethipicum* and *S. melongena* can be successfully transferred in different cultivated genotypes for development of stable CMS-lines in brinjal, which can be of worth use in heterosis breeding programme.

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# VITA

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