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RESEARCH ARTICLE

Inheritance of alloplasmic fertility restoration in eggplant (*Solanum melongena* L.)

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Abstract Cytoplasmic male sterility (CMS) system has been exploited worldwide in field and vegetable crops. In eggplant, alloplasmic CMS lines were developed through interspecific hybridization between *Solanum aethiopicum* L. × *S. melongena* L., while the restorer (R) lines were isolated from the reciprocal cross. The knowledge about inheritance of *Rf* gene is must for its further use in breeding and molecular studies. Therefore, four sets of CMS (D-CMS 291A, D-CMS 99A, D-CMS 5A and D-CMS 72A) and restorer (R 2-1, R 3-4, R 6-2 and R 2596-2) lines were used to develop F_1, F_2 and backcross progenies, to understand the inheritance mechanism. Phenotyping of all the populations and test of goodness of fit revealed involvement of a single dominant gene (*Rf*) for fertility restoration. The visual scoring of flowers for male sterility and fertility was further validated with the tests on pollen stainability, germination and index. Among others, media containing 0.5% agar + 300 ppm calcium nitrate + 5% sucrose + 50 mg/l boric acid + 400 mg/l PEG 4000 furnished the best results for in vitro pollen germination. Differences between and within male sterile and restorer lines were observed for pistil and stamen length and girth, pollen stainability and germination. Stable expression of CGMS and restorer lines in all the generation progenies confirmed their utility in future eggplant breeding programs.

Keywords CMS lines · Restorer gene · Inheritance · Brinjal

Introduction

Eggplant (*Solanum melongena* L.), also known as brinjal and aubergine is an often-cross pollinated vegetable cultivated in tropical and sub-tropical parts of the world (Chowdhury et al. 2011; Dubey et al. 2014). A high range of heterosis has been observed and exploited for yield and other traits in eggplant (Pal and Singh 1949; Mishra 1961; Sambandum 1962; Chadha and Sidhu 1982; Shafeeq et al. 2007; Das et al. 2009; Makani et al. 2013). Hybrid seed for this purpose is produced through hand emasculation and pollination approach, which demands utmost care during cross-pollination process. Genetic tool of male sterility has the potential to ease out the process of hybrid seed production. In eggplant, genic (Phatak et al. 1991) and cytoplasmic male sterility (Khan et al. 2013) have been reported. The use of genic male sterility (GMS) has the limitation of rouging 50% fertile plants, while cytoplasmic male sterility (CMS) has the advantage of 100% sterile progeny for use in hybrid seed production.

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Cytoplasmic male sterility is inherited maternally and exploited widely in crops, where vegetative part is of commercial importance like in onion, carrot, radish, cauliflower and cabbage (Hussain et al. 2018). However, in tomato, chilli and eggplant fruits possess economic value and borne only after restoration of the fertility. CMS is often associated with unusual open reading frames (ORFs) found in mitochondrial genomes (Chase and Babay-Laughnan 2004; Hanson and Bentolila 2004). Many studies (Kruft et al. 2001; Dahan and Mireau 2013; Fujii et al. 2011; Arnal et al. 2014; Barkan and Small 2014; Gaborieau et al. 2016; Melonek et al. 2016) however, have found that few proteins controlled by the nucleus, when expressed in mitochondria alter this process. In majority of the cases, *Rf* genes produce proteins (often PPR) that bind specifically to the CMS conferring transcripts in the mitochondria and promote processing events leading to down-regulation of mitochondrial CMS-inducing proteins and thus restores the fertility (Chen and Liu 2014; Barchi et al. 2019).

The nuclear gene(s) that restore the fertility, altered CMS system into the CGMS (Cytoplasmic genetic male sterility) and possess the potential of commercial exploitation without rouging, hand emasculation and pollination. This system comprised of three lines viz. A (male sterile), B (maintainer) and R (restorer). A-line being complete sterile excludes the requirement of hand-emasculation and is used as female parent along with R-line for hybrid development. Since, fruit development is the outcome of successful sexual fertilization, nuclear gene(s) from R-line are required to alter the mutant organellar transcript(s) of A-line (CMS) to restore the male fertility in their offspring (F_1). Therefore, identification of restorer (*Rf*) gene (s) is the foremost step to establish CGMS system. Punjab Agricultural University has developed series of alloplasmic CMS-lines from a cross of *S. aethiopicum* and *S. melongena* (Garcha and Dhatt 2017). Subsequently, successful attempts were made in identification of fertility restorer lines. However, knowledge about genetics of restorer gene(s) was lacking for further use in breeding and molecular programmes. Thus, present study on inheritance of fertility restoration of alloplasmic male sterile lines of eggplant was conducted.

