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Male-sterility inducing cytoplasmic effects on combining ability in sorghum [*Sorghum bicolor* (L.) Moench]

Belum V. S. Reddy, S. Ramesh, P. Sanjana Reddy and A. Ashok Kumar

Global Theme on Crop Improvement, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324

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Abstract

An investigation was carried out to assess the efficiency of A_2 CMS system in comparison to the widely used A_1 cytoplasm in sorghum for use in hybrid breeding programs at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India during 2002 and 2003 post rainy seasons. Significant cytoplasm effects on *gca* effects of A-lines and on mean performance and *sca* effects of hybrids were detected in only some of the nuclear genetic backgrounds and where detected, the magnitude of effects varied with the trait and was minimal to have any practical significance for any of the traits. The mean days to 50% flowering, plant height and grain yield of A_2 cytoplasm-based hybrids were comparable with those of widely used A_1 cytoplasm-based hybrids. The implications of these results are discussed in relation to opportunities for broadening cytoplasm as well as nuclear genetic base of sorghum hybrid parents.

Key words: Sorghum, cytoplasmic-nuclear male-sterility, iso-nuclear, alloplasmic and combining ability

Introduction

Hybrid cultivar development in sorghum has been possible due to the discovery of workable cytoplasmic-nuclear male-sterility (CMS) designated as A_1 (*milo*) [1]. A large number of CMS-based hybrids have been developed and released/marketed in India and several other countries, and they are based on the single cytoplasm (A_1) except one A_2-based hybrid released in China in 1990's [2]. It is very likely that cytoplasm uniformity of the hybrids might lead to unforeseen out-break of pests and/or diseases as evidenced from devastation of Texas (T) cytoplasm-based corn hybrids due to the outbreak of southern leaf blight disease in 1970 [3]. Cytoplasmic uniformity also limits the scope for diversifying nuclear genetic base of hybrid parents. Therefore, to prevent such eventualities and to broaden the genetic base, the need for CMS diversification of sorghum hybrids was felt long back and as a result, several non-*milo* CMS systems (A_2, A_3 and A_4) were identified and developed [4] for use in hybrid breeding programs. However, utilization of these non-*milo* CMS systems at commercial level depends on factors such as stability of male sterility, maintainer/restorer gene frequency in the germplasm, and the absence of undesirable effects on agronomic traits [5]. The A_2 CMS system discovered by Schertz [4] is stable and sufficient numbers of maintainers/restorers are available on this cytoplasm [5]. In the present study, the effects of A_2 cytoplasm in comparison to A_1 on *gca* effects of A-lines and mean performance and *sca* effects of hybrids in iso-nuclear and allo-plasmic backgrounds were assessed for agronomic traits.

Materials and methods

The materials for the study consisted of six iso-nuclear allo-plasmic (A_1 and A_2) A-lines in six different nuclear genetic backgrounds-ICSA 11, ICSA 26, ICSA 88004, ISA 18757, PMA 17467 and PMA 7061 and three dual R-lines ICSR 92003, ICSR 93001 and ICSR 93031. These were developed at ICRISAT, Patancheru, India in a program to diversify the CMS and nuclear genetic bases of hybrid parents (Reddy *et al.* 2005). The seed parents were crossed with the three dual R-lines in a line × tester mating design to produce 18 hybrid combinations in each of A_1 and A_2 CMS backgrounds. These hybrid combinations were evaluated in a split-split-plot design in three replications with R-lines in main plots, nuclear genetic backgrounds of A-lines in sub

Correspondence author's, e-mail: b.reddy@cgiar.org

plots and cytoplasms in sub-sub plots during 2002 and 2003 post-rainy seasons. Each entry was planted in two rows of four meter length with a spacing of 75 cm between rows and 10 cm between plants within a row at experimental fields of ICRISAT, Patancheru, India. The recommended agronomic practices with protective irrigation were followed to raise a healthy crop. In both the experiments, the data on days to 50% flowering, plant height (m), and seed set (%) upon selfing and open pollination, grain size (g 100^{-1} grains) were recorded on five randomly selected plants in each entry and in each replication, while the data on grain yield were taken on whole-plot basis.

