Influence of Cytoplasmic-nuclear Male Sterility on Agronomic Performance of Sorghum Hybrids

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Introduction

The discovery of a cytoplasmic-nuclear male-sterility (CMS) system (Stephens and Holland 1954) [designated as A_1 (*milo*)] has led to the commercial exploitation of heterosis of F_1 hybrids in sorghum [*Sorghum bicolor* (L.) Moench]. Subsequently, several alternative non-*milo* CMS systems (A_2, A_3 and A_4) were identified and developed (Schertz 1994) for use in hybrid breeding programs to diversify the cytoplasm and nuclear genetic base of sorghum hybrids. Large numbers of A_1-based hybrids (Reddy et al. 2005) and a few A_2-based hybrids (Liu Qing Shan et al. 2000) have been released/marketed for commercial cultivation all over the globe. The evaluation of CMS-based hybrids in relation to those based on male-fertile counterpart cytoplasm would provide an insight into the influence of CMS on agronomic performance. As such studies are lacking, we made an attempt to fill the gap.

Materials and Methods

The test material consisted of isonuclear, alloplasmic male-sterile (A-) lines in 12 nuclear genetic backgrounds (ICSA 11, -17, -26, -37, -38, -42, -88001, -88004, -88005, -18757, PM 17467A and PM 7061A) with A_1 and A_2 CMS systems, and three dual restorer (R-) lines (ICSR 93001, -92003 and -93031). The A-lines selected for the study were diverse in respect of days to flowering and maturity, plant height and grain yield potential. The 12 A-lines with A_1 and A_2 CMS systems were crossed with the three dual R-lines to generate two sets of 36 A × R hybrids. The male-fertile counterparts (B-lines) which maintain the male-sterility of the 12 A_1- and A_2-based A-lines were emasculated and crossed with the same three dual R-lines to obtain 36 B × R crosses. The two sets of 36 A × R (with A_1 and A_2 cytoplasms) and one set of 36 B × R crosses differing only by their cytoplasms were evaluated at ICRISAT-Patancheru, India during the rainy season of 2005. A split-split-plot design (SSPD) with three replications was used. The R-lines were sown in the main plots, the A-lines in the subplots and the cytoplasms in the sub-subplots. Each entry was grown in 4 rows of 2 m length spaced 75 cm apart. The seedlings were thinned to maintain a distance of 10 cm between plants one week after seedling emergence. Recordings were taken of the days to 50% flowering, plant height, grain yield and 100-grain weight (g).

Statistical analysis. Analysis of variance (ANOVA) was carried out as per SSPD. The general combining ability (*gca*) effects of the parents and the specific combining ability (*sca*) effects of the crosses were estimated as per Kempthorne (1957). The significance or otherwise of cytoplasmic differences in respect of *gca* effects of the A-lines and the mean performance and *sca* effects of the hybrids was determined by comparing with the least significant difference (LSD).

Results and Discussion

Variance components. There were significant differences among the A/B-lines (nuclear genotype) for all the traits and among the R-lines for plant height and grain yield, indicating that the selection of the hybrid parents (A/B- and R-lines) for the study (Table 1) was appropriate. The significant mean squares due to the A/B- × R-lines interaction indicated that hybrids differ significantly in their *sca* effects for all the traits. Cytoplasms (A_1, A_2 and B) *per se* appeared to have a significant influence on the expression of hybrids for all the traits, as was evident from the significant mean squares due to cytoplasm. The first-order interaction of cytoplasm with the nuclear genetic background of A-lines or R-lines and the second-order interaction with A-line and R-lines toward variation of isonuclear hybrids was significant for all the traits, suggesting that cytoplasm does have a significant influence on the expression of A-lines and hybrids and that the degree of influence varies with the nuclear genetic background of the A-lines and hybrids for all the traits.

Cytoplasm influence on *gca* effects. The assessment of *gca* effects of hybrid parents is important to judge their suitability for developing hybrids. Cytoplasmic differences for parental *gca* effects were evident in only some of the nuclear genetic backgrounds for all the traits (Table 2). However, the magnitude of cytoplasmic differences varied with the nuclear genetic background of the lines and was too small to have any practical significance, but there also appeared to be no definite trend favoring any particular type of cytoplasm for all the traits except grain yield. For instance, while male-fertile cytoplasm-based lines

ICSA/B 11 and PM 17467A/B were better general combiners for earliness than those based on the male-sterile counterpart cytoplasm, the male-sterile cytoplasm-based line ICSA 37 was a better general combiner for lateness than that based on male-fertile counterpart line. Most of the CMS (A_1- or the A_2)-based lines were better general combiners for grain yield than those based on their male-fertile cytoplasm. The A-lines being male-sterile appear to maximize their fitness in hybrid combinations with the R-lines compared to their counterpart B-lines (with inherent male-fertility). This resulted in superior average performance by A × R crosses compared to B × R crosses for grain yield, which obviously translated into better estimates of the *gca* effects of A-lines compared to those of B-lines.

