Genetics of Fertility Restoration of the A_4 Cytoplasmic-Nuclear Male Sterility System in Pearl Millet

Shashi Kumar GUPTA, Kedar Nath RAI, Mahalingam GOVINDARAJ and Aluri Sambasiva RAO

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India

Abstract: Inheritance of fertility restoration of the A_4 system of cytoplasmic-nuclear male sterility in pearl millet was investigated using six crosses between two diverse male sterile lines (A-lines) and three diverse restorers (R-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, indicated the dominant single-gene control of male fertility restoration. The segregation pattern in BC_1F_2 progenies derived from the fertile BC_1 plants evaluated for one season provided further evidence for the single-gene control. The season did not have much effect on fertility restoration. The information on the single-gene control of fertility restoration will help in diversifying the restorer genetic base of the A_4 CMS system and enhance R-line breeding efficiency in pearl millet.

Keywords: A_4 cytoplasm; fertility restoration; inheritance; male sterility; *Pennisetum glaucum*

The discovery of A_1 cytoplasmic-nuclear male sterility (CMS) at Tifton, Georgia, USA (Burton 1958) initiated the era of hybrid cultivar development in pearl millet [*Pennisetum glaucum* (L). R. Br.], which led to the release of the first grain hybrid in India in 1965 (Athwal 1965). Since then hundreds of commercial hybrids, all of them based on the A_1-CMS system, have been developed and released or commercialized. This dependence on single cytoplasm makes the pearl millet hybrid seed industry vulnerable to disease and insect-pest epidemics, as witnessed in the case of southern leaf blight epidemic caused by *Bipolaris maydis* race T on the Texas cytoplasm-based maize hybrids in the United States (Scheifele *et al.* 1970). This concern necessitated the search for new sources of CMS in pearl millet (Rai *et al*. 2006). Hanna (1989) identified an A_4 CMS system at Tifton, Georgia, USA in a wild grassy *Pennisetum glaucum* (L.) R. Br. subsp. *monodii* (Maire) Brunken. Also, an A_5 CMS system was identified in a pearl millet gene pool (Rai 1995). Among the various CMS systems reported so far, A_4 and A_5 CMS systems were found to have the most stable male sterility (Rai *et al*. 1996, 2001, 2006, 2009). Further, the frequency of maintainers is much higher for the A_4 CMS system than for the A_1 CMS system, and almost all lines are maintainers of the A_5 CMS system (Rai *et al*. 2006). Hence, these two CMS sources provide a much greater opportunity for the genetic diversification of A-lines, and thus a greater opportunity for diversifying the genetic base of hybrids provided more diversity is generated in the restorer lines. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) developed and disseminated 31 diverse and productive A-lines based on the A_4 CMS system during the period 1996–2004 (Rai *et al*. 2006). However, none of them was used by breeders in the public and private sector in India because of the paucity of restorers (R-lines) of this CMS system. Therefore, ICRISAT has now initiated a program focused on the development of A_4 CMS system restorers. It was observed that the fertility restoration ability of the A_4 CMS system restorers is less affected by the genetic background of the A-line than that of the A_1 CMS system restorers (Gupta *et al.* 2010). The understanding of the inheritance of fertility restoration of this CMS system can be useful in devising an effective breeding strategy. Except for a preliminary report (Du *et al.* 1996), there is not much information on the inheritance of fertility restoration of the A_4 CMS system. Therefore, the objective of this study was to undertake a more comprehensive study of the inheritance of fertility restoration of the A_4 CMS system in pearl millet using genetically diverse A- and R-lines.

MATERIAL AND METHODS

Plant material

The basic experimental material consisted of isocytoplasmic A-lines with A_4 cytoplasm in two diverse genetic backgrounds and three previously identified A_4-restorers of diverse parentage (Table 1). The two A_4-lines (81A_4 and ICMA_4 88004) were developed by more than eight backcrosses of 81B and ICMB 88004, respectively, into A_4 cytoplasm.