Statistical analyses

The computed average values for days to 50% flowering, plant height and grain yield in both hybrid and parental trials were subjected to statistical analyses. The data on seed set (%) upon selfing and open pollination were not statistically analyzed as all the hybrids irrespective of cytoplasm background showed over 90% seed set and practically there was no variation in %seed set among the hybrids. The combined analysis of variance (CANOVA) [6] was carried out for days to 50% flowering, plant height, grain size and grain yield considering cytoplasms, nuclear genetic backgrounds of A-lines and R-lines as fixed factors and year as a random factor following split-split-plot design analysis. The error variances in the trials conducted in two years were homogeneous, as revealed by Bartlett's test [7], providing statistical validity to carry out CANOVA.

Though significant, the mean squares due to year x A-line, year x R-line and year x A-line x R-line interactions were of non-cross over type [8]. This is evident from significant and positive rank correlation coefficients between *gca* effects of A-lines and *sca* effects of hybrids estimated in two years. Therefore, the *gca* effects of A-lines and *sca* effects [9] of hybrids were estimated based on the combined data (over the two years) on hybrid performances. Cytoplasm differences for *gca* effects of iso-nuclear A-lines and *sca* effects of iso-nuclear hybrids were tested using critical difference (CD) calculated based on the standard error of mean for cytoplasm factor from the year-wise analysis of variance.

Results and discussion

Variances components

It follows from the CANOVA (Table 1) that both A-lines (nuclear genotype) and R-lines are significantly different from each other for all the traits, justifying the selection of the hybrid parents (A- and R-lines) for the study. The significance of mean squares due to A- and R-lines also indicates that they significantly differ for their gca effects. Similarly, significant mean squares due to A- × R-lines interaction indicate that hybrids differ significantly for their sca effects for all the traits except plant height. Cytoplasm *per se* appeared to have significant influence on the expression of hybrids for only two traits-grain size and grain yield, as evident from significant mean squares due to cytoplasm. In practice, the expression of A-line is determined by the interaction of cytoplasm with nuclear genotype of A-lines and that of hybrids by interaction of A-lines and R-lines. In this context, it is important to note that first-order interaction of cytoplasm with nuclear genetic background of A-lines or R-lines and second-order interaction with A-line and R-lines towards variation of iso-nuclear hybrids was significant only for grain yield, suggesting significant overall influence of cytoplasm on grain yield. Temporal variation (years), which predominantly stem from seasonal variation caused by year-to-year dynamic changes in weather variables [8] had significant influence on the expression of hybrids for all the traits. Seasonal changes over the years appeared to cause significant variation in *gca* effects of A-lines or R-lines and *sca* effects of hybrids from year-to-year for all the traits except days to 50% flowering as indicated from significant mean squares due to year x A-line, year x R-line and year x A-line x R-line interactions. However, the significant and positive rank correlation between *gca* effects of A-lines and *sca* effects of hybrids estimated in two years suggested that year x A-line, year x R-line and year x A-line x R-line interactions were of non-cross over type [8] suggesting that relative ranking of the estimates of *gca* effects of A-lines and *sca* effects of hybrids did not vary over the years for plant height, grain size and grain yield (Table 1).

Cytoplasm effects on gca effects

Although both A_1- and A_2-based A-lines manifested significant *gca* effects in a few of the nuclear genetic backgrounds, cytoplasm did not appear to cause any noticeable influence on *gca* effects for days to 50% flowering, plant height and grain size (Table 2). On the other hand, significant cytoplasm influence on the estimates of *gca* effects of A-lines for grain yield was detected in three of the six nuclear genetic back grounds. However, the magnitude of the cytoplasm differences was small enough to have any practical significance. Thus, the study clearly showed that cytoplasm had limited influence on *gca* effects for any

of the traits investigated. The earlier reports [10] on the absence of cytoplasms (A_1 and A_2) influence on *gca* effects for grain yield lends support to the present findings.

Cytoplasm effects on hybrid mean performance

The results clearly revealed the absence of significant cytoplasm differences in hybrid mean performance for any of the traits under investigation, when average performance of A_1- and A_2-based hybrids as separate groups were examined (Table 3). These results are in conformity with those reported by Moran and Rooney [11]. However, a perusal of pair-wise hybrids mean performances indicated significant cytoplasm differences for grain yield in four of the 18 nuclear genetic backgrounds, although no definite pattern of grain yield with a particular cytoplasm was observed. For example, the hybrids such as ICSA 11 x ICSR 93031, ICSA 18757 x ICSR 93031 and PMA 17647 x ICSR 93031 in A_1 CMS background were significantly superior to those in A_2 CMS background. On the other hand, one A_2 CMS-based hybrid, ICSA 88004 x ICSR 93031 was significantly superior to that in A_1 CMS background. The absence of cytoplasm differences between A_1, A_2 and A_3 [11] and between A_1 and A_3 [12] CMS systems on the basis of average hybrids mean performance, while significant cytoplasm differences on the basis of pair-wise comparison of individual hybrids for grain yield have also been reported by earlier researchers. Such differential trends in cytoplasm differences in some of the nuclear genetic backgrounds could be attributed to the interaction of cytoplasm with nuclear genes of A-lines and of R-lines in these hybrids. However, the distinction between cytoplasm effects and cytoplasm-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 on mitochondrial DNA and the corresponding nuclear restorer genes.