Materials and methods

Plant materials

The present study comprised of four CMS (D-CMS291A, D-CMS 99A, D-CMS 5A and D-CMS72A) and four restorer (R 2-1, R 3-4, R 6-2 and R 2596-2) lines, which were used to develop four F_1, F_2 and BC_1 populations as mentioned in Table 1. CMS lines were developed through inter-specific hybridization between *S. aethiopicum* L. *Aculeatum* group and *S. melongena* L. followed by repeated backcrossing with cultivated genotypes in the Department of Vegetable Science, PAU Ludhiana (Garcha and Dhatt, 2017). Simultaneously, R-lines (restorer) were derived through test crossing from segregating population of *S. melongena* and *S. aethiopicum*. The crosses for F_1s were attempted during spring–summer, 2017 followed by selfing and crossing in subsequent autumn seasons to produce F_2 and BC_1 generations. Progenies so developed were planted for scoring and evaluation of pollen fertility, viability and floral traits during summer, 2018. Total numbers of plants scored for fertility status were 1095 (including 10 each of male sterile and restorer parents, 25 of each F_1, 150 plants of each F_2 and 65 plants each of four BC_1P_2 populations). Simultaneously, the CMS-lines (A-lines) were maintained by crossing with their respective maintainers (B-lines), while the maintainer and restorer lines (R-lines) were maintained by selfing. The plant material was raised in open field conditions as per recommended package of practices (Anonymous 2018) during both years with a maximum and minimum temperature of 39.50 and 11.40 °C, respectively.

Scoring for male fertility and sterility status

To assess the male fertility and sterility status of plants, anthers from freshly opened flowers were dissected to check the absence or presence of pollen grains. Each plant of all populations was assigned a specific number to facilitate the scoring process. The male sterile and fertile plants were distinguished from anther shape and presence of pollen grains at the time of dehiscence between 7 and 10 a.m. The anthers of fertile and sterile flowers of every individual plant in a population were compared to determine normal and shrunken anthers. Anthers having porous tips, fully

Table 1 Detail of alloplasmic CMS lines, restorers and their derived populations

S.No	Male sterile genotypes (A-line)	Maintainer lines (B-line)	Restorer lines (R-line)	Cross combinations	F_1 populations (denoted as)	F_2 populations	BC_1 populations
1	D-CMS291A	D-CMS291B	R 2-1	Cross 1 (C1): D-CMS291A × R2-1	F_1C1	F_2C1	(D-CMS 291A × R2-1) × R 2-1
2	D-CMS99A	D-CMS99B	R 3-4	Cross 2 (C2): D-CMS 99A × R 3-4	F_1C2	F_2C2	(D-CMS99A × R 3-4) × R 3-4
3	D-CMS5A	D-CMS5B	R 6-2	Cross 3 (C3): D-CMS5A × R 6-2	F_1C3	F_2C3	(D-CMS5A × R 6-2) × R 6-2
4	D-CMS72A	D-CMS72B	R 2596-2	Cross 4 (C4): D-CMS72A × R 2596-2	F_1C4	F_2C4	(D-CMS72A × R 2596-2) × R 2596-2

developed lobes filled with pollen grains were termed as normal (Fig. 1b), while without porous tips, shrunken lobes and without pollen grains were termed as sterile (Fig. 1a). SEM (Scanning electron microscope; Hitachi/S-3400 N) was used to confirm the absence or presence of pollen grains in male sterile and fertile flowers. The data so generated for male sterile and male fertile plants in all populations was further analysed to apprehend the inheritance pattern of fertility restoration gene in the restorers understudy.

Assessment of pollen fertility

The visual observations on fertility status of all populations were further substantiated by assessing pollen fertility through pollen stainability, germination and index approach as mentioned below:

Pollen stainability

Pollen stainability with 1.0 percent Aceto-carmine solution was used to determine pollen viability. Flowers from 10 male fertile plants of each population (except CMS lines) were collected before anther dehiscence (before 9:30 a.m.) in Petri-dishes lined with moist blotting paper to avoid pollen dehydration (Franca et al. 2009) and observed under microscope at 10X magnification (using Carl Zeiss Vision Axio Vision software 4.8.2) after staining. Pollen grains stained deep red were recorded as viable and unstained as non-viable (Fig. 1h). Observations recorded on pollen stainability were averaged and calculated in percent as follows:

$$Pollen\ stainability(\%) = \frac{Number\ of\ viable\ pollen\ grains}{Total\ number\ of\ pollen\ grains} \times 100.$$

Pollen germination

Pollen germination plays direct role in fertilization, fruit and seed setting. It is a more practical method to assess pollen viability and was conducted as follows:

Optimization of media for pollen germination To asses pollen germination, eight different growth media combinations varying in agar, calcium nitrate and PEG 4000 (Polyethylene glycol) concentrations were tested (Table 5). Pollen grains collected from restorer line (R 2-1) having highest stainability were used to grow in three replicates. The best performing germination media was further used to assess pollen germination in all the populations. The germination time for pollen grains was kept 3 h as suggested by Guler et al. (1995) and Das et al. (2017).