Hybrid mean performance. A comparison of the overall average performance of A × R (in both A_1 and A_2 backgrounds) and B × R crosses as two separate groups indicated that while there were no differences between them for days to 50% flowering, A × R crosses (in both A_1 and A_2 backgrounds) were significantly taller (by 0.2 m in A_1 and by 0.1 m in A_2 backgrounds) and manifested higher grain yield (by 0.7 t ha^{-1} in A_1 and by 0.9 t ha^{-1} in A_2 backgrounds) than B × R crosses (Table 3). The A × R (only in the A_1 background) crosses had significantly (statistically) larger (by 0.08 g) grains than B × R crosses, though the difference was not visually distinct. However, A × R (A_2 background) crosses were comparable to B × R crosses in terms of grain size. Significant cytoplasmic effects were evident for all the traits when the individual nuclear genetic background of A × R (both in A_1 and A_2) and B × R crosses was examined. For instance, while the A × R (both A_1 and A_2) crosses, besides being early, were taller and possessed larger grains compared to those of B × R crosses in a few nuclear genetic backgrounds, the opposite was true in a few other nuclear genetic backgrounds. In most of the isonuclear genetic backgrounds (26 of the 36), A × R (A_1 and/or A_2) crosses were significantly superior to their counterpart B × R crosses for grain yield (Table 3).

Cytoplasmic influence on *sca* effects. Specific combinations of A- and R-lines with good *gca* effects will remain the essential requirement for the production of superior sorghum hybrids (Duvick 1999). As was observed for *gca* effects, cytoplasmic effects were detected for *sca* effects of hybrids for all the traits only in some of the nuclear genetic backgrounds (data not shown).

As A × R and B × R crosses differ only by the cytoplasmic sterility-inducing genes/factors which are present on the mitochondrial genome, the higher grain yield potential of A × R crosses compared to those of B × R crosses could be attributed to the plieotropic effect of the factors that induce male-sterility or due to the closely linked loci contributing to grain yield. Heterozygosity at the male-sterility/male-fertility loci and/or at linked loci with overdominance effects in A × R crosses in contrast to homozygosity in B × R crosses might also be responsible for the significant difference in performance between A × R and B × R crosses for grain yield. The significant influence of cytoplasmic genes/factors on grain yield in pearl millet (Virk and Brar 1993) lends adequate support to these considerations. However, the distinction between the roles of cytoplasmic factors *per se* and cytoplasm-nuclear genetic interactions is complicated, as the very expression of CMS and its restoration is primarily based on the interaction of genes present on mitochondrial DNA and

Table 1. Analysis of variance of isonuclear alloplasmic (A_1, A_2 and B) sorghum hybrids for agronomic traits, ICRISAT-Patancheru, Andhra Pradesh, India, rainy season, 2005.

Source of variation	Degrees of freedom	Mean sum of squares			
		Days to 50% flowering	Plant height (m)	Grain yield (t ha^{-1})	100-grain weight (g)
Replication	2	53.40	0.32	4.33	0.37
R-line	2	32.40	11.54**	14.77*	1.21
Residual	4	9.10	0.02	1.32	0.21
A/B-line	11	32.86**	0.21**	1.62**	0.30**
R-line × A/B-line	22	11.72**	0.70**	1.71**	0.11**
Residual	66	1.74	0.05	0.42	0.01
Cytoplasm (A_1, A_2 and B)	2	4.11*	0.74**	22.04**	0.08*
R-line × cytoplasm	4	67.43**	1.91**	10.66**	0.14**
A/B-line × cytoplasm	22	8.22**	0.12**	0.81**	0.04**
R-line × A/B-line × cytoplasm	44	4.06**	0.19**	1.22**	0.07**
Residual	144	1.21	0.34	0.29	0.20

*Significant at $P = 0.05$; **Significant at $P = 0.01$.

Table 2. Estimates of general combining ability (*gca*) effects of isonuclear, alloplasmic (A_1 and A_2) sorghum male-sterile lines and their respective maintainer (B-) lines for agronomic traits, ICRISAT-Patancheru, Andhra Pradesh, India, rainy season, 2005.

