Indian Journal of Agricultural Sciences **81** (10): 908–13, October 2011

Agronomic potential of $A_{4(M)}$ cytoplasmic male-sterility system compared to A_1 CMS system of sorghum (*Sorghum bicolor*)

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Received: 18 April 2010; Revised accepted: 20 July 2011

ABSTRACT

An investigation was carried out to compare the $A_{4(M)}$ CMS (cytoplasmic-nuclear male-sterility) system to the widely used A_1 CMS system in sorghum (*Sorghum bicolor* (L.) Moench) for agronomic traits and panicle grain mold resistance (PGMR) score at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India in 2006 and 2007 rainy and postrainy seasons. The cytoplasm *per se* and its first order interaction with A-line seemed to contribute to grain yield, male-fertility restoration % and PGMR during rainy season and male-fertility restoration per cent during postrainy season. The mean days to 50% flowering, plant height and grain yield of $A_{4(M)}$ cytoplasm-based hybrids were comparable with those of A_1 cytoplasm-based hybrids during 2006 and 2007 postrainy seasons while during 2006 rainy season, $A_{4(M)}$ cytoplasm based hybrids in few nuclear backgrounds were significantly superior to A_1 cytoplasm based hybrids for early flowering and grain yield, while in few nuclear backgrounds A_1 cytoplasm-based hybrids were superior. However, the A_1 cytoplasm based hybrids were more tolerant for grain mold. Hence the $A_{4(M)}$ cytoplasm can be used to incorporate genetic diversity in grain sorghum hybrids for grain yield in postrainy season, but its use in rainy season is not recommended, where grain mold poses a problem.

Key words: Allo-plasmic, Cytoplasmic-nuclear male-sterility, Grain mold Male-fertility restoration, Iso-nuclear, Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world (Bantilan *et al.* 2004) and India ranks first with 7.76 m ha under cultivation. However, its productivity (1.0 tonnes/ha) is 60% of the world's average productivity. Grain mold, one of the major constraints to rainy season sorghum production causes both qualitative and quantitative losses. Hybrids are the target materials in sorghum globally as hybrids yield 20 to 30% additional grain and stover over a range of environments compared to open-pollinated varieties. Hybrid sorghum (*Sorghum bicolor* (L.) Moench) seed production relies exclusively on CMS systems and almost all hybrid sorghum seed is produced using the *Milo* (A_1) CMS system. In addition to the A_1, several other cytoplasmic sources, like A_2, A_3, A_4, A_5, A_6, 9E and KS cytoplasms differing from each other and from the A_1 CMS system, were identified (Reddy *et al.* 2010). Apart from them several male-sterile lines have been identified separately in the regions of Maldandi, Guntur and Vizianagaram. They have been tentatively grouped as Indian A_4 types (Sane *et al*. 1996). Among the non-milo cytoplasms, A_2 among the exotics and $A_{4(M)}$ among the Indian sources can be used for practical exploitation in breeding programmes (Kishan and Borikar 1989). Several studies targeted comparison of A_1 and A_2 CMS systems (Moran and Rooney 2003, Reddy *et al.* 2007) and some A_1 with A_3 CMS system (Moran and Rooney 2003). However, the studies related to $A_{4(M)}$ CMS system are limited while utilizing isonuclear lines and common restorers that restore fertility on both of these CMS systems are not reported at all. However, utilization of these non-milo CMS systems at commercial level depends on factors such as stability of male-sterility, restorer gene frequency, effect of male sterile cytoplasm on agronomic traits, and the availability of commercially viable heterosis (Reddy *et al*. 2005). Grain mold is a highly destructive disease of sorghum with production losses ranging from 30% to 100% (Singh and Bandyopadhyay 2000). The testing of alternate cytoplasm for its role in the susceptibility/ tolerance to grain mold before utilization assumes significance.

Based on Ph D thesis submitted to Osmania University, Hyderabad during 2009

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Hence the present investigation was carried out to study $A_{4(M)}$ CMS system *vis a vis* A_1 CMS system for grain yield and agronomic traits, and traits conferring resistance to grain mold.

MATERIALS AND METHODS

Two diverse iso-nuclear allo-plasmic male-sterile cytoplasms, viz. A_1 and $A_{4(M)}$ in six diverse nuclear (maintainers / B-lines) backgrounds viz., ICSB 11, ICSB 37, ICSB 38, ICSB 42, ICSB 88001 and ICSB 88004, thus a total of 12 lines and two restorers (IS 33844-5 and M 35-1-19), that restore fertility in both the cytoplasms were used in the present study. In 2005 postrainy season, 12 A-lines were crossed with two common R-lines to produce 24 hybrids.