Pollen germination (%) Out of eight, best performing media was used to record in vitro pollen germination for all the populations. Pollen grains of freshly opened flowers were dusted using 0.5-inch Camlin paint brush on solidified 5 ml germination media in 20 ml petri-dishes. Germination percentage was recorded after incubation for 3 h at 25 °C under microscope at 10X magnification (using Carl Zeiss

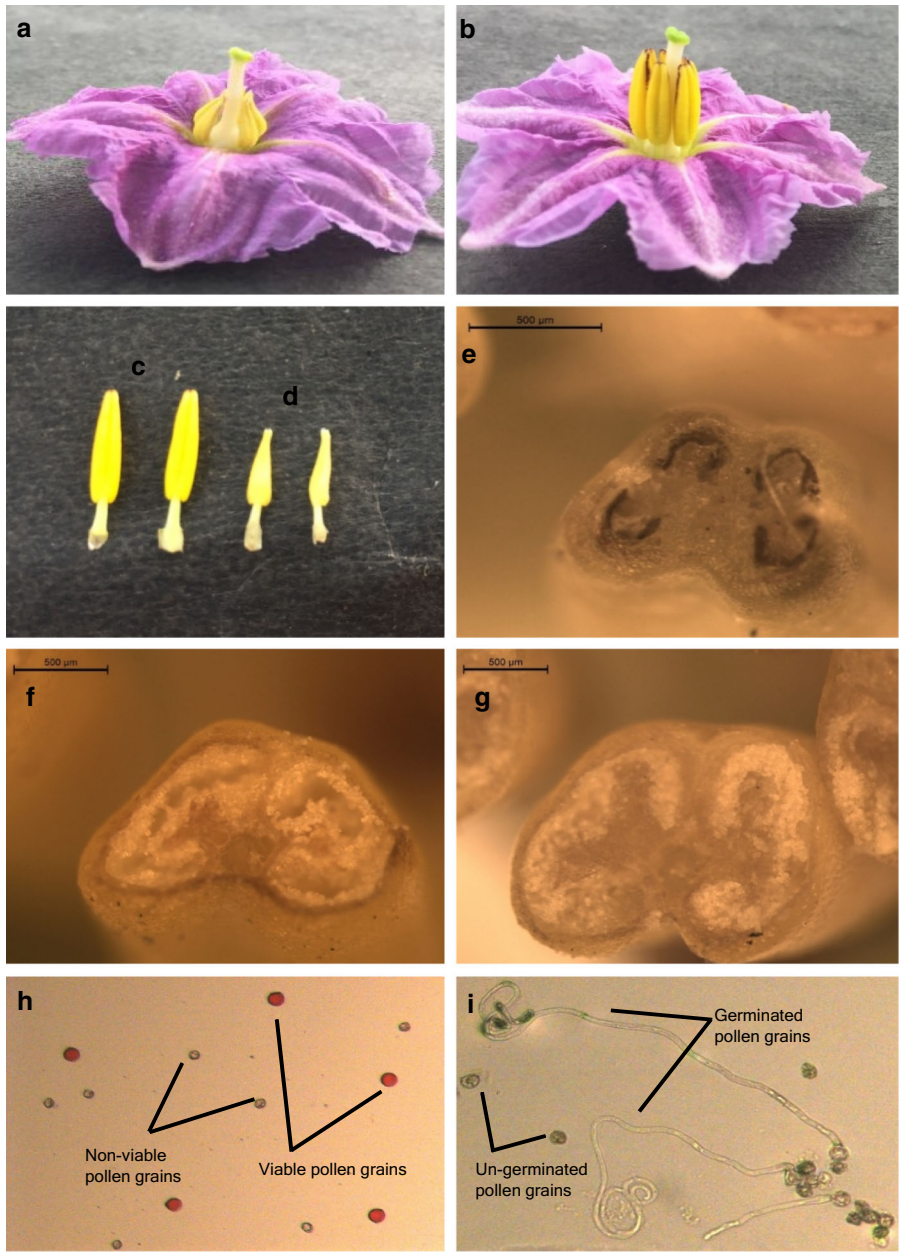

Fig. 1 Male sterile and male fertile flowers **a** male sterile flower, **b** male fertile flower. **c** Anthers of male fertile flower **d** Anthers of male sterile flower, **e** SEM observation of pollen sacs of male sterile flower of D-CMS 291A, **f** SEM observation of pollen sacs of maintainer parent, D-CMS291B, **g** SEM observation of pollen sacs of restorer parent, R2-1, **h** viable and non-viable pollen grains from male fertile flowers, **i** germinated and un-germinated pollen grains from male fertile flowers

Vision Axio Vision software 4.8.2) (Fig. 1i). Pollen tube was considered germinated when the length of pollen tube was at least the size of pollen diameter (Nashilevitz et al. 2009). The pollen germination was recorded in replicates of three from ten individual plants for each population. The plants tagged for pollen stainability were used to record pollen germination. The percent pollen germination was calculated as follows:

$$Germination(\%) = \frac{Number\ of\ germinated\ pollen\ grains}{Total\ number\ of\ pollen\ grains} \times 100.$$

Pollen index

Assessment of Pollen Index (PI) was done through visual scoring of pollen quantity from dissected anthers on 0 to 4 semi-quantitative scale. Restorer line R 2-1 exhibited maximum amount of pollen in its anthers along with better stamen length and girth, therefore, was used as a standard to determine pollen index for all other populations. The pollen index was assigned to different plants on basis of percent pollen with a difference of 0.5. Plants with no pollen grains in their anthers were given PI: **0**; $\leq$ 1% of R 2-1:**0.5**; 2–10% of R 2-1:**1.0**;11–20% of R 2-1:**1.5**; 21–35% of R 2-1:**2.0:**36–50% of R 2-1: **2.5**; 51–70% of R 2-1 as: **3.0**;71–85% of R 2-1: **3.5**, 86–100% R 2-1**:4.0**.

