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Inheritance of A_1 System of Cytoplasmic-Nuclear Male Sterility in Pearl Millet [*Pennisetum glaucum* (L). R. Br.]

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Inheritance of male sterility and fertility restoration of the A_1 system of cytoplasmic- nuclear male sterility in pearl millet was investigated using 12 crosses among three diverse male sterile lines (A-lines) and four diverse restorers (R-lines). Individual plants from R- lines were used to make crosses on A-lines. The segregation pattern of male sterile (S) and male fertile (F) plants observed in F_2 and BC_1 in two seasons at ICRISAT, Patancheru was suggestive more likely of a single-gene control of male sterility and fertility restoration. However, a 3-gene model of male sterility/fertility restoration where dominant alleles at any two of the three duplicate complimentary loci will lead to male fertility could not be ruled out, nor could be ruled out a 2-gene control with duplicate interaction. There was indication of variability even within a highly inbred R-line for fertility restoration gene(s). Depending on the genetic constitution of the R-lines at these loci, even the 3-gene model can lead to single-gene segregation ratios as observed in most of the F_2s and backcrosses, and 2-gene ratios as observed in a few F_2s and backcrosses. The deviations from these expected ratios in some of the crosses influenced by modifiers and environmental conditions generally resulted from the excess of fertile plants in the rainy season or excess of sterile plants in the dry season, the more so in crosses involving an A-line which has been reported to be relatively more unstable for male sterility.

Keywords: *Pennisetum glaucum,* inheritance, A_1 cytoplasm, male sterility, fertility restoration

Introduction

Pearl millet [*Pennisetum glaucum* (L). R. Br.] is one of the major cereals, grown primarily for grain production on more than 26 million ha in the arid and semi-arid tropical regions of Africa and Asia. India is the largest producer of this crop grown on 10 million ha out of 11 million ha in Asia. Pearl millet cultivation in India is dominated by hybrids that occupy about 4.5 million ha. Pearl millet grain hybrids are also grown to a very limited extent in the United States of America. There is a growing interest in pearl millet grain hybrids in some of the African countries. All the grain hybrids are currently based on the A_1 system of cytoplasmic-nuclear male sterility (CMS) that was discovered in 1955 at Tifton, Geor-

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gia in the USA (Burton 1958). The first grain hybrid HB-1 on A_1 CMS system was developed in India in 1965 (Athwal 1965). An understanding of the genetics of male sterility and male-fertility restoration can enhance the efficiency of breeding maintainers (B-lines) and restorers (R-lines). However, even after more than 50 years of extensive utilization, there is little information on the genetics of this CMS system. Based on the fertility restoration behavior of hybrids made on male-sterile lines (A-lines) with the A_1 and two additional CMS sources, Burton and Athwal (1967) hypothesized a single recessive gene responsible for male-sterility and its dominant allele for male-fertility restoration. Siebert (1982) suggested two major dominant complimentary genes with at least one modifier to be controlling male fertility restoration in the A_1 cytoplasm. The objective of this study was to examine the inheritance patterns of male sterility and fertility restoration in a more comprehensive manner, using populations derived by crossing three diverse A-lines based on A_1 CMS system with each of the four diverse R-lines and evaluated in two diverse environments.

Materials and Methods

The basic experimental material consisted of iso-cytoplasmic A-lines with A_1 cytoplasm in three diverse genetic backgrounds and four restorers of diverse parentage. The three A_1-lines (81A, ICMA 88004 and 5054A) were developed by more than eight backcrosses of 81B, ICMB 88004 and 5054B, respectively, into 81A with A_1 cytoplasm. These three A-lines were crossed with each of the four diverse R-lines (L 67B, IPC 511, IPC 804 and IPC 1518) to produce 12 F_1s. Individual plants were used for making plant × plant crosses to produce these F_1s. More than 10 plants of each F_1 were selfed to produce 12 F_2 populations. Bulk pollen from 5–10 plants from each F_1 was used to cross on the respective parental A-lines to produce BC_1 populations. Each F_1 was crossed with bulk pollen from the respective R-line to produce BC_2 population.