Development of experimental populations

The two A-lines (81A_4 and ICMA_4 88004) were crossed with each of the three diverse R-lines (IPC 511, IPC 804 and IPC 1518) to produce six F_1s in the 2009 rainy season. Single plants were used for making plant × plant crosses to produce these F_1s (81A_4 × IPC 511, 81A_4 × IPC 804, 81A_4 × IPC 1518, ICMA_4 88004 × IPC511, ICMA_4 88004 × IPC804, ICMA_4 88004 × IPC 1518). The A-lines were maintained by crossing with their respective B-lines. In the post rainy season of 2009–2010, more than ten plants of each of the six F_1s were selfed to produce F_2 populations. Bulk pollen from five to ten 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. During the 2010 summer season, panicles of five to seven fertile plants in each BC_1 were selfed to produce BC_1F_2 progenies.

Field evaluation and data analysis

Field trials of five parents, six each of F_1s, F_2s, BC_1s, and BC_2s were conducted at ICRISAT, Patancheru, Andhra-Pradesh, India during the summer (March–June) and rainy (July–October) season of 2010, while BC_1F_2 progenies were evaluated in the rainy season of 2010. The parents, F_1, BC_2, and BC_1F_2 populations were evaluated in single-row plots of four meter length with mostly 25–35 plants per plot. Each F_2 population was evaluated in eight-row plots of four meter length with approximately 250–350 plants per plot, and each BC_1 population was evaluated in four rows of four meter length with about 125–150 plants per plot. Pollen shedding of individual plants was used to determine male fertility (F) and sterility (S) reactions of individual plants in all the populations. At full anthesis, plants were scored for pollen shedding between 08:00 and 11:00 h by tapping the inflorescence and observing for the pollen shed. Those shedding pollen were scored as male-fertile (F) and non-shedders as

Table 1. Origin and parentage of B-lines (maintainer counterparts of A-lines) and restorer lines (R-lines) used in inheritance study

Line	Origin	Parentage
B-line		
81B	ICRISAT	ICMB 1: Gamma radiation induced downy mildew resistant selection from Tift 23 D_2B_1
ICMB 88004	ICRISAT	Togo-11-5-2 selection
R-line		
IPC 511	ICRISAT	[(J 934-7 × 700544-7-2=1) × EC 298-2-1]-1-5
IPC 804	ICRISAT	(S10 LB- 30 × LCSN 1225-6-3-1)-1-2-1-1
IPC 1518	ICRISAT	ICRC – F4-146-3

male-sterile (S) (Rai & Hash 1990). Chi-square (χ^2) test was applied to the observed segregation data in F_2, BC_1 and BC_1F_2 populations to test the goodness of fit of various probable genetic ratios. 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 flowering of the first entry to one week after the last entry came to flowering in each season.

RESULTS AND DISCUSSION

Temperatures during the summer season 2010 ranged from 22.6 to 39.7°C (mean 30°C) and relative humidity at 07:00 h ranged from 65 to 92% (mean 77%). During the rainy season 2010, temperatures ranged from 21.1 to 29.4°C (mean 25°C) and relative humidity ranged from 94 to 96% (mean 95%). Thus, the two seasons of field trials represented two contrasting weather environments.

All the restorer parents (R-lines) had all plants fully fertile while the A-lines were fully sterile during both rainy and summer seasons. All the plants in six F_1s and the corresponding six BC_2s were also fully fertile during both the seasons, indicating fertility restoration for the A_4 CMS system to be controlled by dominant gene(s) that are in homozygous state in the present set of restorers. The F_2 population from the 81A_4 × IPC 511 cross segregated for 117 male-fertile (F) and 39 male-sterile (S) plants during the rainy season giving a perfect χ^2 fit to a ratio of 3F:1S (P = 1.00) (Table 2), indicating the dominant monogenic control of fertility restoration. The BC_1 of this cross segregated for 78 male-fertile and 76 male-sterile plants during the rainy season and fitted well to the expected monogenic ratio of 1F:1S (P = 0.87). This segregation pattern repeated during the summer season with a good fit to 3F:1S ratio in F_2 (P = 0.96) and 1F:1S ratio in BC_1 (P = 0.46). The F_2 from the 81A_4 × IPC 804 cross segregated for 210 male-fertile and 65 male-sterile plants during the rainy season and fitted well to the monogenic ratio of 3F:1S (P = 0.60). The BC_1 of this cross segregated for 92 male-fertile and 83 male-sterile plants and gave a good fit to the expected 1F:1S ratio (P = 0.65). This segregation pattern also repeated during the summer season with a good fit to the 3F:1S ratio in F_2 (P = 0.32) and the expected BC_1 ratio of 1F:1S (P= 0.37). In the 81A_4 × IPC 1518 cross the segregation pattern in both seasons had a good fit to 3F:1S ratio in F_2 and 1F:1S ratio in BC_1.

