Vegetable Science (2017) 44 (2) : 101-106

Evaluation of cytoplasmic male sterile (CMS) and maintainer lines for yield and horticultural traits in brinjal (*Solanum melongena* L.)

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Received: September 2017 / Accepted: November 2017

Abstract

The present study was aimed at evaluating six cytoplasmic male sterile lines (A-lines) of *Solanum melongena* L. carrying alloplasmic cytoplasm of *S. aethiopicum* L. along with their fertile counterparts (B-lines) for various yield and horticultural traits. A significant variability was observed among the tested lines. All six cytoplasmic male sterile lines were similar with respective maintainer for the growth traits *viz.*, plant height, plant spread, petiole length, leaf length and leaf width. Five out of seven studied flower traits *viz.*, calyx size, petal length, petal width, pistil length and pedicel length, also displayed no difference between A- and B-lines. However, significant differences were there between A and B-lines for stamen size and days taken for 50% flowering with sterile lines having significantly reduced stamen size and taking more number of daysfor 50% flowering. The yield contributing fruit traits including peduncle length, fruit length, fruit weight, number of fruits plant^{-1} and yield plant^{-1}also displayed non-significant difference between A and B-lines. Similar trend of no difference was displayed by the commercially important colour traits. Theat par performance of newly developed male sterile A-lines with their fertile counterparts provides possibilitiesof further use of these lines in cytoplasmic genetic male sterility system hybrid development programme of brinjal.

Keywords: Brinjal, CMS, A-line, B-line, Heterosis, Hybrid

Introduction

Brinjal, *Solanum melongena* L. (2n=2x=24) is an important vegetable crop cultivated globally over an area of 1.87 mha especially in tropical and sub-tropical regions with production of 50.19 mt (Anonymous 2014). It is known for having good medicinal properties with rich amounts of vitamins and minerals.It can be considered as an 'often cross-pollinated crop' with 2-48 % natural out-crossing being reported (Sambandan 1964). Brinjal has lesser seed requirement per acre and more number of seeds areobtained per fruit therefore; hybrids are being used from long time as they are known to outperform varieties in marketable yields. Traditionally, the manual emasculation and pollination is the method practised for hybrid development, despite availability of male sterility mechanisms like genetic male sterility (Jasmin 1954; Phatak et al. 1991) cytoplasmic male sterility (Fang et al. 1985; Saito et al. 2009; Khan and Isshiki 2010; Khan and Isshiki 2011) and genetically engineered male sterility (Cao et al. 2010; Toppino and Kooiker 2011). The use of male sterility mechanisms could, however, significantly reduce the time and labour required for hybrid seed production.

Out of the different male sterility mechanisms; genetic male sterility being instable under varying environmental conditions and genetically engineered being opposed by anti GM organizations are not preferred. Thus, if fully exploited, the cytoplasmic male sterility (CMS), could provide a viable option. The CMS is a maternal genetic trait resulting from nuclear-cytoplasmic interactions in which higher plants fail to produce functional and viable pollen (Kaul 1988). This male sterility has been successfully used for hybrid seed production with"three-line" (male-sterile line: A-line, maintainer line: B-line and restorer line: R-line) cytoplasmic genetic male sterility (CGMS) system in crops such as maize, rice, sunflower, sorghum, onion and mustard (Banga and Banga 1998). Although, hybrids are produced with the help of restores, the stable male sterile A-lines are must for any successful CGMS system. These male sterile lines in CMS system could be developed with the transfer of alloplasmic cytoplasm from one specie to another as in mustard (Prakash and Chopra 1990), rice (Dalmacio et al. 1995) and pigeon pea (Saxena et al. 2005). But, besides male sterility several unfavourable agronomic traits are also usually related with cytoplasm transfer. To overcome

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these undesirable effects, the backcrosses with the required recurrent parent are made. About five backcrosses are usually considered essential for transfer of required traits as about 98.44% recovery of recurrent parent is expected in fifth backcross generation. Also, in brinjal a wide range is there among fruit sizes *viz.*, round, small or long fruited along with different bearing habits and fruit colours requiring male sterile lines in all these. However, the reports regarding development of A-lines in diverse brinjal genotypes are not widely available. Therefore, present study was focused to study the effect of alien cytoplasm leading to male sterility after five backcross generations with morphological characterization of CMS A-lines and comparing these with their respective maintainers.