Field trials of these 7 parents, 12 F_1s, 12 BC_1s, 12 BC_2s and 12 F_2s were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during the summer (March–June) and rainy (July–October) seasons of 2003. The temperature and relative humidity were recorded from the 35th day to the 70th day of crop growth, which refers to one week prior to the flowering of first entry to one week after the last entry came to flowering in each environment. The mean maximum and minimum temperatures during this period in the (dry) summer season were 36 °C (range 30.4–39.2 °C) and 19.9 °C (range 15.7–23.6 °C), respectively. The mean maximum and minimum temperatures during this period in the rainy season were 30 °C (range 26.4–31.7 °C) and 19.6 °C (range 18–21.4 °C), respectively. During the summer season, the mean relative humidity at 0700 hours was 69% (range 40–95%) and at 1400 hours, it was 31% (range 13–66%). During the rainy season, the mean relative humidity at 0700 hours was 88% (range 80–98%) and at 1400 hours, it was 61% (range 48–76%). The parents, F_1's and BC_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 250–350 plants per plot, and each BC_1 population was

evaluated in four rows of 4 m length with about 125–150 plants per plot. Pollen shedding of individual plants was used to determine male fertility (F) and sterility (S) reaction of individual plants in all the populations. Plants were scored for pollen shedding between 0800 and 1100 hours by tapping the inflorescence and observing for pollen shed. Those shedding pollen were scored as fertile (F) and non-shedders as sterile (S). Chi-square (χ^2) test was applied on the observed segregation data to test the goodness of fit of various probable genetic ratios.

Results

All the 12 F_1s and the corresponding 12 BC_2s had all plants fully fertile during both summer and rainy seasons. The F_2 population from cross 81A × L 67B segregated for 272 male-fertile and 51 male-sterile plants during the summer season and fitted well to a ratio of 54 F: 10 S 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 '$ms_1ms_1ms_2ms_2MS_3MS_3$'. The genotype of restorer parent would be '$MS_1MS_1MS_2MS_2ms_3ms_3$'. The F_1 would be heterozygous at all the three loci ($MS_1ms_1MS_2ms_2MS_3ms_3$), and it would be fertile as found in this study. A plant in the F_2 would be fertile if it possesses dominant alleles at least at two of the any three duplicate-complimentary loci (i.e. $MS_1\_MS_2\_MS_3\_$ / $MS_1\_MS_2\_ms_3ms_3$ / $MS_1\_ms_2ms_2MS_3\_$ / $ms_1ms_1MS_2\_MS_3\_$). According to this genetic model, BC_1 generation should segregate in 3F:1S ratio. The BC_1 of this cross segregated for 114 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 0.65. This segregation pattern was repeated during the rainy season with good fit to a 54F:10S ratio in F_2 ($\chi^2 = 2.42$; $P = 0.12$) and 3F:1S ratio in BC_1 ($\chi^2 = 3.90$; $P = 0.05$).

The F_2 from the cross, ICMA 88004 × L 67B segregated for 294 male-fertile and 20 male-sterile plants during the summer season and fitted well to a ratio of 15F:1S with χ^2 probability of 1.00. Such a segregation pattern would result from the same hypothesized trigenic model that gives a three-gene ratio, if the A-line had same genotype "$ms_1ms_1ms_2ms_2MS_3MS_3$" but the restorer parent were dominant homozygote at all the three loci ($MS_1MS_1MS_2MS_2MS_3MS_3$), which made the F_1 heterozygous only at two loci. The BC_1 of this cross segregated for 130 male-fertile and 31 male-sterile plants and gave a good fit to the expected 3F:1S ratio with χ^2 probability of 0.11. This segregation pattern was repeated during the rainy season with a good fit to 15F:1S ratio in F_2 (χ^2 probability of 1.00). The segregation pattern gave a poor fit to the expected 3F:1S ratio in BC_1 with χ^2 probability of 0.04 due to excess of fertile plants. The F_2 from the cross, 5054A × L67B also gave a good fit to digenic ratio of 15F:1S in F_2 in both the seasons, and to an expected 3F:1S ratio in BC_1 in summer season. In the rainy season BC_1 ratio did not fit to the expected 3F:1S ratio because of the far too excess of fertile plants. This digenic segregation can also be expected from 2-gene duplication interaction model where A-line would be $ms_1ms_1ms_2ms_2$ and R-line would be $MS_1MS_1MS_2MS_2$. The F_2 from cross 81A × IPC 511

Table 1. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1 system A-lines with the restorer parent L-67B in pearl millet, summer and rainy seasons 2003, ICRISAT – Patancheru

