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Inheritance of fertility restoration of A_5 cytoplasmic-nuclear male sterility system in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract

The A_5 cytoplasmic-nuclear male sterility (CMS), the most stable cytoplasm available in pearl millet [*Pennisetum glaucum* (L). R. Br.] is yet to be utilized on large scale by breeders owing to limited fertility restoration in the available germplasm for this cytoplasm. An understanding of the inheritance of fertility restoration can make significant contributions to restorer breeding efficiency of this CMS system. This study investigated inheritance of fertility restoration of this CMS system in which three diverse iso-nuclear A-lines (P_1) were crossed with two A_5 restorer lines (P_2) to produce 4 F_1s and their respective F_2s and two backcrosses. These were evaluated for male sterility (S) and fertility (F) for two seasons at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru. The segregation patterns in the F_2 (54F:10S) and BCP_1 (3F:1S) were broadly suggestive of trigenic inheritance of male fertility restoration, where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration.

Key words: *Pennisetum glaucum,* A_5 CMS, fertility restoration, inheritance

Introduction

Pearl millet (*Pennisetum glaucum* (L). R. Br.) hybrid programs around the world are based almost entirely on the A_1 system of cytoplasmic-nuclear male sterility (CMS) that was discovered and shown to be commercially viable in 1950s (Burton 1958). Since about last two decades, about 80-90 pearl millet hybrids have been cultivated at any point of time in India on about 5-6 m ha, but most of them are based on A_1 CMS system. Also, most of the hybrids cultivated in USA are based on the A_1 CMS system. This dependence on a single cytoplasm makes the hybrid seed industry of a particular crop vulnerable to disease and insect pest epidemics, as witnessed in case of Southern leaf blight epidemic caused by *Maydis bipolaris* race T on the Texas-cytoplasm based maize hybrids in maize in USA (Scheifele et al. 1970).

Hence, attempts were made to search for alternative CMS systems to diversify the cytoplasmic base of pearl millet hybrids, which led to identification of two other viable systems: A_4 CMS system discovered in a germplasm accession of a wild species *Pennisetum glaucum* (L.) R. Br. ssp. *monodii* (Maire) Brunken (Hanna 1989) and A_5 CMS system discovered in a Large-Seeded Gene pool (LSGP) (Rai 1995). Restorer stock of A_5 CMS was identified in LSGP, and was designated as LSGP-R1 (Rai et al. 1995). Later, studies showed the A_5 CMS system to be the most stable CMS system (Rai et al. 2001, 2008) and having the highest frequency of maintainers in diverse composites (Rai et al. 2006). While these two features would provide the greatest opportunity for genetic diversification of seed parents, this CMS system is yet to be utilized by most of pearl millet breeding programs for grain hybrid development due to almost non-existence of restorers in the breeding lines and low frequency of restorers in composites and open pollinated varieties. Considering this, International Crops Research Institute for the Semi-arid tropics (ICRISAT) pioneered the utilization of these two alternative CMS systems, and developed and disseminated 71 A-lines with A_4 cytoplasm and 18 A-lines with A_5 cytoplasm till 2013 (Rai et al. 2015; Yadav

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et al. 2016). Also, ICRISAT initiated a low-key effort about a decade ago to breeding restorer of this CMS system and now several breeding programs worldwide have initiated conversion of their promising pollinator breeding lines into A_5-system restorer line through repeated backcrossing procedures to diversify the genetic base of seed parents (A-lines) and hybrids (Yadav et al. 2012). Recognizing the importance of these new CMS systems, Consultative Group on International Agricultural Research (CGIAR) Research Program on Dryland Cereals (CRP-DC) in its recent strategy to deploy pearl millethybrids in Sub-Sahelian African countries has also emphasized to strengthen hybrid technology based on A_4 and A_5 CMS systems due to their highly stable male sterility (ICRISAT 2014). Also, in a questionnaire based feedback to identify priority research areas in pearl millet, seed industry engaged in hybrid pearl millet research emphasized to enhance greater utilization of A_4 and A_5 CMS systems in hybrid parent development in future (Rai et al. 2012). Hence, considering the emerging needs, the objective of this study was to develop an understanding of the genetics of male sterility and fertility restoration of this alternative and commercially useful new A_5 CMS system with a view to enhance hybrid parents breeding efficiency.