To study the effect of CMS systems on grain yield and agronomic traits and on resistance to grain mold, the 24 hybrids were evaluated in a split-split-plot design in three replications by considering R-lines as main plots, A-lines as sub-plots and cytoplasms as sub-sub-plots in two separate trials during 2006 and 2007 rainy seasons and for agronomic traits during 2006 and 2007 postrainy seasons at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh. Each genotype was sown in two-row plots of 2 m row length; the rows were 75 cm apart and a distance of 15 cm was maintained between plants in a row. Standard crop production and protection practices were followed to raise a healthy crop. The experimental site is located at an altitude of 545 m above mean sea level, latitude of 17.53°N and longitude of 78.27°E. The site received a rainfall of 639 mm during 2006 rainy season and 569 mm during 2007 rainy season during the crop growth period. Data were collected on the two rows for the traits days to 50% flowering (days taken to flower 50% of the plants in a plot), plant height (average height in m from the base of the plant to the tip of the panicle), grain yield (weight in tonnes/ha of the panicles harvested at physiological maturity) and fertility restoration (seed set per cent in the three selfed panicles). To screen for grain mold resistance, sprinkler irrigation was used to provide high humidity from flowering to maturity stages. The hybrids 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 data were subjected to split-split plot analysis using Genstat 10^{th} edition.

RESULTS AND DISCUSSION

Cytoplasmic influence on agronomic traits

Data were analyzed for individual seasons due to significant season × year interaction. During both rainy and postrainy seasons, there were significant differences among the nuclear backgrounds of the A-lines except for days to 50% flowering during postrainy season. R-lines differed significantly for percentage of male-fertility restoration.

Rainy season

The significant mean squares due to cytoplasms *per se* and their first-order interaction with A-line and second-order interaction with A-line and R-line for grain yield suggested the overall influence of cytoplasm on the response of hybrids for grain yield, male fertility restoration per cent and days to 50% flowering while for plant height, the cytoplasm did not show any effect (Table 1). The $A_{4(M)}$ cytoplasm was early to flower by four days in the nuclear background of ICSA 11 × IS 33844-5 and by eight days in the nuclear background of ICSA 11 × M 35-1-19 while the A_1 cytoplasm was early to flower by three days in the nuclear background of ICSA 38 × M 35-1-19 during 2006 rainy season. Such differences were not seen in 2007 rainy season (Table 3). The A_1 cytoplasm was superior to $A_{4(M)}$ cytoplasm by 2.07 to 6.05 tonnes/ha for grain yield in four nuclear backgrounds while $A_{4(M)}$ cytoplasm was superior over the A_1 cytoplasm by 2.29 to 3.86 tonnes/ha in three nuclear backgrounds during 2006 while A_1 cytoplasm was superior to $A_{4(M)}$ cytoplasm by 2.9 tonnes/ha in ICSA 42 × M 35-1-19 background during 2007 (Table 3). The A_1 cytoplasm exhibited greater restoration per cent than $A_{4(M)}$ cytoplasm by 60 to 80% in three iso-nuclear backgrounds during 2006 and by 40 to 90% in six iso-nuclear backgrounds during 2007 (Table 3). For plant height, there was absence of significant differences between cytoplasms in the individual nuclear backgrounds during both the years except for one nuclear background, ICSA 42 × M 35-1-19, where in A_1 based hybrid was taller by 0.5m. Moran and Rooney (2003) reported the absence of cytoplasm (A_1 and A_2) differences for mean days to flowering, plant height and grain yield of iso-nuclear sorghum hybrids.

Postrainy season

During 2006 and 2007 postrainy seasons, cytoplasmic influence was absent on days to 50% flowering, plant height and grain yield. The cytoplasm significantly influenced the restoration per cent through its *per se* effects and its first order interaction with A-line, R-line and second order interaction with A-line and R-line though the effects varied with the years (Table 2). In the individual nuclear backgrounds, there were non-significant differences between the cytoplasms for days to 50% flowering. For plant height, the A_1 cytoplasm based hybrid was taller by 0.4 m than the $A_{4(M)}$ cytoplasm based hybrid in the nuclear background of ICSA 11 × M 35-1-19 while the hybrid based on $A_{4(M)}$ cytoplasm was taller than A_1 cytoplasm by 0.3m in ICSA 38 × M 35-1-19 background during 2006 and by 0.6m in ICSA 42 × M 35-1-19 background during 2007. For grain yield, the A_1 cytoplasm based hybrid was superior over $A_{4(M)}$ based hybrid by 2.6 tonnes/ha in the nuclear background of one

