Effect of genetic background on fertility restoration of pearl millet hybrids based on three diverse cytoplasmic-nuclear male-sterility systems

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Abstract

Isonuclear A-lines with A_1, A_4 and A_5 cytoplasm in five diverse genetic backgrounds (ICMA 88004, ICMA 89111, ICMA 94555, ICMA 96111 and ICMA 96666) crossed with 19 cytoplasm-specific R-lines generated 285 F_1 hybrids, which were evaluated for male fertility/sterility in two contrasting seasons (postrainy season and rainy season) of 2009 at ICRISAT, Patancheru, India. Fertility restoration of hybrids with the A_4 and A_5 cytoplasm was neither affected by the genetic backgrounds nor by the environments as 94 hybrids in each cytoplasmic background were fertile during the postrainy season, while all 95 hybrids were fertile in the rainy season. In case of hybrids with the A_1 cytoplasm, genetic background of the male-sterile lines as well as the environments had strong effect on fertility restoration of hybrids. The largest proportion of hybrids with this cytoplasm (14%) were sterile during the postrainy season, indicating that evaluation of hybrids during the postrainy season may be more efficient to identify those with stable fertility restoration across the environments.

Introduction

In pearl millet (*Pennisetum glaucum*), following the discovery of a commercially viable Tift 23 source of cytoplasmic-nuclear male-sterility (CMS) (Burton 1965), which was later designated as the A_1 system (Burton and Athwal 1967), attempts to diversify the cytoplasmic base of hybrids led to the identification of several additional CMS sources (Rai et al. 2006). Of these, the two CMS sources that represented CMS systems different from the A_1 source and from each other include what has been designated as an A_4 CMS system (Hanna 1989) and an A_5 CMS system (Rai 1995). A comparative study of male sterility of these three CMS systems showed that the A_5 CMS system was most stable across the genetic backgrounds and environments and it had the highest frequency of maintainers. This was followed by the A_4 CMS system, and the A_1 CMS system was least stable among these, and had the lowest frequency of maintainers (Rai et al. 2008). These CMS systems differ very little with respect to their effects on grain yield (Chandra-Shekara et al. 2007, Rai et al. 2008). Thus, for the breeding of male-sterile lines (A-lines), the A_5 system was found most promising, followed by the A_4 and A_1 systems. As far as the usefulness of these three CMS systems in breeding restorer lines (R-lines) is concerned, two questions must be addressed. These are: frequency of restorers in the germplasm, and stability of fertility restoration of hybrids in different genetic backgrounds of A-lines. As a corollary of the maintainer frequency, the restorer frequency, as expected, was found to run in the reverse order: the lowest frequency of restorers of the A_5 system, and highest for the A_1 system. For the conversion of breeding lines into their A_4, and A_5 restorer versions following a simple backcross breeding that has been designed (Rai et al. 2006) has been found rather straightforward, and a large number of otherwise non-restorers have been converted into their A_4 and A_5 restorer versions. The objective of this was to examine the effect of the genetic background of A-lines on the fertility restoration behavior of hybrids based on these three CMS systems.

Material and methods

Isonuclear A-lines with A_1, A_4 and A_5 cytoplasm in five diverse genetic backgrounds (ICMA 88004, ICMA 89111, ICMA 94555, ICMA 96111 and ICMA 96666) were each crossed with 19 R-lines, specific to each of the three CMS systems. This produced 95 hybrids in each CMS system. At the time of crossing, bulk pollen was collected from 8–10 plants of a particular R-line and crossed the same day on all five A-lines with a given cytoplasm. Two staggered plantings of all A-lines at 7 days interval ensured that panicles of all A-lines were available at the right stage for crossing with all the R-lines. All the 285 F_1 hybrids were evaluated during the postrainy (summer) season (8th February planting) and rainy season (9th July planting) of 2009 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Each hybrid was sown in single row plot of 4 m with 60 cm row-to-row spacing in the postrainy season and 75 cm in the rainy season in a single replication. Fifteen days after planting, seedlings were thinned down to 35–40 plants per plot with 10 cm spacing. A basal dose of 100 kg ha^{-1} DAP (diammonium phosphate) was applied before planting, with 100 kg ha^{-1} of urea top dressed 20–25 days after planting. Irrigation was applied at 7–10 days interval during the postrainy season, and as and when required during the rainy season to ensure that there was no moisture stress during the crop growth and development. At 75% anthesis, plots were scored for fertility/sterility on the plot basis using the pollen shedding data taken between 0830 and 1100 hrs. The plots were visually rated as sterile with rating 1 (shrunken anthers and complete lack of pollen shed in all plants), rating 2 (shrunken anthers and complete lack of pollen shed in >90% plants), rating 3 (plump anthers and pollen shed in >90% plants) and rating 4 (plump anthers and pollen shed in all the plants) (Rai and Hash 1990). No test cross hybrid was found where either fertile or sterile plants were in 10–90% range of pollen shed.