Materials and methods

The basic experimental material consisted of three male-sterile (A-lines) with A_5 cytoplasm and two A_5 fertility restorer lines (R-line) of pearl millet [*Pennisetum glaucum* (L). R. Br.]. The three A-lines ($ICMA_5$ 88004, $81A_5$ and $ICMA_5$ 89111) in A_5 cytoplasmic background were developed by more than eight backcrosses of ICMB 88004, 81B and ICMB 89111 respectively, into male-sterile line ICMA-5 (cytoplasm source of A_5). Two of these A-lines ($ICMA_5$ 88004 and 81 A_5) were crossed with an A_5 restorer line LSGP-R1 (derived from LSGP). Also, two A-lines ($ICMA_5$ 88004 and $ICMA_5$ 89111) were crossed with another restorer line PRL-18749. PRL-18749 was developed by backcross breeding of an inbred line [(IPC 107 x SDMV 90031-S1-84-1-1-1) x AIMP 92901 S1-296- 2-1-1-3-B-1)] with LSGP–R1 as a non-recurrent donor parent. Individual plants were used for making plant x plant crosses to produce four F_1s. More than 10 plants of each F_1 were selfed to produce the F_2 populations. Bulk pollen from 5-10 plants from each F_1 was used to cross on the respective parental lines (A- and R-lines) to produce BCP_1 and BCP_2 populations, respectively. Field trials of 4 F_1's, 4 BCP_1's, 4 BCP_2's and 4 F_2's were conducted during the summer (March- June) and rainy (July-October) seasons of 2014 at ICRISAT, Patancheru, Telangana, India. The F_1's and BCP_2 populations were evaluated in single row plots of 4 m length with approximately 30-35 plants per plot. Each F_2 population was evaluated in eight-row plots of 4 m length with approximately 250-300 plants, and each BCP_1 population was evaluated in four rows of 4 m length, with approximately 125-150 plants. Visual observations on pollen shedding of individual plants were used to determine male fertility (F) and sterility (S) reaction in all the populations. Pollen shedding is a standard method followed in pearl millet to recognize two broad categories male and male-sterile (Rai and Hash, 1990).The plants shedding pollen were classified as fertile (F) and non-shedders as sterile (S). Chi-square (c^2) method with Yate's correction factor (Steel and Torrie 1980) was applied on the observed segregation data to test the goodness of fit of various hypothetical ratios.

Results

All the plants in all the four F_1 hybrids and BCP_{2s} (F_1 x R) had fully fertile plants in both summer and rainy season. The F_2 population from the cross $ICMA_5$ 88004 x LSGP-R1 segregated for 229 male-fertile and 41 male-sterile plants during the summer season and had a good fit to a ratio of 54F:10S with χ^2 probability of 1.00 (Table 1). Such a segregation ratio would result from a trigenic model where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration. Thus, we hypothesize that the A-line and its maintainer (B-line) both would have the genotype 'aabbCC'. The genotype of restorer parent would be 'AABBcc'. The F_1 would be heterozygous at all the three loci (AaBbCc), and it would be fertile as found in this study. According to this genetic model, F_2 should segregate in 54F:10S and a plant would be fertile if it possesses dominant alleles at least at two of the any three duplicate-complimentary loci (i.e. A_B_C_/A_B_cc/A_bbC_/aaB_C_), while corresponding BCP_1 generation should segregate in 3F:1S ratio. The BCP_1 of this cross segregated for 96 male-fertile and 42 male-sterile plants during the summer season and fitted well to the expected 3F:1S ratio with χ^2 probability of 0.17. The F_2 of this cross with 409 fertile plants and 40 sterile plants did not fit to 54F:10S ratio in the rainy season whereas BCP_1 had a good fit to the segregation ratio of 3F:1S with χ^2 probability of 1.00. The F_2 segregation of cross $81A_5$ x LSGP-R1 had a good fit to 54F:10S ratio in F_2 and 3F:1S ratio in BCP_1 in both the seasons.

The F_2 population from the cross $ICMA_5$ 88004 x PRL-18749 segregated for 71 male-fertile and 13 male-sterile plants during the summer season and had a good fit to a ratio of 54F:10 S with χ^2 probability of 1.00 (Table 1), and BCP_1 of this cross segregated for 78 male-fertile and 31 male-sterile plants and fitted well to 3F:1S ratio with χ^2 probability of 0.47. The F_2 of the same cross had good fit to 54F:10S ratio in the rainy season with χ^2 probability of 0.69, while BCP_1 had segregation ratio of 79F:15S with low χ^2 probability of 0.06 for 3F:1S segregation. The F_2 segregation of the cross $89111A_5$ x PRL-18749 had a good fit to 54F:10S ratio in F_2 and 3F:1S ratio in BCP_1 in both the seasons.

Table 1. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BCP_1 generations and test of goodness of fit for hypothetical Mendelianratios in four crosses (A_5 CMS-lines x A_5 Restorer parents) of pearl millet, Summer and Rainy seasons of 2014 at ICRISAT – Patancheru.