Table 1 Analysis of variance for grain yield, agronomic traits and grain mold resistance in sorghum across 2006 and 2007 rainy seasons, ICRISAT, Patancheru

Source of variation	df	Days to 50% flowering	Plant height (m)	Grain yield (tonnes/ha)	Restoration (%)	PGMR score
Replication	2	31.05	0.03	15.47	1 122.60	0.97
Year	1	1 182.09*	0.42	259.39*	216.30	330.56**
Residual	2	28.49	0.04	9.50	298.30	1.16
R-line	1	11.45	0.76	1.95	15 336.5*	1.13
Year × R-line	1	30.80	0.00	6.06	78.20	1.52
Residual	4	7.36	0.11	1.88	1 226.60	0.21
A-line	5	12.51**	0.10**	9.35**	1 428.8**	1.23**
Year × A-line	5	4.15	0.01	6.34**	277.30	1.47**
R-line × A-line	5	6.91*	0.10**	2.63	981.2*	0.35*
Year × R-line × A-line	5	2.65	0.03	2.68*	899.1*	0.83**
Residual	40	2.19	0.02	1.12	309.30	0.14
Cytoplasm	1	0.04	0.07	18.72**	23 247.1**	4.25**
Year × Cytoplasm	1	11.39*	0.00	1.02	948.80	1.51**
R-line × Cytoplasm	1	0.30	0.03	5.91	13 223.5**	1.94**
A-line × Cytoplasm	5	7.20**	0.04	10.27**	1 756.9**	0.81**
Year × R-line × Cytoplasm	1	0.08	0.02	0.17	44.50	0.19
Year × A-line × Cytoplasm	5	14.58**	0.01	8.12**	240.40	0.55**
R-line × A-line × Cytoplasm	5	5.58*	0.02	6.86**	808.00	1.67**
Year × R-line × A-line × Cytoplasm	5	4.64*	0.02	2.66	992.0*	0.51**
Residual	48	1.76	0.03	1.53	400.80	0.12

* P<0.05; ** P<0.01

Table 2 Analysis of variance for grain yield and agronomic traits in sorghum across 2006 and 2007 postrainy seasons, ICRISAT, Patancheru

Source of variation	df	Days to 50% flowering	Plant height (m)	Grain yield (tonnes/ha)	Restoration (%)
Replication	2	22.34	0.15	4.63	81.50
Year	1	1 052.23**	0.64**	84.98**	7 810.1*
Residual	2	6.44	0.00	0.49	151.00
R-line	1	26.06*	1.01	5.37	16 673.4**
Year × R-line	1	0.80	0.29	7.49	1 108.40
Residual	4	1.51	0.44	2.00	390.80
A-line	5	5.40	0.26*	5.46**	1 947.3**
Year × A-line	5	2.46	0.02	0.59	1 131.8**
R-line × A-line	5	2.24	0.04	1.53	3 187.5**
Year × R-line × A-line	5	2.90	0.05	4.68*	1 913.5**
Residual	40	2.34	0.07	1.43	160.80
Cytoplasm	1	0.17	0.03	0.49	19 937.4**
Year × Cytoplasm	1	0.85	0.01	1.58	3 964.8**
R-line × Cytoplasm	1	2.52	0.01	0.02	6 866.8**
A-line × Cytoplasm	5	1.74	0.03	1.96	1 925.2**
Year × R-line × Cytoplasm	1	0.06	0.01	0.86	63.50
Year × A-line × Cytoplasm	5	0.64	0.08	1.65	1 761.8**
R-line × A-line × Cytoplasm	5	0.14	0.03	2.74	2 514.3**
Year × R-line × A-line × Cytoplasm	5	1.86	0.20**	2.71	1 641.2**
Residual	48	1.24	0.05	2.36	183.20

*P<0.05; **P<0.01

hybrid, ICSA 88004 × IS 33844-5 during 2007. Pattanashetti *et al.* (2005) also reported the superiority of *milo* hybrids compared to *maldandi* hybrids for most of the agronomic traits including grain yield. Significant differences were observed between the cytoplasms for restoration per cent in several backgrounds. The A_1 cytoplasm-based hybrids had higher restoration per cent (40 to 87%) than $A_{4(M)}$ cytoplasm-based hybrids in three nuclear backgrounds during 2006 and six nuclear backgrounds during 2007 while the $A_{4(M)}$-based hybrid in the nuclear background of ICSA 37 × M 35-1-19 had 50% higher male fertility restoration during 2006 than the A_1 cytoplasm-based hybrid (Table 4).