Phenotypic evaluation of male sterile and restorer lines for floral traits

The floral traits including stamen length and girth (mm), pistil length and girth (mm), sepal and petal length (mm) were recorded from 10 plants of each parent (male sterile and restorer lines) according to DUS (distinctness, uniformity and stability) guidelines by Protection of Plant Varieties and Farmers' Rights Authority, Government of India (PPV&FRA, GOI) and averaged.

Statistical analysis

To test inheritance of fertility restorer gene, generations developed from four cross combinations of male sterile and restorer lines were subjected to goodness of fit against 3:1 ratio using chi-square (Steel and Torrie 1980): $\chi^2 = \sum (O - E)^2/E$; where, O and E are observed and expected frequencies, respectively. The analysis of variance (ANOVA) was implemented on ten replications for pollen stainability and germination using PROC GLM using Statistical Analysis Software System SAS version 9.3 (SAS Institute, Inc 1992; Cary, NC, USA. Comparison of means for any significant variations among germination media, floral traits were performed using Tukey's HSD test at $p \leq 0.05$. The parameters of pollen fertility were subjected to Pearson's correlation coefficient to study the relationship between them. Further, simple linear regression was used to develop scatter plots and to obtain regression equations.

Results

Scoring for male fertility and sterility

The restorers, maintainers, F_{1s} and BC_1 plants for all four cross combinations exhibited normal anthers with adequate amount of fertile pollen grains. However, the F_2 populations of all four cross combinations segregated into male fertile and male sterile plants where, the fertile plants had normal plumpy anthers (Fig. 1b) while, sterile had shrivelled and indehiscent anthers, devoid of pollen grains (Fig. 1a). In addition, the colour of the anthers in sterile plants varied from green to light green from bud stage to anthesis with dead blackish (burnt) tops (Fig. 1d), whereas, the anther colour of fertile plants was found to be yellowish green to yellow from bud stage to anthesis (Fig. 1c). The transverse section of the anthers of mature flowers under SEM confirmed that the pollen sacs of male sterile flowers were deformed, blackish and had no pollen grains (Fig. 1e) as compared to normal fertile flowers filled with pollen grains in maintainer (Fig. 1f) and restorer (Fig. 1g) lines. This confirmed that all the male sterile plants had shrivelled anthers with no pollen grains as compared to the male fertile plants.

Inheritance of fertility restoration

Completely fertile F_1′s (25 plants each) of all four crosses between D-CMS 291A × R 2-1, D-CMS 99A × R 3-4, D-CMS 5A × R 6-2 and D-CMS 72A × R 2596-2 indicated dominant nature of fertility restorer gene. Fertility restoration in all BC_1 progenies (65 plants each) of four crosses further confirmed that fertility restorer gene has monogenic inheritance and, is in homozygous state in their respective restorers. Segregation pattern of fertile: sterile plants in F_2 generation of cross 1 (D-CMS 291A × R 2-1) was 157: 45; in cross 2 (D-CMS 99A × R 3-4) 135: 37; in cross 3 (D-CMS 5A × R 6-2) 114: 30 and in cross 4 (D-CMS 72A × R 2596-2) 108:29 (Table 2). Segregation ratio of all four F_2 generations fitted well in 3:1 ratio of male fertile: male sterile as per test of goodness of fit. Higher table value of $\chi^2$and $p > 0.01$in all four cases confirmed non-significant deviation between the observed and expected

Table 2 Segregation pattern of F_1, F_2 and BC_1 populations

Generation	Fertile	Sterile	Total	Ratio	χ^2	*p*
Cross 1: D-CMS291A × R 2-1						
F_1	25	–	25	–	–	–
F_2	157	45	202	3:1	0.80	0.37
BC_1	65	–	65	–	–	–
Cross 2: D-CMS99A × R 3-4						
F_1	25	–	25	–	–	–
F_2	135	37	172	3:1	1.11	0.29
BC_1	65	–	65	–	–	–
Cross 3: D-CMS5A × R 6-2						
F_1	25	–	25	–	–	–
F_2	114	30	144	3:1	1.34	0.24
BC_1	65	–	65	–	–	–
Cross 4: D-CMS72A × R 2596-2						
F_1	25	–	25	–	–	–
F_2	108	29	137	3:1	1.07	0.30
BC_1	65	–	65	–	–	–

Chi-square table value at 1 df, $\alpha_{0.05} = 3.84$

frequency. Thus, based upon segregating patterns of F_1, F_2 and BC_1 generations, genotype at nuclear locus of male sterile parent can be designated as *rfrf* and restorer as *RfRf* (Table 3).

Assessment of pollen fertility

Pollen stainability (%)

The perusal of data among restorer lines given in Table 4 revealed highest average pollen stainability in R 2-1 (91.38%), followed by R 3-4 (86.25%), R 6-2 (86.21%) and R 2596-2 (82.91%).The pollen stainability for populations (F_1, F_2, BC_1) of all crosses varied from 74.59 to 87.32% in cross 1, 63.61 to 82.09% in cross 2, 66.86 to 83.73% in cross 3 and 63.71 to 68.28% in cross 4.The populations developed from the cross 4 exhibited lowest average pollen stainability in comparison to populations from other three crosses.