Materials and Methods

Experimental site and development of CMS lines: The experiment was conducted at the Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana during 2015-2016. For the development of CMS lines crosses were made in the past years with *S. aethiopicum* as female parent and *S. melongena* cv. Punjab Barsati as male parent. The $F_1$was male sterile which was further used forbackcrossing with six diverse genotypes of various fruit shapes *viz*. round (BR 104), long (BL 12-4, BL 219) and small (SR 93-213, SR 5, P 67). Thus, six CMS lines in three groups were developed. The BC_5 generation CMS A-lines and their maintainers were grown on ridges with spacing of 60 × 45 cm in open field conditions following Randomized Complete Block Design (RCBD) with three replications. All the recommended package of practices was followed to ensure the optimum plant growth. To ensure optimum fruit setting on male sterile CMS lines all flowers on A-lines were pollinated with the help of pollen collected from fertile flowers with the help of Pollen-Collector. The observations were recorded for various vegetative traits {plant height (cm), plant spread (cm), petiole length (cm), leaf length (cm) and leaf width (cm)}and flower traits {calyx size (mm), petal length (mm), petal width (mm), stamen size (mm), pistil size (mm), days to 50% flowering and pedicel length (mm)}. Yield related traits{(peduncle length (cm), fruit length (cm), fruit weight (g), fruit number plant^{-1}, fruit yield plant^{-1} (kg)} along with commercial traitslike various colour traits such as petiole colour, leaf colour, calyx colour, corolla colour and fruit colour were also recorded.

Statistical analysis: Average values per plant for each genotype for each line in each replication for the traits studied were subjected to statistical analysis. The statistical analysis of experimental data was accomplished by Analysis of Variance in Randomized Complete Block Design (RCBD) as per the procedure given by Panse and Sukhatme (1985) using CPCS-1 software (Cheema and Singh 1990).

Results

The effect of alloplasmic cytoplasm on morphological and yield traits in brinjal is not well studied. Introgression of *S. aethiopicum* cytoplasm into *S. melongena* nuclear background resulted into male sterility governed by cytoplasm with some adverse effects on floral, vegetative and marketable traits. The adverse effects were minimized with the help of backcrossing uptoBC_5 generation in six diverse genotypes of brinjal. These BC_5 generation CMS lines are studied along with their fertile counterparts/maintainers for various traits.The analysis of variance for eighteen quantitative characters in six genotypes manifested thatmean sum of squares for all the traits except petal length in the CMS lines and their maintainers differed significantly (P=0.05) demonstrating ample variability for advanced lines development (Table 1).

Table 1: Analysis of variance with respect to 18 characters of A- and B-lines combined

Source	Replications	Genotypes	Error
Degree of freedom	2	11	22
Plant height	433.27*	266.83*	34.91
Plant spread	41.39	254.28	45.47
Petiole length	0.52	0.85*	0.15
Leaf length	0.37	4.52*	0.55
Leaf width	0.54	5.39*	0.54
Calyx size	0.42	8.08*	0.70
Petal length	2.92	2.25	1
Petal width	0.21	2.02*	0.32
Stamen size	0.42	14.72*	0.48
Pistil size	0.27	4.41*	0.40
Days to 50% flowering	22.58	100.98*	12.58
Pedicel length	0.35	7.01*	0.58
Peduncle length	0.12	1.24*	0.83
Fruit length	0.62	25.87*	0.28
Fruit girth	1.84	6.23*	0.10
Fruit no. plant^{-1}	16.29	587.14*	16.34
Fruit weight	82.14	8839.78*	26.88
Yield plant^{-1}	0.15	0.50*	0.76

*Significant at 5% level

Growth traits: The estimation of vegetative/growth traits is very important especially in CMS lines as these lines generally outgrow the fertile counterparts because of lesser fruit setting obtained with artificial pollination. But in our study non-significant differences were manifested between A- and B-lines for all the vegetative traits studied *viz.*, plant height, plant spread, petiole length, leaf length and leaf width (Table 2). However, significant differences were present among all lines with maximum plant height and leaf width in line SR 93-213A (81.06 cm); plant spread (86.07cm) and leaf length

Table 2: Growth traits in CMS and maintainer lines of brinjal

Genotype	Plant height (cm)	Plant spread (cm)	Petiole length (cm)	Leaf length (cm)	Leaf width (cm)
BR 104 A	66.70	86.07	3.48	15.79	10.35
BR 104 B	64.36	85.72	3.45	15.12	10.13
BL 219A	55.80	64.09	3.73	14.50	8.45
BL 219 B	44.87	60.39	3.96	13.27	8.15
BL 12-4 A	62.85	81.28	4.16	12.74	7.98
BL 12-4 B	63.08	80.12	4.14	12.19	7.56
SR 93-213 A	81.06	79.67	4.55	15.73	11.33
SR 93-213 B	77.31	72.00	4.50	15.20	11.30
SR 5A	68.78	64.33	3.37	14.47	9.18
SR 5 B	62.24	62.61	3.31	13.40	9.02
P 67 A	71.55	75.23	4.74	15.61	10.79
P 67 B	63.29	69.72	4.68	14.97	10.57
LSD (5%)	10.0	11.42	0.66	1.26	1.25
CV (5%)	9.07	9.18	9.70	5.15	7.71