Cytoplasm effects on sca *effects*

Although, studies on the effects of cytoplasm on hybrid

Table 1. CANOVA of iso-nuclear allo-plasmic male-sterile lines, their common restorers and hybrids in sorghum during 2002 and 2003 post-rainy seasons at ICRISAT, Patancheru

Source	df	Mean sum of squares			
		Days to 50% flowering	Plant height (m)	Seed size (g 100^{-1})	Grain yield (t ha^{-1})
Year	1	361.7*	19221.0**	13.0**	265.5*
Residual	4	32.8	1086.7	0.1	2.6
R-line	2	656.8**	73162.2**	4.0**	2.4*
Year x R-line	2	4.1	1199.8*	0.1	2.3*
Residual	8	6.8	174.8	0.1	0.3
A-line	5	55.2**	3673.1**	2.6**	17.2**
Year x A-line	5	4.7	741.1*	0.3**	4.4**
R-line x A-line	10	9.2**	253.6	0.3**	2.7**
Year x R-line x A-line	10	5.9	326.5	0.1*	1.7**
Residual	60	3.1	272.0	0.03	0.5
Cytoplasm	1	0.6	100.8	0.2*	1.5*
Year x Cytoplasm	1	0.5	250.1	0.12	1.3*
R-line x Cytoplasm	2	1.4	17.3	0.01	2.8**
A-line x Cytoplasm	5	1.1	310.1	0.01	1.1**
Year x R-line x Cytoplasm	2	2.7	9.6	0.01	0.9*
Year x A-line x Cytoplasm	5	1.9	63.7	0.04	0.3
R-line x A-line x Cytoplasm	10	1.8	281.4	0.03	1.6**
Year x R-line x A-line x Cytoplasm	10	2.6	295.3	0.04	1.2**
Residual	72	2.0	164.3	0.04	0.3

*Significant at P = 0.05 level; **Significant at P = 0.01 level

Table 2. Estimates of mean *gca* effects of iso-nuclear sorghum male-sterile lines as influenced by their cytoplasm during 2002 and 2003 post-rainy seasons

A-lines	Days to 50% flowering		Plant height (m)		Grain size (g 100^{-1} grains)		Grain yield (t ha^{-1})	
	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2
ICSA 11	-2.38**	-2.15**	2.41	3.24	0.35**	0.29**	-0.02[a]	-0.51**
ICSA 26	0.51	0.62	-5.09	-3.15	-0.02	-0.1	0.03	-0.17
ICSA 88004	0.84*	0.84*	8.79**	10.74**	0.07	0.04	0.23	0.73**[a]
ISA 18757	-0.38	0.29	10.74**	2.41	0.16**	0.06	-0.95**[a]	-1.42**
PMA 17467	1.34**	1.01**	7.69*[a]	-1.76	-0.44**	-0.52**	0.44**	0.24
PMA 7061	-0.27	-0.27	-19.82**	-16.2**	0.05	0.04	0.77**	0.64**
SE (gi)	0.39		3.13		0.05		0.14	
CD (A_1 - A_2)	1.08		8.68		0.13		0.38	

a = Significant cytoplasm differences; *Significant at P=0.05 level; **Significant at P=0.01 level

mean performance for various traits are useful, *sca* effects of iso-nuclear hybrids would be more revealing since they reflect differential interaction of cytoplasms with nuclear genes of A-lines as well as R-lines and it is this interaction in higher magnitude and desired direction that results in superior hybrid performance. Specific combinations of A- and R- lines with good *sca* effects will remain the essential requirements for the production of superior hybrids [13]. In the present study, significant cytoplasm differences were detected only for grain yield in a few nuclear genetic backgrounds. However, the magnitudes of *sca* effects of hybrids within cytoplasm groups as well as magnitude of cytoplasm differences were small to have any practical significance (Table 3). Further, no definite trend in *sca* effects favoring any particular cytoplasm was noticed. For example, A_2-based hybrids in two nuclear genetic backgrounds (ICSA 11 x ICSR 93001 and ICSA 26 x ICSR 93001) were superior to those of A_1-based hybrids. On the contrary, A_1-based hybrids in two nuclear genetic backgrounds (ISA 18757 x ICSR 93001 and PMA 7061 x ICSR 92003) were superior to those of A_2-based hybrids. These results suggested limited influence of cytoplasm on *sca* effects for all the four traits under study. The absence of cytoplasm effects on *sca* effects for grain yield has also been reported earlier [10].