Cytoplasmic influence on resistance to grain mold

The PGMR score is an indicative of grain mold resistance in sorghum. It varied across the years. A-lines tested differed significantly for PGMR score and also significant differences were found in the hybrids as indicated by significant mean squares due to A-line × R-line. The significant mean squares due to cytoplasms *per se* and their first-order interaction with A-line (varied across years), R-line and second-order interaction with A-line and R-line (varied across years) for PGMR score suggested the overall influence of cytoplasm on the response of hybrids for grain mold resistance (Table 1). During 2006, the hybrids based on A_1 cytoplasm in four nuclear backgrounds were superior over the hybrids based on $A_{4(M)}$ cytoplasm by a score of 0.7 to 2.0 (the latter group had higher score meaning more susceptible) while the hybrids based on $A_{4(M)}$ cytoplasm in three nuclear backgrounds were superior over the hybrids based on A_1 cytoplasm by a score of 1.0 to 1.3. During 2007, the hybrids based on A_1 cytoplasm in six nuclear backgrounds were superior over the hybrids based on $A_{4(M)}$ cytoplasm by a score of 0.7 to 1.5 (Table 3). Stack and Pedersen (2003) reported that the A_1 cytoplasm exhibited slightly lower grain mold incidence than A_2 (64 versus 70%). In contrast, Reddy *et al.* (2006) reported the

Table 3 Mean performance of A_1 and $A_{4(M)}$ cytoplasms in 12 nuclear backgrounds for grain yield and agronomic traits and for grain mold resistance in sorghum across 2006 and 2007 rainy seasons, ICRISAT, Patancheru

Year	R-line	A-line	Days to 50% flowering		Plant height (m)		Grain yield (tonnes/ha)		Restoration (%)		PGMR score	
Cytoplasm			A_1	$A_{4(M)}$	A_1	$A_{4(M)}$	A_1	$A_{4(M)}$	A_1	$A_{4(M)}$	A_1	$A_{4(M)}$
2006	IS 33844-5	ICSA 11	69	**65a**	2.8	2.7	**6.29a**	3.56	93	80	5.0	**4.0a**
		ICSA 37	70	69	3.0	3.0	6.93	6.26	87	90	4.0	4.0
		ICSA 38	71	69	3.0	3.0	4.45	**8.31a**	90	87	3.7	4.0
		ICSA 42	70	71	3.0	3.0	6.12	**8.58a**	77	87	**3.0a**	3.7
		ICSA 88001	68	68	3.0	3.0	8.09	6.78	87	90	4.0	4.0
		ICSA 88004	67	68	3.0	3.0	**6.67a**	4.60	90	90	5.0	**4.0a**
	M 35-1-19	ICSA 11	74	**66a**	3.0	2.8	**9.87a**	3.82	**90a**	10	4.0	4.0
		ICSA 37	68	68	2.6	2.6	**6.46a**	4.01	**87a**	27	**4.0a**	4.7
		ICSA 38	**69a**	72	2.9	2.9	6.59	8.18	90	90	**3.0a**	4.0
		ICSA 42	69	71	**2.9a**	2.4	8.81	7.09	87	60	3.3	3.7
		ICSA 88001	68	67	2.9	2.9	7.21	7.38	90	90	5.3	**4.0a**
		ICSA 88004	68	69	2.9	2.9	6.33	**8.62a**	**87a**	10	**3.0a**	5.0
2007	IS 33844-5	ICSA 11	62	64	2.9	2.9	3.50	3.22	90	60	**6.7a**	7.5
		ICSA 37	66	64	3.0	3.0	5.12	3.60	90	90	6.2	6.5
		ICSA 38	64	65	3.1	3.1	4.27	4.43	90	90	6.9	6.5
		ICSA 42	63	64	3.1	3.0	3.99	2.74	90	90	**6.3a**	7.7
		ICSA 88001	63	64	3.1	3.1	4.64	5.71	90	90	6.9	7.3
		ICSA 88004	63	64	3.2	3.1	4.82	3.33	**100a**	60	7.0	6.9
	M 35-1-19	ICSA 11	62	63	2.9	2.9	3.59	1.98	**87a**	42	7.4	7.9
		ICSA 37	62	63	2.9	2.9	4.65	3.33	**90a**	0	**6.3a**	7.8
		ICSA 38	63	63	3.0	2.9	5.13	4.38	**90a**	30	**6.5a**	7.3
		ICSA 42	63	63	2.9	2.7	**4.1a**	1.20	**83a**	38	**7.3a**	8.0
		ICSA 88001	62	63	3.0	3.0	5.98	4.84	90	83	6.8	6.7
		ICSA 88004	62	62	2.8	3.0	3.85	4.22	**90a**	45	**7.1a**	7.9
LSD at $P \leq 0.05$ (between two cytoplasms at the same levels of R-line, A-line and year)			2.2		0.3		2.0		33.0		0.6	