Pollen germination (%)

Optimization of germination media To test germination, media was standardized using pollen of restorer line R 2-1. Among eight different combinations, M_5 media containg 0.5% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 400 mg/l PEG 4000 gave maxiumum germination of the pollen grains (36.96%), followed by M_6, M_8, M_7, M_2, M_4 and M_3 (Table 5).

Table 3 Hypothetical genotype of the different populations developed and used in the study

Sr. no	Population	Behaviour	Genotype	
			Nucleus	Cytoplasm
1	Male sterile parents-P_1 (D-CMS291A, D-CMS99A, D-CMS5Aand D-CMS72A)	Male sterile	*rfrf*	Sterile
2	Maintainer lines- (D-CMS291B, D-CMS99B, D-CMS5B and D-CMS72B)	Male fertile	*rfrf*	Fertile
3	Restorer parents- P_2 (R 2-1, R 3-4, R 6-2 and R 2596-2)	Male fertile	*RfRf*	Fertile
4	F_1	Male fertile	*Rfrf*	Sterile
5	F_2	Male fertile	male fertile: *RfRf*/ *Rfrf*	Sterile
		Male sterile	male sterile: *rfrf*	
6	BC_1	Male fertile	*RfRf* /*Rfrf*	Sterile

Table 4 Pollen fertility status of F_1, F_2 and BC_1 populations

Sr. no	Population	Pollen stainability (%)	Pollen germination (%)	Pollen index (PI)
Cross 1: D-CMS291A × R 2-1				
1	D-CMS291A(P_1)	–	–	0
2	R 2-1 (P_2)	91.38 ± 0.87	42.44 ± 2.30	4.00
3	D-CMS291A × R 2-1 (F_1)	74.59 ± 1.84	14.75 ± 2.52	3.10
4	F_2 (male fertile plants)	79.70 ± 2.01	11.47 ± 1.26	3.05
5	BC_1 [(D-CMS291A × R 2-1) × R 2-1]	87.32 ± 1.34	19.8 ± 2.04	3.50
Cross 2: D-CMS99A × R 3-4				
1	D-CMS99A(P_1)	–	–	0
2	R 3-4 (P_2)	86.25 ± 0.59	36.78 ± 1.84	3.50
3	D-CMS99A × R 3-4 (F_1)	63.61 ± 1.43	10.37 ± 0.98	2.90
4	F_2 (male fertile plants)	79.58 ± 1.68	8.00 ± 0.69	3.20
5	BC_1 [(D-CMS99A × R 3-4) × R 3-4]	82.09 ± 0.55	5.15 ± 0.20	3.10
Cross 3: D-CMS5A × R 6-2				
1	D-CMS5A(P_1)	–	–	0
2	R 6-2 (P_2)	86.21 ± 0.72	42.11 ± 2.74	4.00
3	D-CMS5A × R 6-2 (F_1)	66.86 ± 2.86	8.76 ± 0.94	3.05
4	F_2 (male fertile plants)	83.73 ± 0.75	11.75 ± 2.02	3.05
5	BC_1 [(D-CMS5A × R 6-2) × R 6-2]	82.93 ± 0.83	7.51 ± 0.37	3.00
Cross 4: D-CMS72A × R 2596-2				
1	D-CMS72A(P_1)	–	–	0
2	R 2596-2 (P_2)	82.91 ± 0.59	19.43 ± 1.59	3.00
3	D-CMS72A × R 2596-2 (F_1)	63.71 ± 1.88	5.45 ± 0.37	2.50
4	F_2 (male fertile plants)	68.28 ± 1.97	5.85 ± 0.42	2.30
5	BC_1 [(D-CMS72A × R 2596-2) × R 2596-2]	67.91 ± 1.62	7.62 ± 0.86	2.60

Pollen germination (%) in the populations Pollen germination for all populations of four cross combinations is presented in Table 4. Restorer R 2-1 exhibited maximum pollen germination (42.44%) followed by R 6-2 (42.11%), R 3-4 (36.78%) and R 2596-2 (19.43%). Average pollen germination of F_2 and BC_1 populations of cross 1 was 11.47 to 19.80%, cross 2 was 5.15 to 10.37%, cross 3 was 7.51 to 11.75% and cross 4 was 5.45 to 7.62%, respectively. As of restorer line R-2596-2, its derived F_2 and BC_1 populations showed lowest pollen germination. The restorers having high pollen stainability expressed higher pollen germination, thereby indicating a positive direct relationship between the two (Fig. 2a).

Pollen index

The male sterile plants were given a pollen index of 0 due to absence of pollen grains in anthers. Among restorer parents, R 2-1 and R 6-2 had highest pollen index followed by R 3-4 and R 2596-2 (Table 4). The pollen index for other populations for all the four crosses varied from 3.05 to 3.50 in cross 1, 2.90 to 3.10 in cross 2, 3.00 to 3.05 in cross 3 and 2.30 to 2.60 in cross 4. Here also, F_2 and BC_1 populations of cross 4 exhibited lowest values.