Conclusions

Significant cytoplasm effects on *gca* effects of A-lines and on mean performance and *sca* effects of hybrids were detected in only some of the nuclear genetic backgrounds and where detected, the magnitudes of effects varied with the trait and were minimal to have any practical significance for any of the traits. The results have also demonstrated that the use of *gca* effects of A-lines and hybrids mean performance and *sca* effects criteria are complementary for discerning cytoplasm differences. Therefore, it appears that A_2 CMS system is comparable to A_1 CMS system for harnessing heterosis in sorghum. The use of A_2 CMS system for commercial exploitation has also been advocated by Moran and Rooney [11]. It may, however, be noted that the line × tester analysis used in the study is based on fixed-effects model and the present results are applicable only to the material used in the study and some variation in the results might be observed if a different set of materials are evaluated.

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References

1. **Stephens J. C. and Holland P. F.** 1954. Cytoplasmic male sterility for hybrid sorghum seed production. Agron. J., **46**: 20–23.
2. **Liu Qing Shan, Ping Jun Ai, Li Tuan Yin and Zhang Fu Yao.** 2000. New grain sorghum cytoplasmic male-sterile line A_2 V_4 A and F_1 hybrid Jinza No.12 for Northwest China. International Sorghum and Millets Newsletter, **41**: 31–32.
3. **Tatum L. A.** 1971. The southern corn leaf blight epidemic. Science, **171**: 1113-1116.
4. **Schertz K. F.** 1994. Male-sterility in sorghum: Its characteristics and importance. *In:* Use of molecular markers in sorghum and pearl millet breeding for

Table 3. Estimates of mean performance and sca effects of iso-nuclear sorghum hybrids as influenced by male-sterility inducing cytoplasms