Cross	Seasons	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	P
			F	S	F	S	F	S		
81A-P8* × L67B-P3	Summer	F_2	272	51	54	10	273	50	0.00	1.00
		BC_1	114	42	3	1	117	39	0.21	0.65
	Rainy	F_2	339	49	54	10	327	61	2.42	0.12
		BC_1	141	64	3	1	154	51	3.90	0.05
ICMA88004-P14 × L67B-P1	Summer	F_2	294	20	15	1	294	20	0.00	1.00
		BC_1	130	31	3	1	121	40	2.54	0.11
	Rainy	F_2	339	22	15	1	338	23	0.00	1.00
		BC_1	177	41	3	1	164	55	4.13	0.04
5054A-P8 × L67B-P2	Summer	F_2	282	27	15	1	290	19	2.85	0.09
		BC_1	147	27	3	1	131	44	2.85	0.09
	Rainy	F_2	384	22	15	1	381	25	0.35	0.55
		BC_1	187	35	3	1	167	56	9.61	<0.01

* Plant number

Table 2. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1 system A-lines with the restorer parent IPC 511 in pearl millet, summer and rainy seasons 2003, ICRISAT – Patancheru

Cross	Seasons	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	P
			F	S	F	S	F	S		
81A-P5 × IPC511-P1	Summer	F_2	184	57	3	1	181	60	0.23	0.63
		BC_1	97	76	1	1	86.5	86.5	2.31	0.13
	Rainy	F_2	258	77	3	1	251	84	0.72	0.39
		BC_1	115	72	1	1	93.5	93.5	9.43	<0.01
ICMA88004-P8 × IPC511-P2	Summer	F_2	220	73	3	1	220	73	0.00	1.00
		BC_1	68	77	1	1	72.5	72.5	0.44	0.51
	Rainy	F_2	250	79	3	1	247	82	0.17	0.68
		BC_1	55	68	1	1	61.5	61.5	1.63	0.20
5054A-P4 × IPC511-P3	Summer	F_2	224	44	54	10	226	42	0.77	0.79
		BC_1	104	57	3	1	121	40	8.75	<0.01
	Rainy	F_2	367	77	54	10	375	69	0.87	0.35
		BC_1	104	51	3	1	116	39	4.75	0.03

segregated for 184 male-fertile and 57 male-sterile plants during the summer season and fitted well to 3F:1S ratio with χ^2 probability of 0.63, and to an expected 1F:1S ratio in the BC_1 with a χ^2 probability of 0.13 (Table 2). Such a segregation pattern could have resulted from the same hypothesized trigenic model suggested above, but in this case, the genotype of the restorer line would be either '$MS_1MS_1ms_2ms_2MS_3MS_3$' or '$ms_1ms_1MS_2MS_2$ MS_3MS_3', which would make the F_1 heterozygous at only one locus. But such segregation pattern can also result simply from a single-gene control of male sterility and fertility restoration.

In the rainy season also, the F_2 of this cross gave a good fit to a 3F:1S ratio, but its corresponding BC_1 did not fit to 1F:1S ratio due to excess of fertile plants. In the cross, ICMA 88004 × IPC 511, the segregation pattern had a good fit to 3F:1S ratio in F_2 and 1F:1S ratio in BC_1 in both the seasons. In the cross, 5054A × IPC 511, the segregation pattern gave a good fit to a trigenic ratio of 54F: 10S in the F_2 in both the seasons.

In the cross, 81A × IPC 804, the segregation pattern had a good fit to 3F:1S ratio in F_2 in the rainy season and to 1F:1S ratio in BC_1 in both seasons (Table 3). The F_2 segregation of this cross did not fit to 3F:1S ratio in summer season due to far excess of male-sterile plants. The F_2 of the cross, ICMA 88004 × IPC 804 gave good fit to 3F:1S ratio in F_2 in both seasons and to 1F:1S ratio in the summer season. The segregation in BC_1 in the rainy season did not fit to the expected 1F:1S ratio due to far excess of fertile plants. In cross 5054A × IPC 804, the segregation patterns did not fit the expected ratios of any gene model both in the F_2 and BC_1 populations in both the seasons.

