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Allelic relationship between restorer genes for A_1 and A_4 CMS systems in pearl millet

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Abstract

Understanding the allelic complementation of restoration among the stable restorer lines will contribute to robust pearl millet restorer breeding program. Thus, present study aimed to test allelic relationship between four diverse restorer lines each for A_1 and A_4 cytoplasmic male sterility (CMS) systems to find linkage between restorer genes carried by different restorers of each CMS systems. Six testcrosses were developed for each CMS system by crossing each of A_1 and A_4 male sterile lines (81A_1 and 81 A_4) with all possible combination of F_1s among four A_1 restorers and A_4 restorers, respectively. Almost all the plants in these testcrosses showed complete male fertility indicating presence of same fertility restoration gene (allelic) is present in all the four restorer lines of A_1 CMS system. Similar results were found for A_4 CMS system. This single gene system within A1 and A4 provides the opportunity to breeders to incorporate this allelic restorer gene into any of the advanced lines for its rapid conversion into new restorer lines besides to search for new restorer gene for future diversification.

Key words: Allelism, fertility restoration, male sterility, *Pennisetum glaucum*

Introduction

Hybrid development in pearl millet (*Pennisetun glaucum* (L.) R. Br) began with the discovery of A_1 cytoplasmic male sterility (CMS) system at Tifton, Georgia, USA (Burton, 1958). Later, many other CMS systems were discovered, but A_4 and A_5 CMS systems were found to be more stable than A_1 CMS system (Rai et al. 2009) The A_4 CMS system was identified at Tifton, USA in a wild grass *Pennisetum glaucum* subsp. *monodii* (maire) Brunken (Hanna, 1989). In the past, most of ICRISAT-bred populations have shown 65-92% restoration for A_1 CMS whereas A_4 CMS system reported restoration frequency of 30-72% (Rai et al. 2006). The highest restoration frequency for A_1 CMS system led to its utilization in most of the breeding programs in public and private sector resulting into release of most hybrids till date on this single CMS in USA and India. Recently utilization of alternative CMS systems, especially A_4 CMS has increased, to diversify the cytoplasmic base for hybrids. Precise knowledge of genetics of fertility restoration is an essential for restorer breeding program and also important to know any intergenic interactions. Earlier studies reported single dominant gene to be responsible for the inheritance of fertility restoration of A_1 and A_4 cytoplasm though possibility of di- and tri-genic inheritance of fertility restoration was also proposed earlier for A_1 CMS system (Yadav et al. 2010; Gupta et al. 2012). Till date, ICRISAT had developed about 170 seed-parents, of which about 130 are based on A_1 and A_4 CMS systems, and also developed about 80 restorers since 2006 (Rai et al. 2009). However, there has been no study in pearl millet to understand the allelic complementation of restoration or other kinds of non-allelic genetic interaction among the stable restorer lines. The information of same fertility restoration gene would require no additional back crossing while disparity of fertility restorer genes can facilitate pyramiding of more than one fertility restoration gene in desirable genetic backgrounds. Therefore, objective of the present study was to determine allelic relationship between restorer genes present in diverse set of restorer lines of A_1 CMS and A_4 CMS systems.

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Materials and methods

Four diverse restorer lines (R-line) of A_1 CMS system namely IPC 511, IPC 804, ICMR 08777 and ICMR 08999 were crossed in a half-diallel fashion to generate six (R × R) F_1s; similarly four A_4 CMS restorer lines (IPC 804, IPC 1518, ICMR 08777 and ICMR 08999) were crossed in similar fashion to generate six (R × R) F_1s in 2012 summer season. All these R lines (IPC 511, IPC 804, PIC 1518, ICMR08777 and ICMR 08999) were restorers of both A_1 and A_4 CMS systems (Table 1). First three restorers were used in the study R × R F_1s were made using bulk pollen collected from 10-15 plants of the pollen parent. During 2012 rainy season, four restorers of A_1 CMS system and their six R × R F_1s were testcrossed on $81A_1$, to generate 6 three-way F_1 testcrosses [A_1 x (R_1 × R2)] and 4 parental F_1 testcrosses (A × R), respectively. Similarly, for A_4 CMS system, 6 R × R F_1 hybrids and their four parents were testcrossed on $81A_4$ CMS line to generate 6 three-way F_1 testcrosses and 4 parental testcrosses, respectively, using bulk pollen from each plot.

These three-way F_1s and parental F_1s were evaluated for fertility and sterility reactions in three crop seasons (summer and rainy season of 2013; and summer season of 2014) at ICRISAT, Patancheru. The parental F_1s were sown in two-row plots of 4 m length (to get approximately 50-60 plants), whereas the three-way F_1s were grown in 6 row plots of 4m length with approximately 200-250 plants. Data on fertility and sterility (F/S) reaction of individual plants in each plot of parental and three-way F_1s was recorded following the pollen shedding method in all three crop seasons. Pollen shedding is a standard method followed in pearl millet to recognize two broad categories male fertile and male-sterile (Rai and Hash 1990). Plants were scored for pollen shedding between 8 to 11 am by tapping the main panicle to observe pollen clouds (yellow powdery structure). Each plant was tagged depending on the pollen cloud into three different classes *i.e*., fertile (F), sterile (S) or F shy (very thin light yellow powder). Since the traces of pollen were light yellow and less dense powder which was detectable to the naked eyes during the pollen shed scoring, self-seed set (SSS) was recorded as an additional measure of male fertility in all the F shy plants (shy pollen shedders), and selfed seed set data at maturity was considered to categorize them as fertile (F) and sterile (S) plants.