Hybrids	Days to 50% flowering				Plant height (m)				Grain size (g 100^{-1} grains)				Grain yield (t ha^{-1})			
	Mean		*sca* effects		Mean		*sca* effects		Mean		*sca* effects		Mean		*sca* effects	
	A_1	A_2	A_1	A_2	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	71.3	72.2	–1.2	–0.6	1.8	1.7	7.7	3.6	3.4	3.3	0.1	0.0	3.7	4.1	–0.2	0.6**[a]
ICSA 11 × ICSR 92003	73.3	74.3	0.0	–0.4	1.8	1.7	–5.6	–2.5	3.6	3.5	0.1	0.0	4.0	3.3	0.2	0.3
ICSA 11 × ICSR 93031	69.2	68.0	0.9	0.9	2.2	2.3	–0.3	1.9	3.7	3.7	0.0	–0.1	3.9[a]	2.9	0.1	-0.2
ICSA 26 × ICSR 93001	75.5	75.2	0.3	–0.9	1.6	1.6	1.9	–2.3	3.1	2.9	0.1	0.0	4.2	4.1	–0.8**	0.7**[a]
ICSA 26 × ICSR 92003	76.0	76.5	0.4	0.2	1.7	1.8	1.6	3.7	3.2	3.1	–0.1	0.1	3.7	3.3	–0.4	0.2
ICSA 26 × ICSR 93031	71.0	71.2	0.3	0.2	2.2	2.2	–7.5	–2.0	3.3	3.3	–0.2*	–0.1	4.0	3.9	–0.3	–0.3
ICSA 88004 × ICSR 93001	76.7	76.7	–0.1	0.7	1.8	1.8	–1.9	–8.5	3.1	3.0	0.1	0.1	4.3	4.4	0.0	–0.3
ICSA 88004 × ICSR 92003	77.2	77.2	–0.3	0.0	1.9	1.9	3.1	4.5	3.0	3.2	0.0	0.0	4.6	4.9	–0.4	–0.7**
ICSA 88004 × ICSR 93031	69.7	69.7	0.5	0.5	2.3	2.3	3.4	0.6	3.8	3.6	–0.3**	–0.1	3.6	4.7[a]	0.2	0.1
IS 18757 × ICSR 93001	74.8	74.3	–0.8	–0.6	1.8	1.7	–11.9*	7.3[a]	3.3	3.1	0.1	0.2*	2.4	3.2	1.5**[a]	0.1
IS 18757 × ICSR 93031	74.7	75.5	–0.3	–0.7	1.8	1.9	3.7	0.6	3.4	3.4	0.1	0.0	4.7[a]	2.8	–0.3	0.0
IS 18757 × ICSR 93031	70.3	72.0	0.3	0.9	2.5	2.2	2.0	–3.2	3.4	3.3	–0.1	–0.1	2.0	1.5	–0.1	–0.1
PM 17647 × ICSR 93001	76.7	76.2	1.35*	0.0	1.8	1.7	–5.9	5.0	2.5	2.5	–0.1	–0.1	4.0	4.4	0.2	–0.3
PM 17647 × ICSR 92003	76.8	76.2	0.3	0.4	1.9	1.8	2.5	–2.0	2.8	2.6	–0.1	–0.1	4.2	4.3	0.2	0.3
PM 17647 × ICSR 93031	71.5	71.7	–1.37*	–1.37*	2.3	2.2	–3.1	–2.5	3.0	2.9	0.3**	0.1	4.9[a]	3.7	–0.3	0.2
PM 7061 × ICSR 93001	75.0	74.8	0.5	1.52*	1.4	1.5	10.0	–5.0	2.9	2.9	–0.2**	–0.2**	4.3	4.2	–0.8**	–0.8**
PM 7061 × ICSR 92003	75.8	76.5	0.0	0.5	1.6	1.6	–5.3	–4.2	3.2	3.1	0.0	0.0	4.8	4.6	0.7**[a]	–0.2
PM 7061 × ICSR 93031	69.3	68.8	–0.6	–1.1	2.1	2.2	5.5	5.2	3.8	3.7	0.2**	0.2**	4.9	4.8	0.4	0.5*
Mean	74	74			1.9	1.9			3.25	3.18			3.92	3.79		
CD (Mean/S_{ij})	1.35		0.68		10.85		5.43		0.16		0.08		0.47		0.24	
CD (A_1-A_2) (P = 0.05)	2.65		1.87		21.27		15.04		0.31		0.22		0.92		0.65	

a = Significant cytoplasm differences; *= Significant at P=0.05; **P=0.01 level

developing countries. Witcombe J.R. and Duncan R.R. (eds.), Proc. Intl. Conf. Genet. Improvement. Overseas Development Administration (ODA) Plant Sciences Research Conference, 29 March–1 April 1993, Norwich, UK, ODA, UK, pp. 35–37.

5. **Reddy B.V. S., Ramesh S. and Ortiz R.** 2005. Genetic and Cytoplasmic-Nuclear Male Sterility in Sorghum. Plant Breeding Reviews, **25**: 139-172.
6. **Steel R. G. D. and Torrie J. H.** 1980. Principle and Procedures of statistics. 2nd edition, McGraw-Hill Co., New York.
7. **Bartlett M. S.** 1937. Some examples of statistical methods of research in agriculture and applied biology. J. Roy. Stat. Soc., **4**: 137-170.
8. **Annicchiarico P.** 2002. Genotype x environment interaction-challenges and opportunities for plant breeding and cultivar recommendations. FAO Plant Production and Protection Paper, pp. 115.
9. **Kempthorne O.** 1957. An Introduction to Genetic Statistics. John Wiley & Sons Inc., New York.
10. **Williams-Alanis H. and R. Rodriguez-Herrera.** 1994. Combining ability on iso-genic sorghums in A_1 and A_2 cytoplasms. International Sorghum and Millets Newsletter, **35**: 75.
11. **Moran J. L. and Rooney W. L.** 2003. Effect of cytoplasm on the agronomic performance of grain sorghum hybrids. Crop Sci., **43**: 777-781.
12. **Lee R. D., Johnson B. E., Eskridge K. M. and Pedersen J. F.** 1992. Selection of superior female parents in sorghum utilizing A_3 cytoplasm. Crop Sci., **32**: 918-921.
13. **Duvick D. N.** 1999. Heterosis: feeding people and protecting natural resources. *In:* Genetics and exploitation of heterosis in crops (Coors J.G. and Pandey S. eds.), ASACSSA-SSSA, Madison, WI, pp. 19-29.