Genetic Enhancement and Breeding

Effects of Cytoplasmic-nuclear Male-sterility Systems on Sorghum Grain Mold Development

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Introduction

Hybrid cultivar development in sorghum [*Sorghum bicolor* (L.) Moench] became possible with the discovery of cytoplasmic-nuclear male-sterility (CMS) designated as A_1 (*milo*) (Stephens and Holland 1954). Since then large numbers of CMS-based hybrids have been developed and commercialized in countries having a well-developed seed industry, including India and China. Commercial hybrids worldwide are currently based on the A_1 CMS system. However, hybrids based on a single CMS system with narrow nuclear genetic diversity of both male-sterile (A-) lines and restorer (R-) lines often become vulnerable to insect pests and diseases as was evident from the outbreak of southern corn leaf blight on hybrids based on a Texas cytoplasm in 1970 (Tatum 1971). It has been shown that the A_2 CMS system is a good alternative to the A_1 system in terms of the agronomic performance of hybrids (Moran and Rooney 2003; Reddy et al. 2005). However, commercial utilization of non-*milo* CMS systems depends on several factors including their effects on agronomic traits, and their responses to major diseases and insect pests. In the present study, the effects of A_2 cytoplasm on grain mold development are assessed in comparison to A_1 and their implications for diversification of CMS-based hybrid parents and their hybrids are discussed.

Materials and Methods

The experiment was conducted with two sets of diverse isonuclear, alloplasmic A-lines each in six nuclear genetic backgrounds with A_1 and A_2 CMS systems. Set I consisted of ICSA 17, -37, -38, -42, -88001 and -88005; and Set II of ICSA 11, -26, -88004, -18757, PM 17467A and PM 7061A. Each of the six A-lines was crossed with three R-lines (ICSR 93001, -92003 and -93031) to generate 36 hybrids in each set. These hybrids were screened for grain mold reaction under field conditions during the rainy season of 2004 at ICRISAT, Patancheru, Andhra Pradesh, India. Sprinkler irrigation was used to provide high humidity during the flowering to grain maturity stages. The experiment used a completely randomized block design with two replications. Each entry was sown in two rows of 4 m with a spacing of 75 cm between rows and 10 cm between plants within a row. The hybrids and their parents were scored for grain mold severity (panicle grain mold rating, PGMR) at physiological maturity on 10 tagged panicles in each plot using a 1–9 scale, where 1 = no mold, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75%, 9 = >75% grains colonized by grain mold fungi. The threshed grain mold rating (TGMR) was also taken on bulked grains from the same 10 tagged panicles per plot using the same 1–9 scale.

Statistical analysis. The computed mean PGMR and TGMR scores were used for analysis of variance (ANOVA) and for estimation of the general combining ability (*gca*) of the parents, and the specific combining ability (*sca*) and mid-parent heterosis of the crosses (Kempthorne 1957). The cytoplasmic differences for *gca* of A-lines and *per se* responses and *sca* effects of hybrids for PGMR and TGMR were tested for critical difference (CD). The difference between A_1- and A_2-based hybrids for mid-parent heterosis was tested using the paired t-test.

Results and Discussion

Variance components. The significant mean squares due to A-lines in both sets — except for PGMR in Set I — indicated substantial variability for responses to grain mold infection (ANOVA not presented). The nonsignificant mean squares due to A-lines × cytoplasm and R-lines × cytoplasm interactions for PGMR and TGMR indicated that the absence of cytoplasmic effects on grain mold infection is irrespective of nuclear genetic backgrounds in A-lines and their hybrids in both sets.

Cytoplasm effects on *gca* effects. The assessment of the *gca* effects of hybrid parents is important in order to

Table 1. Estimates of general combining ability (*gca*) of sorghum isonuclear alloplasmic (A_1 and A_2) A-lines (Sets I and II) for panicle grain mold rating (PGMR) and threshed grain mold rating (TGMR), ICRISAT-Patancheru, India, rainy season, 2004.