Phenotypic evaluation of parental lines for floral traits

It was observed that male sterile plants differ from the male fertile not only for absence/presence of pollen grains, but also for other floral traits.The perusal of

Table 5 Pollen germination (%) of restorer R 2-1 in different media

Sr. no	Medium	Composition	Germination (%)
1	Medium 1 (M_1)	5% sucrose + 4 ppm boron	9.68 ± 0.35[e]
2	Medium 2 (M_2)	1% agar + 50 mg/l boric acid + 5% sucrose	17.55 ± 0.23[d]
3	Medium 3 (M_3)	0.3% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 200 mg/l PEG 4000	5.32 ± 0.33[f]
4	Medium 4 (M_4)	0.4% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 300 mg/l PEG 4000	17.40 ± 0.33[d]
5	Medium 5 (M_5)	0.5% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 400 mg/l PEG 4000	36.96 ± 0.48[a]
6	Medium 6 (M_6)	0.6% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 400 mg/l PEG 4000	26.10 ± 0.37[b]
7	Medium 7 (M_7)	0.8% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 400 mg/l PEG 4000	22.16 ± 0.37[c]
8	Medium 8 (M_8)	1.0% agar + 50 mg/l boric acid + 5% sucrose + 300 ppm calcium nitrate + 400 mg/l PEG 4000	25.95 ± 0.25[b]

Critical value at 5% = 1.72, ± indicates the standard error to mean germination per centage

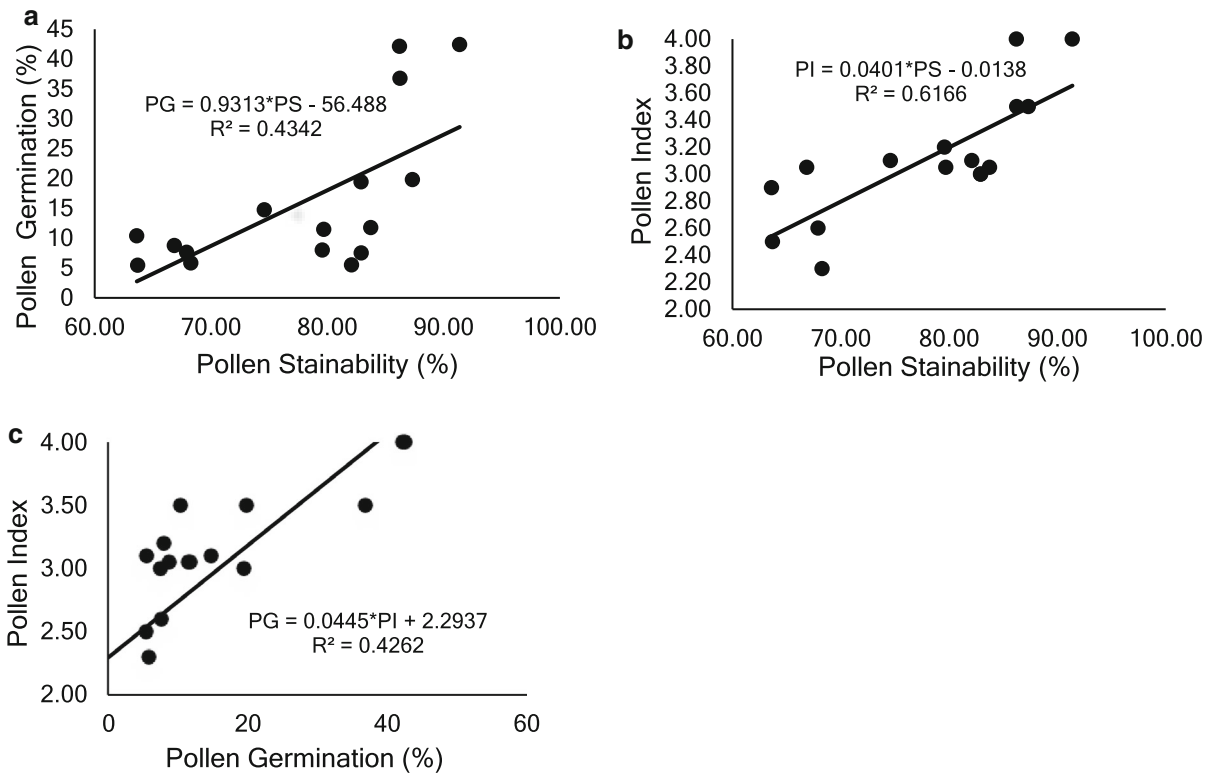

Fig. 2 Regression analysis depicting relation among pollen fertility factors in all populations: **a** regression analysis between the pollen stainability and germination (%), **b** regression analysis between the pollen stainability and pollen index among the populations, **c** regression analysis between the pollen germination (%) and pollen index among the populations

data for floral organs revealed that all the restorers plants had larger stamen length, pistil length and petal length than male sterile lines,except sepal length. Further, among all the restorers, R 2-1 had comparatively larger stamen length and girth, pistil length and girth, petal length and sepal length, than other three restorers. The comparison of means for all floral characters have been presented in Fig. 3.