in (15.79 cm) BR 104A and petiole length in P 67A (4.74 cm). The results are demonstrating that the CMS lines outperform the maintainers in growth confirming the fact of excessive growth of sterile lines, however the growth was not statistically proven for difference between A and B-lines. The long fruited genotypes had comparatively lesser growth with minimum difference between sterile and respective maintainers among all the genotypes as plant heightand spread was lowest in BL 219 while leaf length and leaf width was lowest in BL 12-4.

Flower traits: Seven flowering traits were studiedwhere significant differences were observed except petal length (Table 3). However, the sterile and maintainer lines of all six genotypes were at par with oneanother except for stamen size and days to 50% flowering. Among various traits calyx size (18.39 mm) was biggest in SR 5A line. Petal width (13.36 mm), pistil size (18.43 mm) were highest in BL 12-4B and pedicel length (19.30 mm) was highest in BL 12-4A genotype while all lines were at par for petal length. Among the traits where A and B-lines of genotypes were dissimilar, the reason becomes the alloplasmic cytoplasm of *S. aethiopicum* as the major effect of this cytoplasm is the reduction of stamen size with no pollen grains (Khan and Isshiki 2010) and the same was evident from present study as all A-lines displayed significant reduction in stamen size from their respective maintainers (Figure 1). For the trait of days taken for 50% flowering, the male sterile lines of four genotypes took more number of days than their respective maintainer lines with no difference only in two genotypes *viz.*, BL 219 and SR 93-213. The diversity among the genotypes in floral traits in the selected six genotypes could be viewed from Table 3.

Fruit traits: Yield is the most important aspect ofany vegetable breeding programme and in brinjal, yield is manifested from its fruit characteristics. In the present study significant differences were reported for all the five fruit traits studied (Table 4). However, A- and B-lines of all genotypes displayed non-significant results for peduncle length, fruit length, fruit weight and fruit yield plant^{-1}. While in case of fruit girth and fruit number plant^{-1} also, all genotypes except SR 5 displayed non-significant results between respective A and B-lines. The values confirm the isogenic levels of A- and B-lines after five generations of backcrossing. Among all the lines, the fertile lines had better fruit traits than their sterile counterparts. The long fruited genotype BL 12-4B was found to be having maximum fruit length (14.09 cm) and fruit yield plant^{-1} (2.72 kg) while its A-line had biggest

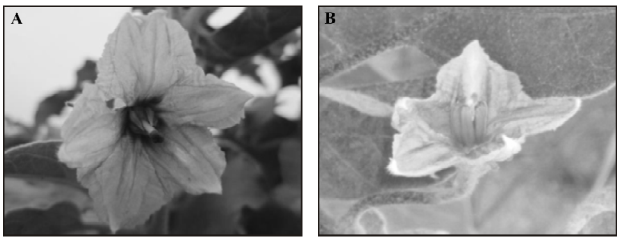

Figure 1: A- Shrivelled stamen size of BL 219 A; B- Normal stamen size of BL 219 B

Table 3: Floral traits in CMS and maintainer lines of brinjal

Genotype	Calyx size (mm)	Petal length (mm)	Petal width (mm)	Stamen size (mm)	Pistil size (mm)	50% flowering (days)	Pedicel length (mm)
BR 104 A	16.48	16.93	11.48	7.25	15.07	53.33	14.95
BR 104 B	17.07	17.73	11.93	10.78	15.47	42.67	15.33
BL 219A	15.77	15.99	10.87	7.98	16.86	49.33	16.66
BL 219 B	15.57	16.46	11.01	10.19	16.31	47.67	16.73
BL 12-4 A	14.08	18.21	12.88	9.19	17.59	53.00	19.30
BL 12-4 B	14.32	19.00	13.36	13.59	18.43	40.67	19.26
SR 93-213 A	18.16	16.65	11.03	8.19	16.71	57.00	18.23
SR 93-213 B	18.10	16.87	11.16	13.47	16.61	53.67	18.50
SR 5A	18.39	17.42	11.38	7.83	15.13	47.00	18.83
SR 5 B	18.13	17.66	11.42	10.97	15.59	39.67	19.20
P 67 A	14.68	16.35	10.78	8.12	14.20	55.67	16.91
P 67 B	14.78	16.68	10.91	11.90	15.10	47.33	16.88
LSD (5%)	1.41	NS	0.96	1.18	1.08	6.0	1.29
CV (%)	5.13	5.83	4.92	6.98	3.95	7.25	4.34