The letter 'a' and the value in bold indicates the significant cytoplasm differences for the trait in that nuclear background and also the superiority of that cytoplasm

Table 4 Mean performance of A_1 and $A_{4(M)}$ cytoplasms in 12 nuclear backgrounds for grain yield and agronomic traits and for grain mold resistance in sorghum across 2006 and 2007 postrainy seasons, ICRISAT, Patancheru, India

Year	R-line	A-line	Days to 50% flowering		Plant height (m)		Grain yield (tonnes/ha)		Restoration (%)	
			A_1	$A_{4(M)}$	A_1	$A_{4(M)}$	A_1	$A_{4(M)}$	A_1	$A_{4(M)}$
2006	IS 33844-5	ICSA 11	69	69	2.4	2.4	6.0	4.0	90	87
		ICSA 37	70	71	2.5	2.5	4.8	5.4	87	87
		ICSA 38	71	69	2.6	2.5	3.4	4.1	80	90
		ICSA 42	72	72	2.4	2.4	4.9	3.3	90	83
		ICSA 88001	70	68	2.7	2.8	5.3	5.8	90	90
		ICSA 88004	69	70	2.5	2.5	3.9	4.8	90	87
	M 35-1-19	ICSA 11	68	68	**2.3a**	1.9	5.0	3.6	**87a**	0
		ICSA 37	69	69	2.1	2.4	4.8	3.6	40	**90a**
		ICSA 38	69	69	2.2	**2.5a**	5.1	5.1	83	90
		ICSA 42	69	70	2.3	2.0	4.7	4.6	**86a**	16
		ICSA 88001	69	69	2.4	2.5	4.7	5.9	83	90
		ICSA 88004	69	71	2.3	2.3	4.6	3.2	**60a**	0
2007	IS 33844-5	ICSA 11	76	76	2.2	2.2	5.3	4.6	80	77
		ICSA 37	76	75	2.1	2.3	4.4	6.5	77	77
		ICSA 38	76	76	2.1	2.4	5.2	6.7	73	77
		ICSA 42	76	75	2.5	2.2	6.4	6.3	80	87
		ICSA 88001	75	75	2.5	2.4	7.2	6.7	**77a**	3
		ICSA 88004	75	75	2.4	2.3	**6.0a**	3.4	**73a**	27
	M 35-1-19	ICSA 11	74	74	2.1	2.3	6.1	6.9	**80a**	7
		ICSA 37	74	75	2.3	2.1	7.2	6.0	**47a**	7
		ICSA 38	75	74	2.2	2.3	6.3	7.0	**87a**	0
		ICSA 42	75	75	1.7	**2.3a**	5.2	5.3	77	63
		ICSA 88001	74	74	2.4	2.3	7.4	7.4	90	83
		ICSA 88004	75	75	2.3	2.2	6.5	7.4	**75a**	0
LSD at $P \leq 0.05$ (between two cytoplasms at the same levels of R-line, A-line and year)			1.8		0.4		2.5		22	

The letter 'a' and the value in bold indicates the significant cytoplasm differences for the trait in that nuclear background and also the superiority of that cytoplasm

similar effects of A_2 cytoplasm compared with A_1 cytoplasm in contributing for resistance to grain mold.

The results revealed that during rainy seasons of 2006 and 2007 the influence of cytoplasm varied with the nuclear background of A-line or A-line x R-line for days to 50% flowering and grain yield though there was no cytoplasmic influence on plant height. During the postrainy seasons of 2006 and 2007, the hybrids based on $A_{4(M)}$ cytoplasm were comparable to the hybrids based on A_1 cytoplasm for days to flowering, plant height and grain yield. However, for restoration %, A_1 cytoplasm-based hybrids were superior to $A_{4(M)}$ cytoplasm-based hybrids during both rainy and postrainy seasons. The A_1 cytoplasm-based hybrids were superior to $A_{4(M)}$ cytoplasm-based hybrids for grain mold resistance. Thus, the $A_{4(M)}$ cytoplasm compares well to the A_1 cytoplasm for agronomic traits, especially during postrainy season and its further exploitation depends on the further identification/breeding for high yielding R-lines and A-lines with other desirable traits important for post rainy season adaptation.

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