	PGMR[1]		TGMR[1]	
Parent	A_1	A_2	A_1	A_2
Set I				
ICSA 17	–0.12	–0.09	0.33	–0.33
ICSA 37	–0.39	–0.54	–0.83	–0.67
ICSA 38	0.35	–0.85	0.33	–0.50
ICSA 42	–0.19	0.78	0.00	1.00
ICSA 88001	0.55	0.25	0.67	0.17
ICSA 88005	0.25	0.01	0.00	–0.17
CD (g_i) ($P = 0.05$)	1.42		1.32	
CD (A_1–A_2) ($P = 0.05$)	2.01		1.86	
Set II				
ICSA 11	–0.59	–0.74*	–0.46	–0.96
ICSA 26	1.14**	0.93**	1.04	0.88
ICSA 88004	–1.09**	–1.04**	–1.46*	–1.29*
ICSA 18757	–1.94**	–1.94**	–2.46**	–2.46**
PM 17467A	2.39**	2.34**	2.88**	2.38**
PM 7061A	0.31	0.21	1.04	0.88
CD (g_i) ($P = 0.05$)	0.62		1.10	
CD (A_1–A_2) ($P = 0.05$)	0.88		1.56	

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

1. Mean of two replications, 10 panicles per replication, based on a 1–9 scale, where 1 = no mold, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75%, 9 = >75% molded grain.

judge their suitability for developing hybrids because the mean performance of parental lines need not always be a good indicator of their *gca* effects. In the present study, none of the A-lines in Set I, irrespective of its CMS background, showed significant *gca* effects for PGMR and TGMR (Table 1). In Set II, although most of the A-lines in both the CMS backgrounds showed significant *gca* effects for PGMR and TGMR, the differences between A_1- and A_2-based A-lines were not significant. Thus, it appears that the *gca* effects of both A_1- and A_2-based A-lines in both sets were comparable for responses to grain mold infection.

Effects of cytoplasm on grain mold reaction in hybrids. Cytoplasmic effects were not significant when grain mold scores were averaged over the hybrids in both sets (Tables 2 and 3). These results are in congruence with those reported by Stack and Pedersen (2003). Although differences (statistically nonsignificant) between A_1- and A_2-based hybrids were observed in a few nuclear genetic backgrounds, there were no definite trends favoring any of the CMS systems. For example, in Set I, while A_1-based hybrids in two genetic backgrounds, ICSA 88001 × ICSR 93001 and ICSA 42 × ICSR 92003, showed higher grain mold resistance (GMR) — as is evident from their PGMR scores — than the respective hybrids based on the A_2 CMS system, A_2-based hybrids in two genetic backgrounds, ICSA 88001 × ICSR 92003 and ICSA 38 × ICSR 92003, showed higher GMR than the respective hybrids based on the A_1 CMS system (Table 2). Similar nuclear genotype-dependent CMS effects were observed in Set II (Table 3). Stack and Pedersen (2003) too reported nuclear genotype-dependent CMS (A_1 and A_2) effects on GMR. Such CMS effects on GMR could be attributed to the interaction of the cytoplasm with the nuclear genes of the R-lines in these hybrids. However, the distinction between cytoplasm effects and cytoplasmic-nuclear interactions is complicated. This is not surprising considering that the very differentiation of CMS types is primarily based on the interaction of genes present in mitochondrial DNA and the corresponding nuclear restorer genes (Mackenzie 2005).

Cytoplasmic influence on *sca* effects and heterosis. Estimates of the *sca* effects of both A_1- and A_2-based hybrids were comparable, and cytoplasmic effects were absent in all the nuclear genetic backgrounds in both sets. There was no apparent difference between the A_1- and A_2-based hybrids for mid-parent heterosis as was revealed by the paired 't' test. While cytoplasmic differences in the estimates of mid-parent heterosis were noticed in some of the nuclear genetic backgrounds in both sets (Tables 2 and 3), there were no definite trends in favor of any cytoplasm.

Table 2. Male-sterility-induced cytoplasmic effects on specific combining ability (*sca*) and mid-parent heterosis for panicle grain mold rating (PGMR) and threshed grain mold rating (TGMR) in sorghum isonuclear hybrids in A_1 and A_2 CMS backgrounds (Set I), ICRISAT-Patancheru, India, rainy season 2004.