Table 4: Fruit traits in CMS and maintainer lines of brinjal

Genotype	Peduncle length (cm)	Fruit length (cm)	Fruit girth (cm)	Fruit no. plant^{-1}	Fruit weight (g)	Fruit yield (kg pl^{-1})
BR 104 A	3.80	7.67	7.58	8.73	183.76	1.67
BR 104 B	3.77	7.69	7.57	10.96	186.88	2.07
BL 219A	3.61	10.27	4.32	28.66	62.91	1.85
BL 219 B	3.56	10.16	4.84	34.61	64.96	2.26
BL 12-4 A	5.11	13.84	3.14	38.33	63.77	2.57
BL 12-4 B	4.91	14.09	3.23	41.48	65.76	2.72
SR 93-213 A	3.40	6.59	3.95	44.46	31.49	1.42
SR 93-213 B	3.43	7.05	3.82	50.16	32.22	1.65
SR 5A	3.11	5.57	4.49	44.87	33.26	1.53
SR 5 B	3.01	5.35	5.06	52.82	36.81	1.95
P 67 A	3.54	7.20	4.83	38.50	55.12	2.12
P 67 B	3.48	7.12	5.23	43.26	56.35	2.30
LSD (5%)	0.49	0.89	0.54	6.84	8.78	0.47
CV (%)	7.74	6.14	6.55	11.10	7.12	13.70

peduncle length (5.11 cm). The fruit girth (7.58 cm) was highest in round shaped BR 104A while its B-line had highest fruit weight (186.88 g). Maximum number of fruits plant^{-1}(52.82) were found in small round fruited SR 5B.

Colour traits: Along with various quantitative traits the qualitative traits especially colour traits are also of upmost importance as per consumer preference. The various colour traits of all the lines were similar for respective sterile and maintainer lines displaying effectiveness of backcross method. Again variability in genotypes could be verified from Table 5. As three colours of fruits were present with BR 104, and SR 5 displaying violet blue; BL 219, BL 12-4 and SR 93-213 grated purple while purple coloured fruits were displayed by P 67. Similarly variability was there among genotypes for other colour traits *viz.* petiole colour, leaf colour, corolla colour and calyx colour. Despite variability in genotypes the colour of all sterile lines was similar to their respective maintainers for all traits studied.

Discussion

CMS is an extensively used male sterility system for heterosis exploitation in many field and horticultural crops. The basic principle of CMS is nuclear and cytoplasmic interactions where cytoplasm does not allow producing functional pollen grains. Inbreds are basic necessity for hybrid development programme due to trueness to type and stability provided by them, therefore development of uniform CMS inbred lines identical to their respective maintainers is of utmost importance. For the development of CMS lines the *S. aethiopicum* cytoplasm derived male sterility is one of the many alloplasmic male sterility systems available in brinjal (Khan and Isshiki 2010). Because, a variety of fruit types are available in brinjal, innovation and effective utilization of diverse CMS lines becomes important task for breeders. Therefore, in the current research, six new alloplasmic male sterile lines of brinjal with *S. aethiopicum* cytoplasm were developed and evaluated for their performance along with their maintainers.

Phenotypically, the A-lines in all six genotypes were similar to their respective B-lines for most of the traits. Variability if present was limited only toa couple of flowering traits. Our study found no difference between A- and B-lines for growth traits such as plant height and spread, leaf length and width but these were in contrast to earlier findings in Brassica species by Pathania et al. (2003) and Rao et al. (1994) where excessive growth in CMS lines compared to their fertile counterparts were reported. Apart from the vegetative traits the A and B-lines were statistically at par for most

Table 5: Colour traits in CMS and maintainer lines of brinjal

Genotype	Petiole colour	Leaf colour	Corolla colour	Calyx Colour	Fruit colour
BR 104 A	Green	Grayed purple	Violet	Violet	Violet blue
BR 104 B	Green	Grayed purple	Violet	Violet	Violet blue
BL 219A	Green	Green	Violet	Green	Grayed purple
BL 219 B	Green	Green	Violet	Green	Grayed purple
BL 12-4 A	Green	Green	Violet	Green	Grayed purple
BL 12-4 B	Green	Green	Violet	Green	Grayed purple
SR 93-213 A	Yellow green	Green	Violet	Green	Grayed purple
SR 93-213 B	Yellow green	Green	Violet	Green	Grayed purple
SR 5A	Green	Green	Violet	Green	Violet blue
SR 5 B	Green	Green	Violet	Green	Violet blue
P 67 A	Violet blue	Grayed purple	Violet	Purple	Purple
P 67 B	Violet blue	Grayed purple	Violet	Purple	Purple