Stamen length and girth

The restorer R 2-1, R 3-4, R 6-2 and R 2596-2 showed higher stamen length and girth than male sterile parents (D-CMS 291A, D-CMS 99A, D-CMS 5A and D-CMS 72A), due to presence of well developed pollen grains in the anthers. In male sterile lines non-significant differences were observed for stamen length, but stamen girth varied from 0.81 to 0.88 mm ($p < 0.05$). The restorer lines varied from 10.60–11.67 mm and 1.41–1.56 mm for stamen length and girth, respectively (Fig. 3a, b).

Pistil length and girth

The pistil length of male sterile parents was smaller (14.82–15.79 mm) than the restorers (16.02–16.26 mm). But girth of males sterile (0.90–1.00 mm) was at par of restorers (0.94–0.97 mm) (Fig. 3c, d).

Petal length

The perusal of data indicated significantly higher petal length in D-CMS 99A, than other three CMS lines. Restorers R 2-1, R 3-4, R 6-2 exhibited non-significant differences among themselves for petal length except R 2596-2,which was significantly less (Fig. 3e).

Sepal length

The male sterile parents were larger (10.23–11.28 mm) in sepal size than restorer lines (10.24–10.71 mm) (Fig. 3f).

Correlation and regression analysis between the pollen fertility attributes

Correlation analysis showed that pollen stainability is positively and significantly correlated with pollen germination (r = 0.78) and pollen index (r = 0.78). Pollen germination and pollen index (r = 0.96, $p \leq 0.05$, 0.01) too were positively and significantly correlated. The linear scatter plot pattern of regression also confirmed a significant positive correlation among the three attributes ($p \leq 0.05$) viz., pollen stainability and pollen germination ($R^2 = 0.434$) (Fig. 2a); pollen stainability and pollen index ($R^2 = 0.616$) (Fig. 2b); pollen germination and pollen index ($R^2 = 0.426$) (Fig. 2c).

Discussion

CMS lines evades hand emasulation and pollination process and, lowers the cost of hybrid seed production. In those crops where seeds or fruits are the commercial product, restoration of fertility in F_1 generation is must. Similarly, restoration of fertility is needed for commercial exploitation of hybrids in eggplant. In present study, four alloplasmic CMS lines viz. D-CMS 291A, D-CMS 99A, D-CMS 5A and D-CMS 72A exhibited complete sterility during both autumn and spring–summer seasons and thus were confirmed stable for male sterility. Likewise, their maintainer counterparts and restorer lines showed stable pollen fertility throughout the study period. This stability confirmed that these lines could be used in hybrid development programmes in future. Along with stability of parental lines, knowledge on inheritance of fertility restorer gene(s) is also important for further transfer in diverse genetic backgrounds and molecular studies.

Chi square analysis fitted the segregation pattern of the four F_2 populations in 3:1 ratio (male fertile: male sterile), which indicated that fertility restoration is governed by a single dominant gene. The restoration of fertility in hybrid progenies as well as in back crosses populations of all the four cross combinations indicated homozygous nature of the fertility restorer gene. The present results are in agreement with Saito et al. (2009) expressing a single dominant gene for fertility restoration in CMS system of *S. grandifolium* cytoplasm. On contrary, Khan and Isshiki (2010, 2011) and Hasannunahar et al. (2012) reported two dominant fertility restorer genes in their populations having cytoplasms of *S. aethiopicum* L. Aculeatum group, *S. anguivi* and *S. grandifolium*. This