Results and discussion

The complete fertility restoration observed in all the four A × R crosses for both A_1 and A_4 CMS systems across three seasons confirmed the fertility restoration ability of restorer parents involved in this study (Tables 2 and 3). This confirmed the A_1 and A_4 restorer parents used in this study had corresponding nuclear alleles for fertility restoration in A_1 and A_4 sterile cytoplasm,

Table 1. Origin and parentage of genetic materials used in this allelism study

Line	Origin	Parentage/description	CMS/restoration	Reference(s)
81A (ICMA 1)	ICRISAT	Tift 23D2A cytoplasm source (A_1) backcrossed to 81B which is a induced downy mildew resistant selection from Tift 23D2B	A_1 and A_4	Rai et al. (1996)
IPC 511	ICRISAT	[(J 934-7 x 700544-7-2-1) x EC 298-2-11 -1-5	A_1 and A_4	Rai et al. (1996, 2001); Yadav et al. (2010); Gupta et al. (2012)
IPC 804	ICRISAT	(S 10LB-30 x LCSN 1225-6-3-1)-1-2-1-1	A_1 and A_4	Rai et al. (1996, 2001); Yadav et al. (2010); Gupta et al. (2012)
IPC 1518	ICRISAT	ICRC-F4- 146-3	A_1 and A_4	Rai et al. (1996); Yadav et al. (2010); Gupta et al. (2012)
ICMR 08777	ICRISAT	[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-18-2-2-4-1-B	A_1 and A_4	ICRISAT (2008)
ICMR 08999	ICRISAT	JBV 3 S1-18-2-2-1-3-2	A_1 and A_4	ICRISAT (2008)

Table 2. Segregation pattern for fertility restoration in segregating three-way testcross F_1 progenies for A_1 CMS system in pearl millet, 2013 summer and rainy seasons, and 2014 summer season, ICRISAT, Patancheru

Testcross	Summer 2013			Rainy 2013			Summer 2014		
	F	S	Total	F	S	Total	F	S	Total
Parental testcross									
81 A_1 x IPC 511	57	0	57	41	0	41	50	0	50
81 A_1 x IPC 804	41	0	41	44	0	44	46	0	46
81 A_1 x ICMR 08777	54	0	54	32	0	32	55	0	55
81 A_1 x ICMR 08999	52	0	52	43	0	43	52	0	52
(R x R) F_1 testcross									
81 A_1 x (IPC 511 × IPC 804)	146	0	146	134	0	134	136	1	137
81 A_1 x (IPC 511 × ICMR 08777)	156	0	156	149	0	149	161	0	161
81 A_1 x (IPC 511 × ICMR 08999)	156	0	156	145	1	146	146	0	146
81 A_1 x (IPC 804 × ICMR 08777)	143	1	144	147	1	148	158	2	160
81 A_1 x (IPC 804 × ICMR 08999)	161	1	162	145	4	149	143	4	147
81 A_1 x (ICMR 08777 × ICMR 08999)	167	3	170	140	0	140	140	0	140

F = Fertile; S = Sterile

respectively.

All the plants of six F_1s ($81A_1 \times F_1$ of two A_1 restorers) had shown almost complete fertility in all three seasons, indicating that IPC 511, IPC 804, ICMR 08777 and ICMR 08999 possess identical restorer genes for A_1 CMS restoration (Table 2). All the plants in three of the six testcrosses showed complete fertility in 2013 summer season, while three plants out of 167 in $81A_1$ × (ICMR 08777 × ICMR08999), and only one plant each was found sterile in $81A_1$ × (IPC 804 × ICMR08777) and in $81A_1$ × (IPC 804 × ICMR08999) out of 143 and 161 plants, respectively. In 2013 rainy season, all plants in three testcrosses showed complete fertility while 1 to 4 partially sterile plants were observed in other three testcrosses $81A_1$ × (IPC 511 × ICMR08999), $81A_1$ × (IPC 804 × ICMR08777) and $81A_1$ × (IPC 804 × ICMR08999). Similar results were again observed in 2014 summer season. The plants in testcross $81A_1$ x (IPC511 × ICMR08777) showed completely fertile plants across three seasons while testcross $81A_1$ × (IPC 511 × IPC 804), $81A_1$ × (IPC 511 × ICMR08999) and $81A_1$ × (ICMR 08777 × ICMR08999) showed completely fertile plants across two seasons. Such minor fluctuations in segregation pattern of fertile and sterile plants may occur due to differences in weather conditions such as temperature and relative humidity across seasons and years, as also earlier reported in other cereals such as rice (Govinda Raj and Virmani, 1986) and wheat (Ali et al. 2011).