	Days to 50% flowering			Plant height (m)			Grain yield (t ha^{-1})			100-grain weight (g)		
Parent	A_1	A_2	B	A_1	A_2	B	A_1	A_2	B	A_1	A_2	B
ICSA/B 11	–0.89*	–0.78	–2.89**ab	0.05	0.10	–0.01	–0.15a	0.03b	–1.07**	0.11*	0.19**	0.28**a
ICSA/B 17	0.00	–0.11	0.00	–0.05	0.03	–0.05	0.07	0.42*b	–0.44*	0.06	0.03	0.13**
ICSA/B 26	0.22	0.44	–0.89*b	–0.10	–0.07	–0.14*	0.26a	0.39*b	–1.09**	–0.04	–0.03	0.00
ICSA/B 37	0.67	0.56	4.78**ab	0.05a	–0.10	–0.15*	–0.24	0.80**b	–0.31	–0.02	0.04	0.02
ICSA/B 38	1.11**	1.33**	2.22**	0.07a	0.21**b	–0.20**	0.63**	0.97**b	0.11	0.01	0.11*	0.00
ICSA/B 42	0.56	–0.56	–0.22	0.14*a	–0.14*	–0.25**	0.09	0.53**	0.00	0.08	0.00	0.15**b
ICSA/B 88001	–0.78	–0.78	–1.89**	0.07	0.15*	0.07	0.64**a	0.28b	–0.81**	–0.16**	–0.20**	–0.00ab
ICSA/B 88004	1.0*	1.33**	0.78	0.22**	0.22**	0.07	0.09	0.14	0.13	–0.05	0.09	0.00
ICSA/B 88005	0.00	–0.56	–1.0*	–0.02	–0.18**	–0.06	0.52**a	0.11b	–0.54**	–0.04	–0.02	–0.05
IS 18757 A/B	0.22	–0.11	–0.56	0.10	0.13*	0.10	–0.15a	0.31b	–0.71**	–0.01	0.21**b	0.01
PM 17467A/B	–0.67	–1.0*	–2.44**b	–0.02	0.02	–0.16*	0.22	0.06b	–0.77**	–0.20**	–0.25**	–0.19**
PM 7061A/B	0.78	0.44	–0.33	0.30**a	–0.08b	–0.32**	–0.18	0.25b	–0.58**	–0.06	–0.15**	–0.02
CD (g_i) ($P = 0.05$)		0.81			0.13			0.39			0.10	
CD (g_i) ($P = 0.01$)		1.06			0.17			0.51			0.13	
CD (A_1/A_2 & B) ($P = 0.05$)		1.14			0.18			0.55			0.14	

*Significant at $P = 0.05$; **Significant at $P = 0.01$.
a = Significant difference between A_1 and B cytoplasms.
b = Significant difference between A_2 and B cytoplasms.
ab = Significant difference between A_1, A_2 and B cytoplasms.

Table 3. Mean performance of isonuclear, alloplasmic (A_1, A_2 and B) sorghum hybrids for agronomic traits, ICRISAT-Patancheru, Andhra Pradesh, India, rainy season, 2005.