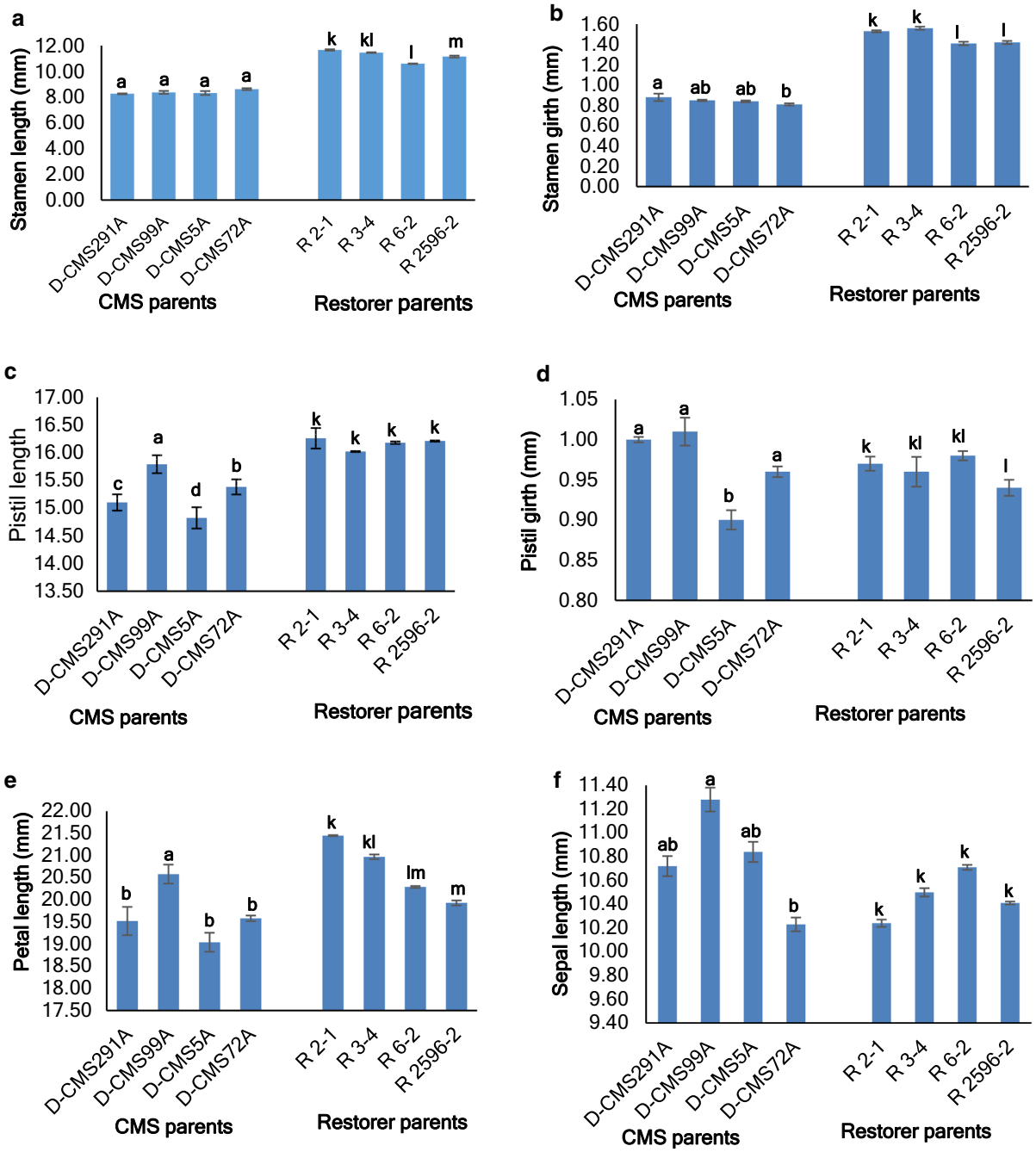

Fig. 3 Phenotypic evaluation of alloplasmic CMS and restorer lines for floral traits **a** Stamen length (mm), **b** Stamen girth (mm), **c** Pistil length (mm), **d** Pistil girth (mm), **e** Petal length (mm), **f** Sepal length (mm). Groups have been assigned using Tukey's HSD test at $p \leq 0.05$ to show significantly different means (CMS: a–d; Restoreres: k–n)

deviation could be attributed to differences and variation in the genetic material used in the studies.

Pollen stainability (%) conducted to substantiate visual scoring of all the F_1 populations revealed and accentuated the fertility restoration potential of all four restorers under study. Though the pollen stainability achieved in F_1s was lower than their respective restorer parents and backcross progenies. This could

be due to more contribution of restorer parent in the back-cross populations. Similar, observations in F_1 hybrids and backcross generations between *Solanum melongena* L. and its related species were reported by Rajasekaran (1970, 1971), Rangasamy and Kadambavanasundaram (1974), Khan and Isshiki (2010) and Hasnunnahar et al. (2012). Pollen quality plays direct role in fertilization, fruit set and ultimately, seed set. Testing for pollen germination provides a practical test of pollen viability. Varied responses of pollen germination reported by various workers could be due to lack of proper pollen growth medium (Khan and Isshiki 2011; Hasnunnahar et al. 2012). However, in our case, addition of calcium and PEG 4000 in pollen germination media significantly improved pollen germination under in vitro conditions. Calcium maintained cationic balance in pollen and pollen tip growth during germination and tube elongation, respectively while, PEG (Polyethylene glycol) regulated the permeability of plasma membrane and provided stability to the pollen tube membrane during pollen germination (Read et al. 1993; Steinhorst and Kudla 2013; Jayaprakash 2018). Similar trend as of pollen stainability was maintained in pollen germination, wherein, restorer parents had the highest pollen germination than their subsequent generations (Khan and Isshiki 2009, 2010; Hasnunnahar et al. 2012; Khan et al. 2014). This further accentuated the fertility restoration potential of restorers under study. Among four restorers, R 2-1 was adjudged as the best on the basis of highest stamen length, stamen girth, pollen stainability, pollen germination and pollen index. The amount of pollen and its development is linked with the stamen size (Pinheiro-Costa et al. 2018). As the restorer parent plays major role in developemnt of heterotic hybrids, therefore, higher pollen amount and pollen fertility should be ensured to have higher percentage of fruit setting.

Conclusion

It can be concluded that single dominant gene (*Rf*) is responsible for fertility restoration of alloplasmic cytoplasm in eggplant. This information would be useful in transferring fertility restorer gene into diverse genetic backgounds for development of F_1 hybrids. This would further be helpful in molecular tagging and characterization of fertility restorer gene to hasten the hybrid breeding programs of eggplant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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