	PGMR[1]		*sca* effects		Mid-parent heterosis		TGMR[1]		*sca* effects		Mid-parent heterosis	
Isonuclear hybrids	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2
ICSA 17 × ICSR 93001	3.6	3.9	–0.14	0.08	–9.43	–3.14	5.0	4.0	0.33	0.00	–13.0	–30.4
ICSA 37 × ICSR 93001	3.2	3.2	–0.28	–0.18	–24.26	–25.44	3.5	3.5	0.00	–0.17	–30.0	–30.0
ICSA 38 × ICSR 93001	3.1	2.9	–1.16	–0.16	–23.27	–28.30	3.5	3.5	–1.17	-0.33	–30.0	–30.0
ICSA 42 × ICSR 93001	4.7	5.1	1.03	0.41	–2.08	5.21	5.0	5.5	0.67	0.17	–13.0	–4.3
ICSA 88001 × ICSR 93001	3.4	4.2	–1.01	0.09	–31.66	–15.58	4.5	4.5	–0.50	0.00	–28.0	–28.0
ICSA 88005 × ICSR 93001	4.8	4.5	0.69	0.63	21.52	13.92	5.0	4.5	0.67	0.33	0.0	–10.0
ICSA 17 × ICSR 92003	3.9	3.3	0.21	–0.43	–12.50	–26.14	4.5	3.5	0.00	–0.33	–30.8	–46.2**
ICSA 37 × ICSR 92003	2.8	2.7	–0.63	–0.53	–40.86	–41.94	2.5	3.0	–0.83	–0.50	–56.5**	–47.8**
ICSA 38 × ICSR 92003	5.4	3.0	1.24	0.09	21.59	–31.82	6.0	3.5	1.50	–0.17	4.3	–39.1
ICSA 42 × ICSR 92003	2.9	5.1	–0.73	0.56	–45.45	–2.39	3.5	6.0	–0.67	0.83	–46.2**	–7.7
ICSA 88001 × ICSR 92003	5.9	3.5	1.54	–0.51	8.33	–35.19	6.0	4.5	1.17	0.17	–14.3	–35.7
ICSA 88005 × ICSR 92003	3.7	3.3	–0.36	–0.48	–16.57	–24.57	3.5	3.5	–0.67	–0.50	–39.1*	–39.1*
ICSA 17 × ICSR 93031	8.2	8.6	–0.07	0.35	67.18	76.41	9.0	9.0	–0.33	0.33	16.1	16.1
ICSA 37 × ICSR 93031	8.9	8.5	0.90	0.70	72.68	65.85	9.0	9.0	0.83	0.67	28.6	28.6
ICSA 38 × ICSR 93031	8.6	7.6	–0.08	0.07	76.41	54.87	9.0	9.0	–0.33	0.50	28.6	28.6
ICSA 42 × ICSR 93031	7.9	8.2	–0.30	–0.97	37.72	42.98	9.0	9.0	0.00	–1.00	16.1	16.1
ICSA 88001 × ICSR 93031	8.4	9.0	–0.53	0.42	42.13	53.19	9.0	9.0	–0.67	0.17	9.1	9.1
ICSA 88005 × ICSR 93031	8.3	8.2	–0.33	–0.15	70.10	69.07	9.0	9.0	0.00	0.17	28.6	28.6
Mean	5.4	5.2	–	–	–	–	5.9	5.8	–	–	–	–
CD (within cytoplasm)/ CD (S_{ij}) ($P = 0.05$)	0.61		2.46		–		0.73		2.28		–	–
CD (A_1–A_2) (at same levels of nuclear genotype and R-lines)/ CD (S_{ij}–S_{kj}) ($P = 0.05$)	2.80		3.48		–		2.72		3.23		–	–
Paired 't' test probability	–		–		0.5		–		–		–	0.5

*Significantly different from zero at $P = 0.05$. **Significantly different from zero at $P = 0.01$.

1. Mean of two replications, 10 panicles per replication, based on a 1–9 scale, where 1 = no mold, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75%, 9 = >75% molded grain.

Table 3. Male-sterility-induced cytoplasmic effects on specific combining ability (*sca*) and mid-parent heterosis for panicle grain mold rating (PGMR) and threshed grain mold rating (TGMR) in sorghum isonuclear hybrids in A_1 and A_2 CMS backgrounds (Set II), ICRISAT-Patancheru, India, rainy season 2004.