In case of three-way testcrosses of A_4 CMS system, plants in all six testcrosses [$81A_1 \times F_1$ of two A_4 restorers) had shown almost complete fertility across three seasons, indicating IPC 804, IPC 1518, ICMR 08777 and ICMR 08999 to be sharing identical restorer genes among them for restoring fertility for A_4 sterile cytoplasm (Table 3). However, one testcross $81A_4$ × (IPC 804 × ICMR 08999) had exceptionally higher number of (10) sterile plants out of total 140 plants in 2013 summer season, though it reported only one sterile plant in other two seasons (2013 rainy and 2014 summer), which might be due to some error in recording pollen fertility in 2013 summer season. Hence, all the testcrosses had almost all fertile plants across seasons indicating that all the restorers under study possessed same fertility restorer gene of A_4 cytoplasm.

The newly developed restorer parent ICMR 08999 had 1 to 4 sterile plants across season in crosses involving A_1 cytoplasm, while it had 1 to 10 sterile plants across seasons in crosses involving A_4 cytoplasm. However, this sterility pattern was not consistent across the seasons, as few sterile plants noticed for sterility in summer season were not observed in other two season (Table 3) for A_4 system, while few sterile plants observed from crosses between IPC 804 and ICMR 08777 and ICMR 08999 for A_1

Table 3. Segregation pattern for fertility restoration in segregating three-way testcross F_1 progenies for A_4 CMS system in pearl millet, 2013 summer and rainy seasons, and 2014 summer season, ICRISAT, Patancheru

Testcross	Summer 2013			Rainy 2013			Summer 2014		
	F	S	Total	F	S	Total	F	S	Total
Parental testcross									
81A_4 x IPC 1518	58	0	58	46	0	46	NA	NA	NA
81A_4 x IPC 804	56	0	56	43	0	43	43	0	43
81A_4 x ICMR 08777	55	0	55	19	0	19	54	0	54
81A_4 x ICMR 08999	56	0	56	37	0	37	39	0	39
(R x R) F_1 testcross									
81 A_4 x IPC 1518 × IPC 804)	121	0	121	141	0	141	120	0	120
81 A_4 x (IPC 1518 × ICMR 08777)	124	0	124	109	0	109	NA	NA	NA
81 A_4 x (IP IPC 1518 × ICMR 08999)	133	0	133	93	1	94	97	0	97
81 A_4 x (IPC 804 × ICMR 08777)	122	0	122	93	1	94	98	0	98
81 A_4 x (IPC 804 × ICMR 08999)	130	10	140	131	1	132	135	1	136
81 A_4 x (ICMR 08777 × ICMR 08999)	137	0	137	119	0	119	141	0	141

NA, data not available due to very low seed quantity for planting

system, were found to be sterile in summer season as well as in rainy season (Table 2). This could be due to higher influence of genetic background of the A-lines and genetic × environment interaction for A_1 CMS system of fertility restoration than the A_4 CMS system. In general, the presence of such low frequency of sterile plants in these crosses might be due to accumulation of modifiers having negative effect on fertility restoration genes in heterozygous condition. Also, the higher temperatures and lower relative humidity may enhance the expression of modifiers for sterility. Similar reports were found earlier in pearl millet (Yadav, 2005; Gupta et al. 2010) and other major crops like maize (Duvick, 1956, Kheyr-Pour et al. 1981), rice (Govinda Raj and Virmani, 1986; Ramalingam et al. 1995) and wheat (Ali et al. 2011). Thus, overall results of this study showed that same gene is present in all the four restorer lines for A_1 and A_4, respectively. This result is concomitant with the earlier study in pearl millet which reported single dominant gene to be responsible for inheritance of fertility restoration of A_1 and A_4 cytoplasm (Yadav et al. 2010 and Gupta et al. 2012). Though earlier study in pearl millet reported possibilities of two or three genes responsible for restoration of A_1 cytoplasm (Yadav et al. 2010), but absence of sterile plants in segregating generations of A_1 restorers involved in this study indicated the presence of same restorers gene across all four A_1 restorers. Further, the presence of same fertility restorer gene across diverse restorers allows breeders to utilize any of the potential line in breeding program to initiate the development of new restorer lines of A_4 CMS system in future. With the advent of genomic technology, this study merits furtherance's of mapping to localize these restorer genes or genomic regions for wider application in pearl millet hybrid parent improvement. With advancement in genomic tools, molecular markers have also been used in other crops for the mapping and tagging of fertility restorer genes and classification of pollen parents into different fertility restorers (Ahmadikhan et al. 2007; Shah et al. 2012; Ghara et al. 2012; Revathi et al. 2013). Such diagnostic markers are yet to be generated and validated in different genetic backgrounds for early identification of effective restorers in pearl millet.

Authors' contribution

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

Declaration

The authors declare no conflict of interest.

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