Table 2. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and the test of goodness of fit for hypothetical Mendelian ratios in crosses of 81A_4 with the restorer parents IPC 511, IPC 804 and IPC 1518 in pearl millet, rainy and summer seasons 2010, Patancheru

Cross	Season	Generation	No. of plants observed		Expected ratio		χ^2	P
			F	S	F	S		
81A_4 × IPC 511	rainy	F_2	117	39	3	1	0.00	1.00
		BC_1	78	76	1	1	0.03	0.87
	summer	F_2	94	31	3	1	0.00	0.96
		BC_1	36	30	1	1	0.55	0.46
81A_4 × IPC 804	rainy	F_2	210	65	3	1	0.27	0.60
		BC_1	92	83	1	1	0.46	0.50
	summer	F_2	179	51	3	1	0.98	0.32
		BC_1	82	71	1	1	0.79	0.37
81A_4 × IPC 1518	rainy	F_2	156	44	3	1	0.96	0.33
		BC_1	37	40	1	1	0.12	0.73
	summer	F_2	142	58	3	1	1.71	0.19
		BC_1	69	77	1	1	0.44	0.51

P – probability

The F_2 from the $ICMA_4$ 88004 × IPC 511 cross segregated for 176 male-fertile and 54 male-sterile plants during the rainy season and fitted well to 3F:1S ratio (P = 0.59), but its corresponding BC_1 did not fit to 1F:1S ratio due to the excess of fertile plants (Table 3). However, the segregation pattern of this cross during the summer season had a good fit to both F_2 ratio of 3F:1S (P = 0.40) and BC_1 ratio of 1F:1S (P = 0.20). Such a segregation pattern in a different genetic background, represented by $ICMA_4$ 88004, indicated the dominant monogenic control of fertility restoration again. The $ICMA_4$ 88004 × IPC 804 cross had a good fit to the F_2 ratio of 3F:1S in rainy season (P = 0.51) and in summer season (P = 0.37) and also a good fit to the expected 1F:1S ratio in BC_1 in both the rainy and summer season (P = 0.07). The segregation pattern in F_2 of the $ICMA_4$ 88004 × IPC 1518 cross had a good fit in rainy season (P = 0.81), but its corresponding BC_1 did not fit to 1F:1S ratio due to the excess of fertile plants. In this cross, there was neither a good fit to 3F:1S in F_2 nor to 1F:1S ratio in BC_1 segregation in summer season due to the excess of fertile plants. The segregation pattern in this set of crosses revealed that out of the six cases of F_2s (three F_2s evaluated in two seasons), five cases had a good fit to 3F:1S ratio. However, out of the six cases of BC_1, only three cases had a good fit to 1F:1S ratio. In all these four cases (1 case of F_2 and three cases of BC_1) not conforming to single-gene segregation, there was an excess of fertile plants. Of these, two cases of deviation as observed in the rainy season could result from relatively lower temperatures and higher humidity that enhances the expression of modifiers for fertility restoration in pearl millet (Rai & Hash 1990). 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. Genetic studies in maize (*Zea mays*) (Singh & Laughman 1972), sorghum (*Sorghum bicolor*) (Tripathi *et al.* 1985) and rapeseed (*Brassica napus*) (Pahwa *et al.* 2004) have shown a considerable effect of the genetic background and environments on the CMS inheritance. However, the excess of fertile plants as observed in the two remaining cases in the summer season could not be explained.

The segregation pattern observed in BC_1F_2 progenies derived from three to seven BC_1 fertile plants of each of the six crosses showed all the progenies having a good fit to 3F:1S ratio, which is expected from the dominant monogenic control of fertility restoration (Table 4).