of flower traits also. These finding was again in contrast to many reports of Brassica species *viz.*, the similarity in calyx size was in contrast to the findings of Yang et al. (2005), petal length and width to the Dey et al. (2010) and the pistil length to the Malik et al. (1999). However, in brinjal no negative effect of alloplasmic cytoplasm on pistil length is reported by Khan and Issihiki (2010) demonstrating similarity with present report. The major part affected with alloplasmic cytoplasm leading to sterility is stamen size and presence or absence of pollen grains. We reported the smaller stamen size in A-lines which was in line with the findings of Khan and Issihiki (2010) who also reported shrivelled and smaller anthers with no pollen grains. The other variable flower trait reported was days to 50% flowering; where four out of six genotypes displayed significant late flowering in A-lines. The delayed flowering in male sterile lines of various crops is also reported by Dhatt and Singh (1999) and Dhatt and Gill (2000).

For all the fruit traits *viz.* peduncle length, fruit length, fruit girth and fruit yield plant^{-1}the CMS lines were found similar to their respective maintainers. However, difference was there between A- and B-lines of two genotypes among number of fruits plant^{-1} and fruit weight. The reduced fruit setting was also reported by Prohens et al. (2012) in backcross generations produced by crossing of *S. melongena* with *S. aethiopicum.* For the commercially important colour traits all genotypes displayed similarity in A- and B-lines marking the effective transfer of nucleus to sterile lines. Overall, with the only major difference of pollen absence in shrivelled anthers, these newly developed CMS lines could be called as isogenic to the respective maintainer lines. The results indicated that genetic backgrounds of A- and B-lines became identical after five generations of backcross except difference in fertility. The same could be verified from the estimation that about 98.48% of genome is transferred until BC_5 generation. These isogenic CMS lines developed in our study could be used as effective A-lines in hybrid seed production. In conclusion, six new CMS A-lines with varying fruit characteristics were bred and characterized for various horticultural and yield traits. The future studies could prevail around development of restorers for these lines and exploiting heterosis.

सारांश

वर्तमान अध्ययन *सोलेनम मेलोजेना* एल. के कोशिका द्रव्यी नरबन्ध्य वंशक्रमों (ए–लाइनों) जिसमें सोलेनम इथियोपिकम एल.के ऐलोप्लाज्मिक साइटोप्लासम को लेकर विभिन्न उपज और उद्यानिकी लक्षणों के लिए अपने उपजाऊ समकक्षों (बी–लाइन) का मूल्यांकन किया गया। वंशक्रमों के मध्य महत्वपूर्ण परिवर्तनशीलता पाया गया। सभी छः कोशिका द्रव्यी नरबन्ध्य वंशक्रम के विकसीत गुणों जैसे कि पौधों की ऊँचाई, पौधे का प्रसार, वृन्त की लम्बाई, पत्ती की लम्बाई और पत्ती की चौड़ाई के लिए समकक्षों के समान थी। सात अध्ययनित पुष्प घटकों में से पाँच अर्थात् बाहय दलपुन्ज आकार, पत्ती की लंबाई, पत्ती की चौड़ाई, गर्भकेसर की लम्बाई और पुष्पवृन्त की लम्बाई में ए और बी–लाइनों की तुलना में कोई अंतर नहीं पाया गया। जबकि, पुंकेसर आकार के लिए ए और बी–लाइनों के बीच महत्वपूर्ण अंतर थे और 50 प्रतिशत फूलों के लिए बन्ध्य वंशक्रम में काफी कम पुंकेसर आकार और 50 प्रतिशत फूलों के लिए अधिक दिनों की संख्या में वृद्धि थी। पुष्पवृन्त की लंबाई, फल की लंबाई, फल का वजन, फलों की संख्या/पौधे और उपज/पौधे सहित फलों के गुणों में योगदान के लिए ए और बी–लाइनों के बीच भी अंतर महत्वपूर्ण नहीं थे। व्यवसायिक रूप से महत्वपूर्ण रंग लक्षणों हेतु भी कोई अन्तर नहीं पाया गया। नव विकसित बन्ध्य ए–लाइनों के उनके उपजाऊ समकक्षों के साथ–साथ कोशिका द्रव्यी अनुवांशिकता नरबन्धय प्रणाली संकार विकास कार्यक्रम में इन पंक्तियों के आगे उपयोग की सम्भावनाएं प्रदान करता है।

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