In cross 81A × IPC 1518, there were indications of one-gene segregation, but the lack of fit resulted generally from the excess of fertile plants in the rainy season and sterile plants in the summer season, except for the unexpected excess of fertile plants in BC_1 in the summer season (Table 4). In cross ICMA 88004 × IPC 1518, segregation patterns gave a good fit to 3F:1S ratio in F_2 in the rainy season and to 1F:1S ratio in BC_1 in both seasons. The lack of fit to the expected 3F:1S ratio to the F_2 in the summer season resulted from far excess of sterile plants. In cross 5054A × IPC 1518, the segregation pattern was not consistent. For instance, while the F_2 segregation in the summer season gave a good fit to a 3F:1S ratio in the F_2, it did not fit the expected 1F:1S ratio in the BC_1. Similarly, while the F_2 segregation of this cross gave a good fit to a 54F:10S ratio in the F_2 in the rainy season, it did not fit the expected 3F:1S ratio in the BC_1.

Discussion

The overall segregation pattern of male-sterile (S) and male-fertile (F) plants in populations derived from crosses between the two relatively more stable A-lines (81A and ICMA 88004) and three diverse R-lines (IPC 511, IPC 804 and IPC 1518) was indicative of a single-gene segregation for male-sterility/fertility restoration, generally giving a good χ^2 fit to the expected 3F:1S ratio in F_2 and 1F:1S ratio in BC_1 populations. Out of 12 cases of F_2s from these crosses (6 F_2s evaluated in two seasons), only three cases that did not have a good fit to an expected 3F:1S ratio were all in the summer season where excess of male-sterile plants were observed. Similarly, out of 12 cases of the BC_1 from these

Table 3. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1 system A-lines with the restorer parent IPC 804 in pearl millet, summer and rainy seasons 2003, ICRISAT – Patancheru

Cross	Seasons	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	P
			F	S	F	S	F	S		
81A-P3 × IPC804-P4	Summer	F_2	179	95	3	1	205	69	13.66	<0.01
		BC_1	74	84	1	1	79	79	0.51	0.48
	Rainy	F_2	231	81	3	1	234	78	0.15	0.69
		BC_1	105	83	1	1	94	94	2.35	0.13
ICMA88004-P5 × IPC804-P1	Summer	F_2	217	79	3	1	222	74	0.45	0.50
		BC_1	88	72	1	1	80	80	1.41	0.24
	Rainy	F_2	293	116	3	1	307	102	2.46	0.11
		BC_1	129	93	1	1	111	111	5.52	0.02
5054A-P3 × IPC804-P3	Summer	F_2	197	95	54	10	246	46	61.95	<0.01
		BC_1	113	54	3	1	125	42	4.79	.03
	Rainy	F_2	273	67	54	10	287	53	3.99	0.05
		BC_1	164	38	3	1	152	50	3.80	0.05

Table 4. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1 system A-lines with the restorer parent IPC 1518 in pearl millet, summer and rainy seasons 2003, ICRISAT – Patancheru

Cross	Seasons	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	P
			F	S	F	S	F	S		
81A-P1 × IPC1518-P3	Summer	F_2	212	92	3	1	228	76	4.49	0.03
		BC_1	99	57	1	1	78	78	10.78	<0.01
	Rainy	F_2	289	76	3	1	274	91	3.39	0.06
		BC_1	89	63	1	1	76	76	4.11	0.04
ICMA88004-P3 × IPC1518-P2	Summer	F_2	197	129	3	1	245	81	36.91	<0.01
		BC_1	93	77	1	1	85	85	1.32	0.25
	Rainy	F_2	245	74	3	1	239	80	0.55	0.46
		BC_1	64	56	1	1	60	60	0.41	0.52
5054A-P2 × IPC1518-P1	Summer	F_2	228	67	3	1	221	74	0.82	0.36
		BC_1	120	48	3	1	126	42	0.96	0.33
	Rainy	F_2	190	37	54	10	192	35	0.04	0.84
		BC_1	115	22	3	1	103	34	5.37	0.02

crosses, only four cases did not have a good fit to an expected 1F:1S ratio, of which three cases were in the rainy season where excess of fertile plants were observed. Such deviations could likely result from the relatively lower temperatures and higher humidity enhancing the expression of modifiers for fertility restoration in the rainy season, while higher temperatures and lower relative humidity would enhance the expression of modifiers for sterility in the summer season. The effects of these modifiers could be inconsistent, depending on the genetic backgrounds of the segregating populations with the major genes for male sterility/fertility restoration present. Genetical studies in maize *(Zea mays)* (Singh and Laughnan 1972), sorghum (*Sorghum bicolor*) (Tripathi et al. 1985), rice *(Oryza sativa)* (Govinda and Virmani 1988), rapeseed *(Brassica napus)* (Pahwa et al. 2004), and pepper (*Capsicum annum* L.) (Wang et al. 2004) have shown considerable effect of the genetic background and environments on the CMS inheritance.