Cross	Season	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	Probability
			F	S	F	S	F	S		
$ICMA_5$ 88004 P4 × LSGP-R1	Summer	F_2	229	41	54	10	228	42	0.00	1.00
		BCP_1	96	42	3	1	103	35	1.87	0.17
	Rainy	F_2	409	40	54	10	379	70	15.10	<0.01
		BCP_1	72	24	3	1	72	24	0.00	1.00
$81A_5$ P1 × LSGP-R1	Summer	F_2	258	35	54	10	247	46	2.74	0.10
		BCP_1	107	39	3	1	109	37	0.15	0.70
	Rainy	F_2	355	62	54	10	352	65	0.13	0.72
		BCP_1	177	58	3	1	176	59	0.00	1.00
$ICMA_5$ 88004 P4 × PRL-18749	Summer	F_2	71	13	54	10	71	13	0.00	1.00
		BCP_1	78	31	3	1	82	27	0.52	0.47
	Rainy	F_2	156	26	54	10	154	28	0.16	0.69
		BCP_1	79	15	3	1	70	24	3.63	0.06
$ICMA_5$ 89111 P1 × PRL-18749	Summer	F_2	167	28	54	10	165	30	0.15	0.70
		BCP_1	70	26	3	1	72	24	0.13	0.72
	Rainy	F_2	176	13	54	10	159	30	2.79	0.09
		BCP_1	79	25	3	1	78	26	0.01	0.92

F = Fertile; S = Sterile

Discussion

F_1 hybrids and BCP_{2s} (F_1 x R) in all the four crosses had fully fertile plants in both summer and rainy season, indicating male-fertility to be dominant over male-sterility for A_5 cytoplasm. The F_2 population from the cross $ICMA_5$ 88004 x LSGP-R1 segregated for 229 male-fertile and 41 male-sterile plants during the summer season and had a good fit to a ratio of 54F:10 S with χ^2 probability of 1.00 (Table 1), expected of a trigenic inheritance where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration. According to this hypothesized trigenic genetic model, BCP_1 (A x F_1) generation should segregate in a 3F:1S ratio. The BCP_1 of this cross segregated for 96 male-fertile and 42 male-sterile plants during the summer season and fitted well to the expected 3F:1S ratio with χ^2 probability of 0.17. The F_2 of this cross with 409 fertile plants and 40 sterile plants did not fit to 54F:10S ratio in the rainy season due to excess of fertile plants whereas BCP_1 had a good fit to the expected segregation ratio of 3F:1S with χ^2 probability of 1.00. The F_2 segregation of cross $81A_5$ x LSGP-R1 had a good fit to 54F:10S ratio in F_2 and 3F:1S ratio in BCP_1 in both the seasons. Such type of trigenic inheritance where any two of the three dominant duplicate-complimentary genes restorer fertility was also reported for A_4 (M) CMS system in sorghum (Reddy et al. 2010).

The F_2 population from the cross $ICMA_5$ 88004 x PRL-18749 segregated for 71 male-fertile and 13 male-sterile plants during the summer season and had a good fit to a ratio of 54F:10 S with χ^2 probability of 1.00 (Table 1), and BCP_1 of this cross segregated for 78 male-fertile and 31 male-sterile plants and fitted well to the expected 3F:1S ratio with χ^2 probability of 0.47. The F_2 of the same cross had good fit to 54F:10S ratio in the rainy season with χ^2 probability of 0.69, while BCP_1 had segregation ratio of 79F:15S with low χ^2 probability of 0.06 due to more number of fertile plants. The F_2 segregation of the cross $89111A_5$ x PRL-18749 had a good fit to 54F:10S ratio in F_2 and 3F:1S ratio in BCP_1 in both the seasons.

For A_5 CMS system, out of total of 8 cases of F_2s from these crosses (4 F_2s evaluated in two seasons), seven cases had good fit to 54F:10S ratios and all the 8 cases of BCP_1s from these crosses had good fit to the expected 3F:1S ratios. The one case of non-fitness of F_2 ratio was observed in rainy season in ICMB 88004 backgrounds where excess of male fertile plants were present. Such deviations resulting from the relatively lower temperatures and higher humidity may enhance the expression of modifiers for fertility restoration in the rainy season as reported earlier (Rai et al. 2001; Yadav et al. 2010) in pearl milletand in maize (Duvick 1959). Influence of genotypic backgrounds on the expression of fertility restorer genes has also been reported in *Brassica napus* (Pahwa et al. 2004) and in rice (Govinda and Virmani 1988).

The overall segregation patterns of male sterile (S) and fertile (F) plants in populations derived from crosses between the three A_5-lines and two A_5 R-lines for the A_5 CMS system with 54F:10S ratio in F_2 and 3F:1S ratio in BCP_1 populations indicated three-gene segregation for male-sterility/fertility restoration where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration.Earlier, as one to three genes were found responsible for fertility restoration of A_1 cytoplasm (Yadav et al. 2010), and single gene for A_4 cytoplasm (Gupta et al. 2012), the results of this study reporting trigenic inheritance of A_5 cytoplasm suggests to plan investigations in future to reveal allelism between fertility restoring genes of three different CMS systems (A_1, A_4 and A_5) in pearl millet.

Authors' contribution

Conceptualization of research (SKG, KNR); Designing of the experiments (SKG, DVY, MGR, VNK); Contribution of experimental materials (KNR, SKG); Execution of field/lab experiments and data collection (MG, DVY, MB); Analysis of data and interpretation (SKG, MG, DVY, VNK); Preparation of manuscript (SKG, MG).

Declaration

The authors declare no conflict of interest.

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