	PGMR[1]		*sca* effects		Mid-parent heterosis		TGMR[1]		*sca* effects		Mid-parent heterosis	
Isonuclear hybrids	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2
ICSA 11 × ICSR 93001	3.6	4.1	–0.44	0.21	–13.25	–1.20	4.5	4.0	–0.25	–0.25	–28.00	–36.00
ICSA 26 × ICSR 93001	7.2	6.5	1.38*	0.89	66.28**	50.00**	7.5	7.0	1.25	0.92	20.00	12.00
ICSA 88004 × ICSR 93001	2.7	3.1	–0.89	–0.54	–26.90*	–15.86	3.5	3.0	–0.25	–0.92	–44.00**	–52.00**
ICSA 18757 × ICSR 93001	2.0	2.0	–0.69	–0.69	–37.98**	–37.98**	2.0	2.0	–0.75	–0.75	–60.00**	–60.00**
PM 17467A × ICSR 93001	7.9	8.3	0.93	1.33*	55.12**	61.95**	9.0	9.0	0.92	1.42	24.14	24.14
PM 7061A × ICSR 93001	4.5	3.8	–0.44	–1.04	–6.25	–20.83*	6.0	5.0	–0.25	–1.08	–17.24	–31.03
ICSA 11 × ICSR 92003	3.5	2.7	–0.16	–0.81	–29.29**	–45.45**	3.5	3.0	–0.71	–0.71	–39.13*	–47.83**
ICSA 26 × ICSR 92003	4.7	4.2	–0.69	–1.02	–7.84	–18.63*	5.0	4.5	–0.71	–1.04	–13.04	–21.74
ICSA 88004 × ICSR 92003	3.4	3.0	0.24	–0.21	–23.16*	–32.20**	3.0	3.0	–0.21	–0.38	–47.83**	–47.83**
ICSA 18757 × ICSR 92003	2.0	2.0	–0.31	–0.31	–50.31**	–50.31**	2.0	2.0	–0.21	–0.21	–55.56**	–55.56*
PM A 17467 × ICSR 92003	8.4	8.2	1.76**	1.66**	41.77**	39.24**	9.0	9.0	1.46	1.96*	33.33	33.33
PM 7061A × ICSR 92003	4.1	4.8	–0.46	0.29	–26.79**	–15.18*	6.0	6.0	0.29	0.46	–11.11	–11.11
ICSA 11 × ICSR 93031	3.0	2.8	0.60	0.60	–47.79**	–50.44**	4.0	3.5	0.96	0.96	–42.86**	–50.00**
ICSA 26 × ICSR 93031	3.4	4.0	–0.69	0.13	–41.38**	–31.03**	4.0	4.5	–0.54	0.13	–42.86**	–35.71
ICSA 88004 × ICSR 93031	2.5	2.7	0.65	0.75	–51.22**	–48.29**	2.5	3.5	0.46	1.29	–64.29**	–50.00**
ICSA 18757 × ICSR 93031	2.0	2.0	1.00	1.00	–57.67**	–57.67**	2.0	2.0	0.96	0.96	–65.22**	–65.22**
PM 17467A × ICSR 93031	2.7	2.3	–2.69**	–2.99**	–60.00**	–65.28**	4.0	2.5	–2.38*	–3.38**	–50.00**	–68.75**
PM 7061A × ICSR 93031	4.2	3.9	0.90	0.75	–34.13**	–38.10**	4.5	5.0	–0.04	0.63	–43.75**	–37.50*
Mean	4.0	3.9	–	–	–	–	4.6	4.4	–	–	–	–
CD (within cytoplasm)/ CD (S_{ij}) ($P = 0.05$)	0.15		1.07		–		0.18		1.91		–	
CD (A_1–A_2) (at same levels of nuclear genotype and R-lines)/CD (S_{ij}–S_{kj}) ($P = 0.05$)	1.31		1.52		–		1.68		2.7		–	
Paired 't' test probability	–		–		0.5		–		–		0.1	

*Significantly different from zero at $P = 0.05$. **Significantly different from zero at $P = 0.01$.

1. Mean of two replications, 10 panicles per replication, based on a 1–9 scale, where 1 = no mold, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75%, 9 = >75% molded grain.

Conclusions. By and large, cytoplasms did not show significant influence on *gca* of A-lines and the mean performance of hybrids, and *sca* for grain mold infection. Though cytoplasmic effects on mid-parent heterosis were observed in some of the nuclear genetic backgrounds, there were no definite trends in favor of any cytoplasm. Considering the comparable performance of A_1- and A_2-based hybrids for agronomic traits and for reaction to grain mold, it appears that the A_2 system offers an immediate option for the much-needed CMS diversification for breeding hybrids. Although the present results are based on a good number of appropriate genetic materials with a wide spectrum of genetic variability for agronomic traits, it is necessary to repeat the experiment to validate the findings, given that plant responses to grain mold infection and development depend on several weather variables during the grain-filling and maturity stages (Thakur et al. 2003).

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