	Days to 50% flowering			Plant height (m)			Grain yield (t ha^{-1})			100-grain weight (g)		
Hybrids	$A_1 \times R$	$A_2 \times R$	$B \times R$	$A_1 \times R$	$A_2 \times R$	$B \times R$	$A_1 \times R$	$A_2 \times R$	$B \times R$	$A_1 \times R$	$A_2 \times R$	$B \times R$
ICSA/B 17 × ICSR 92003	67[a]	68	70	3.2[a]	3.2[b]	2.3	2.2	2.9[b]	2.3	3.0	2.6	2.8
ICSA/B 88001 × ICSR 92003	66	66	67	3.3[a]	3.2[b]	2.4	3.0[a]	2.4[b]	1.9	2.8[a]	2.8[a]	2.5
PM 17467A/B × ICSR 92003	65[a]	67	67	2.3	2.5	2.4	2.8[a]	1.7	2.3[b]	2.6	2.5	2.6
ICSA/B 11 × ICSR 93001	69	70	64[ab]	2.3	2.2	2.3	3.5[a]	3.5[b]	1.1	2.7	2.8	3.2[ab]
ICSA/B 17 × ICSR 93001	70	69	67[ab]	1.9	2.2	2.3[a]	2.9	3.6[b]	2.8	2.6	2.7	2.8
ICSA/B 26 × ICSR 93001	72	73	66[ab]	2.3	2.2	2.1	4.6[a]	4.8[b]	1.0	2.8	2.7	2.7
ICSA/B 37 × ICSR 93001	70[a]	70[b]	73	2.3	2.1	2.2	3.3[a]	4.5[b]	2.3	2.6	2.4	2.5
ICSA/B 38 × ICSR 93001	68	69	69	2.3	2.3	2.1	4.4[a]	4.5[b]	3.7	2.7	2.8[a]	2.5
ICSA/B 42 × ICSR 93001	68	67	68	2.2	2.2	2.1	3.5[a]	3.1[b]	2.8	2.8	2.6	2.8
ICSA/B 88001 × ICSR 93001	67	67	65[b]	2.4	2.4	2.4	2.7[a]	2.4[b]	2.1	2.5	2.4	2.8[ab]
ICSA/B 88004 × ICSR 93001	72	73	67[ab]	2.2	2.3	2.3	4.0[a]	3.6[b]	2.1	2.5	2.8	2.6
ICSA/B 88005 × ICSR 93001	68	67	65[ab]	2.3	2.3	2.4	3.2[a]	3.4[b]	2.3	2.5	2.4	2.6[b]
IS 18757A/B × ICSR 93001	71	69	66[ab]	2.3[a]	2.3[b]	1.9	3.1[a]	3.5[b]	1.9	2.6	2.9[a]	2.4
PM 17467A/B × ICSR 93001	70	68	65[ab]	2.5	2.5	2.3	3.0[a]	3.4[b]	1.6	2.5	2.4	2.5
PM 7061A/B × ICSR 93001	69	69	66[ab]	2.4[a]	2.3	2.0	2.6[a]	2.5[b]	1.6	2.5	2.3	2.8[ab]
ICSA/B 11 × ICSR 93031	67	67	65[ab]	2.9	3.1	3.0	1.7[a]	2.1[b]	1.3	3.0	3.0	2.9
ICSA/B 17 × ICSR 93031	67	66	67	2.6	2.5	3.1[ab]	2.9[a]	2.6[b]	1.5	2.8	3.0	3.0
ICSA/B 26 × ICSR 93031	68	67	67	3.1	3.2	3.0	2.0[a]	2.3[b]	1.4	2.6	2.7	2.7
ICSA/B 37 × ICSR 93031	68[a]	67[b]	72	2.5	2.4	2.9[ab]	1.6	3.6[b]	1.7	2.7	2.8	2.9
ICSA/B 38 × ICSR 93031	70	70	70	2.4	2.7	2.8[ab]	3.0[a]	3.8[b]	1.8	2.6	2.7	2.9[ab]
ICSA/B 42 × ICSR 93031	68	69	67	2.6	2.2	2.9	3.0[a]	4.8[b]	1.6	2.8	2.5	3.0[ab]
ICSA/B 88001 × ICSR 93031	68	68	66[ab]	2.4	2.7	3.2[ab]	4.0[a]	3.8[b]	1.4	2.5	2.5	2.9[ab]
ICSA/B 88005 × ICSR 93031	69	68	66[ab]	2.4	2.0	2.9[ab]	3.7[a]	2.0[b]	1.3	2.6	2.9	2.9[a]
IS 18757A/B × ICSR 93031	67	69	68	3.3	3.4	3.6	1.5	3.0[b]	1.3	2.9	3.0	3.0
PM 17467A/B × ICSR 93031	66	66	64[ab]	3.0	2.8	2.7	2.6[a]	2.8[b]	1.6	2.5	2.6	2.6
PM 7061A/B × ICSR 93031	68	68	67	3.2[a]	3.2	2.9	2.4[a]	3.2[b]	1.4	2.9	2.9	2.8
Mean	68	68	68	2.7[a]	2.6[b]	2.5	2.8[a]	3.0[b]	2.1	2.7[a]	2.7	2.8
LSD (between overall mean of A_1/A_2 & B hybrids) ($P = 0.05$)	0.30			0.05			0.15			0.04		
LSD (between cytoplasms at same levels of A- and R-lines) ($P = 0.05$)	1.78			0.30			0.31			0.23		
CV (%)	1.60			7.10			20.70			5.20		

a = Significant difference between A_1 and B cytoplasms.
b = Significant difference between A_2 and B cytoplasms.
ab = Significant difference between A_1/A_2 and B cytoplasms.

the corresponding nuclear restorer genes (Frei et al. 2004). Systematic investigation is necessary to identify the cytoplasmic factors that contribute to grain yield by manipulating the mitochondrial genome.

Conclusions

Male-sterility inducing cytoplasms (A_1 and A_2) do have a significant influence on agronomic traits including grain yield, but only in some nuclear genetic backgrounds. The CMS-based A-lines and hybrids were significantly better than those based on their male-fertile counterparts for grain yield in terms of their *gca* effects and mean performance, respectively. However, it is to be noted that these results are based on limited data and need confirmation by multi-year and/or multilocation evaluation.

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