The segregation patterns observed in this study are more likely to arise due to single-gene control system. However, the same segregation pattern arising from a 3-gene control system where R-lines with specific genetic constitution are involved cannot be ruled out, although it appears unlikely because (i) only one out of the three crosses with a specific plant from the same and only one R-line gave 3-gene ratio and (ii) for that to happen the constitution of A-line need to be '$ms_1ms_1ms_2ms_2MS_3MS_3$ (not very likely to apply to B-lines with diverse pedigree unless the frequency of MS_3 gene is very high in the germplasm), and the R-lines should have dominant alleles at the first two loci and recessive allele at the third locus. In fact, 2-gene control system with duplicate interaction may appear to be a more likely situation than the 3-gene control system hypothesized in this study because two F_2 populations out of three derived from cross 81A × L 67B (where different plants of the R-lines were involved in crosses) had a good χ^2 fit to the expected 15F:1S ratio in both seasons and 3F:1S expected ratio in BC_1 in the summer season. During the rainy season, there was significant deviation from the expected 3F:1S ratio in the rainy season due to excess of fertile plants in this season as also observed in some of the BC_1 populations of other crosses. Under the 2-gene control hypothesis with duplicate interaction, the A-line with a genetic constitution of $ms_1ms_1ms_2ms_2$ and the R-line with a genetic constitution of $MS_1MS_1MS_2MS_2$ will give 15F:1S ratio in the F_2 and 3F:1S ratio in the BC_1 population. Under this hypothesis, R-line being dominant homozygote only at one of the two loci will then give a single-gene inheritance pattern of 3F:1S in the F_2 and 1F:1S in the BC_1 populations as observed in this study. How often will one encounter 2-gene segregation patterns or 1-gene inheritance patterns will depend on the frequency of dominant restorer alleles at these two loci in pearl millet germplasm. In case, the frequency of restorer allele at one of the two loci is very low to rare, one would find most of the studies producing the results of 1-gene inheritance under the 2-gene control system. Siebert (1982) reported two major dominant complimentary genes for fertility restoration of A_1 cytoplasm in pearl millet.

Highly variable and inconsistent segregation patterns across the crosses and the environments were obtained where 5054A was involved as the female parent. It gave a 2-gene fit to 15F:1S ratio in the F_2 in both the seasons and to BC_1 ratio of 3F:1S in one season when crossed with L 67B, 3-gene fit to 54F:10S ratio in F_2 in both seasons, but lack of fit

to the expected 3F:1S ratio in the BC_1 when crossed with IPC 511, lack of fit to any of these three hypothetical ratios in both populations and both seasons when crossed with IPC 804, and good fit to 3F:1S ratio in the summer season and to 54F:10S ratio in the rainy season in F_2, but lack of fit to the expected ratios in both seasons in BC_1 when crossed with IPC 1518. Of the three male-sterile lines used in this study, 5054A has been found to be most unstable for its male sterility as reflected in the relatively higher frequency of pollen shedders observed in this line (Rai et al. 2008). Thus, for a valid interpretation of the genetics of male sterility/fertility restoration, it is important that A-lines with stable male sterility be used in crosses. It is likely that unstable male sterility of 5054A with pollen shedding behavior may lead to excess of fertile plants in both generations, which may convert a 3F:1S ratio in F_2 and 1F:1S ratio in BC_1 to a 54F:10S ratio in F_2 and 3F:1S ratio in BC_1. In this respect, it is important to note that 81A also throws low frequency of pollen shedders and ICMA 88004 is the most stable A-line. If the 3-gene model of inheritance, although it appears unlikely, is found to hold in future studies, then this study also showed that morphological uniformity of an R-line is not necessarily a reliable indicator of the genetic purity of the line with respect to traits such as fertility restoration in highly cross-pollinated crops like pearl millet. Therefore, even in highly inbred lines, individual plants should be used for making crosses for genetical studies. An earlier study in pearl millet (Hash et al. 2006) showed the existence of significant variability for resistance to downy mildew [*Sclerospora graminicola* (Sacc.) Schroet.] even in highly inbred lines of pearl millet.

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