Results and discussion

During the rainy season, all 95 hybrids each with A_4 and A_5 cytoplasm were fully fertile, indicating that genetic background of A-lines had no effect on the fertility restoration of hybrids (Table 1). During the postrainy season, 1 hybrid with each of these cytoplasm turned male-sterile, and 3 hybrids with the A_5 cytoplasm and 5 hybrids with A_4 cytoplasm had >90% of plants that were fertile. From a practical standpoint, it has two implications. First, in terms of seed set and productivity, it does not matter whether a hybrid of pearl millet has all plants fully fertile, or a majority of fully fertile plants. Second, if the full fertility of hybrid is desired, then such variability within the hybrid is indicative of the variability for fertility restoration ability within the restorer line, implying that by 1–2 generations of single plant selection within the R-line can lead to uniformity and thus to produce an improved version that will produce fully fertile hybrid.

In case of hybrids based on the A_1 cytoplasm, even in the rainy season, five hybrids were fully sterile. In the postrainy season, as many as 13 hybrids were sterile, and these included those five hybrids which were sterile in the rainy season. This showed large seasonal effect on the

Table1. Number of hybrids with different pollen shedding scores during postrainy season (D09) and rainy season (R09) 2009 at ICRISAT, Patancheru, India.

Cytoplasm	Pollen shedding score[1]	ICMA 89111		ICMA 94555		ICMA 96111		ICMA 96666		ICMA 88004		Total	
		D09	R09	D09	R09	D09	R09	D09	R09	D09	R09	D09	R09
A_1	1	2	0	0	0	7	2	0	0	4	3	13	5
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	2	0	1	0	3	0
	4	17	19	19	19	12	17	17	19	14	16	79	90
A_4	1	0	0	0	0	1	0	0	0	0	0	1	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	2	0	0	0	3	0	5	0
	4	19	19	19	19	16	19	19	19	16	19	89	95
A_5	1	0	0	1	0	0	0	0	0	0	0	1	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	0	0	0	0	0	2	0	0	0	3	0
	4	18	19	18	19	19	19	17	19	19	19	91	95
Total	1	2	0	1	0	8	2	0	0	4	3		
	2	0	0	0	0	0	0	0	0	0	0		
	3	1	0	0	0	2	0	4	0	4	0		
	4	54	57	56	57	47	55	53	57	49	54		

1. Score 1 = Shrunken anthers and complete lack of pollen shed in all plants, 2 = Shrunken anthers and complete lack of pollen shed in >90% plants, 3 = Plump anthers and pollen shed in >90% plants and 4 = Plump anthers and pollen shed in all the plants.

fertility restoration behavior of R-lines in hybrids with this cytoplasm. During the flowering period, daily maximum temperature varied between 29.4 and 38.8°C (mean of 35.7°C) and relative humidity early morning between 59 and 91% (mean of 75.3%) in 2009 postrainy season; while in the rainy season, the corresponding daily maximum temperature was 25.9–33.0°C (mean of 29.6°C) and relative humidity 80–98% (mean of 92.6%) (Table 2). Thus, relatively lower temperature and higher humidity during the rainy season might have led to enhanced expression of fertility restoration. Earlier studies on pearl millet (Rai and Hash 1990, Yadav et al. 2010) and maize (*Zea mays*) (Duvick 1959) also indicated that lower temperature and higher humidity represent more favorable environment for fertility restoration of hybrids. Thus, it would appear that postrainy season provides a much more reliable test environment to identify stable R-lines and fertile hybrids in all three CMS systems, more so in case of the A_1 CMS system. On two male-sterile lines (ICMA 94555 and ICMA 96666), all the hybrids were fully fertile in both seasons. The largest deviation from this expected pattern was observed for hybrids based on male-sterile line ICMA 96111 that had seven sterile hybrids during the postrainy season, followed by ICMA 88004 that had 4 hybrids sterile in the postrainy season, indicative of high genetic × environment interaction in the background of these two genotypes. Thus, in the A_1 CMS system, fertility restoration of hybrids was highly influenced by the genetic background of the A-lines. Twelve out of 19 pollinators had fertile hybrids on all the five different backgrounds of A_1 cytoplasm. From the same set of 7 pollinators which gave sterile hybrids on ICMA 96111, 4 and 2 pollinators had sterile hybrids on 88004A and ICMA 89111 respectively in postrainy season and 3 pollinators had sterile hybrids on 88004A in rainy season, indicating that some pollinators had cytoplasmic-genetic effect on the fertility restoration. Also, all hybrids found sterile in the postrainy season included those that were sterile in the rainy season as well. This would imply that ICMA 96111 would be a more efficient tester to identify R-lines with stable fertility restoration across the environments and genetic backgrounds of A-lines. Similar effect of female parent genotype on fertility restoration has also been reported in rice (*Oryza sativa*) (Govinda and Virmani 1998) and rapeseed (*Brassica napus*) (Pahwa et al. 2004).

Further studies in this direction based on more A-lines of diverse origin and hybrid evaluation conducted across more diverse environments (including season and locations) are likely to identify A-line(s) and environment(s) that may potentially enhance the efficiency of breeding R-lines with stable fertility restoration ability.

Table 2. Temperature and relative humidity during 36 to 56 days after planting in two test seasons in 2009 at ICRISAT, Patancheru, India.

Weather variable		Summer season	Rainy season
Max. daily temp. (°C)	Mean	35.7	29.6
	Range	29.4–38.8	25.9–33.0
Min. daily temp. (°C)	Mean	19.8	22.5
	Range	15.5–22.4	20.5–24.5
Relative humidity (%) at 0700 hrs	Mean	75.3	92.6
	Range	59–91	80–98
Relative humidity (%) at 1400 hrs	Mean	24.5	70.7
	Range	14–42	52–95

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