The F_2s and BC_1s had a similar segregation pattern across both rainy and summer seasons in ten out of the 12 cases (six F_2s and six BC_1s evaluated in two

Table 3. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and the test of goodness of fit for hypothetical Mendelian ratios in crosses of $ICMA_4$ 88004 with the restorer parents IPC 511, IPC 804 and IPC 1518 in pearl millet, rainy and summer seasons 2010, Patancheru

Cross	Season	Generation	No. of plants observed		Expected ratio		χ^2	P
			F	S	F	S		
$ICMA_4$ 88004 × IPC 511	rainy	F_2	176	54	3	1	0.28	0.59
		BC_1	100	69	1	1	5.69	0.02
	summer	F_2	122	47	3	1	0.71	0.40
		BC_1	66	52	1	1	1.66	0.20
$ICMA_4$ 88004 × IPC 804	rainy	F_2	214	65	3	1	0.43	0.51
		BC_1	122	95	1	1	3.36	0.07
	summer	F_2	215	63	3	1	0.81	0.37
		BC_1	92	69	1	1	3.29	0.07
$ICMA_4$ 88004 × IPC 1518	rainy	F_2	73	23	3	1	0.06	0.81
		BC_1	50	28	1	1	5.00	0.03
	summer	F_2	178	35	3	1	8.34	0.00
		BC_1	80	51	1	1	6.42	0.01

P – probability

Table 4. Segregation for male-fertile (F) and male-sterile (S) plants in BC_1F_2 progenies and the test of goodness of fit for 3F:1S segregation ratio in crosses of two A_4 CMS A-lines with three restorer parents in pearl millet, rainy season 2010, Patancheru

Cross	Progenies	No. of plants observed		χ^2	*P*
		F	S		
$81A_4$ × IPC 511	1	44	13	0.15	0.70
	2	36	11	0.06	0.80
	3	41	14	0.01	0.94
	4	10	3	0.03	0.87
	5	44	15	0.01	0.94
	6	35	10	0.19	0.67
	7	46	16	0.02	0.88
$81A_4$ × IPC 804	1	21	8	0.10	0.75
	2	19	8	0.31	0.58
	3	17	8	0.65	0.42
	4	14	4	0.07	0.79
	5	24	8	0.00	1.00
	6	19	7	0.05	0.82
$81A_4$ × IPC 1518	1	19	9	0.76	0.38
	3	12	5	0.18	0.67
$ICMA_4$ 88004 × IPC 511	1	43	16	0.14	0.71
	2	30	5	2.14	0.14
	3	44	15	0.01	0.94
	4	56	18	0.02	0.89
	5	31	12	0.19	0.66
$ICMA_4$ 88004 × IPC 804	1	47	17	0.08	0.77
	2	42	15	0.05	0.82
	3	43	15	0.02	0.88
	4	40	12	0.10	0.75
	5	30	4	3.18	0.07
	6	39	16	0.49	0.48
	7	20	8	0.19	0.66
$ICMA_4$ 88004 × IPC 1518	1	34	13	0.18	0.67
	2	46	13	0.28	0.60
	3	37	11	0.11	0.74
	4	27	9	0.00	1.00
	5	33	10	0.07	0.79
	6	40	10	0.67	0.41

P – probability

seasons) indicating that the season did not have much effect on fertility restoration of the A_4 CMS system. This was also evidenced by the two remaining cases (BC_1 of $ICMA_4$ 88004 × IPC 511 in rainy season, and F_2 of $ICMA_4$ 88004 × IPC 1518 in summer season) where segregation distortion was observed in one case in either of both seasons. The overall segregation patterns of male sterile (S) and fertile (F) plants in populations derived from crosses between the A-lines ($81A_4$ and $ICMA_4$ 88004) and three diverse R-lines (IPC 511, IPC 804 and IPC 1518) provided the evidence of dominant single-gene segregation for fertility restoration with 3F:1S ratio in F_2 and 1F:1S ratio in BC_1 populations. Du *et al.* (1996) also reported a single dominant gene for fertility restoration of A_4 cytoplasm in pearl millet, based on the study on a single cross evaluated in a single season. An earlier study found the dominant monogenic control of fertility restoration of the A_1-system of cytoplasmic-nuclear male sterility also in pearl millet (Yadav *et al.* 2010). The single-gene control of fertility restoration in the A_4 CMS system, as revealed in this study, will help breeders to easily diversify the genetic base of restorers due to ease in the restorer breeding procedure, which in turn will help to diversify the cytoplasmic base of pearl millet hybrids.

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Corresponding author:

Shashi Kumar Gupta, Ph.D., International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 502 324 Patancheru, Andhra Pradesh, India
e-mail: s.gupta@cgiar.org