Alternative cytoplasmic male sterility systems in sorghum and their utilization

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Introduction

In sorghum, Stephen and Holland (1954) discovered cytoplasmic male sterility (CMS) designated as *milo* cytoplasm in the progenies of a cross between two cultivars, *milo* and combine *kafir*, with *milo* as the female and *kafir* as the male. Male-sterile plants to the extent of 25% were observed in the F_2 generation of the above cross when *milo* was used as female and not as male. The male-sterile segregants from this cross produced male-sterile hybrids when crossed with the *kafir* parent and fully fertile hybrids when crossed with the *milo* parent. Thus, it was recognized that *kafir* could be used as a maintainer of this source of CMS. Since the progeny received the cytoplasm from the female, it was hypothesized that the *milo* parent had a male sterility-inducing cytoplasm and dominant genes for pollen fertility, whereas the combine *kafir* parent contained a normal (fertile) cytoplasm but the recessive male-sterile genes. All progenies of the *milo*×combine *kafir* cross contained *milo* (sterility-inducing) cytoplasm, but those that also inherited the homozygous recessive genes from the *kafir* parent were male-sterile. The male-sterile plants in the *milo*×combine *kafir* cross were used as females in repeated backcrossing with *kafir* as the male parent. At the end of seven backcrosses, the entire genome of *kafir* was transferred into the *milo* cytoplasm. This resulted in two morphologically similar versions of the combine *kafir* (CK 60) parent: a male-sterile combine *kafir* (CK 60A) and a male-fertile combine *kafir* (CK 60B). The male-sterile lines are designated as A-lines and their maintainer lines as B-lines. Subsequently, several sources of CMS systems (A_1, A_2, A_3, A_4, A_5 and A_6) (Table 13) were discovered.

Inheritance of fertility restoration in CMS systems

The inheritance of fertility restoration is dependent on the specific combinations of cytoplasms and nuclear genes. Fertility restoration is controlled by a single gene in

some combinations (eg, A_1) but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm (Schertz 1994).

Segregating progenies with A_1 cytoplasm in F_2 generation showed that a single gene was responsible for fertility restoration of A_1 male-sterile cytoplasm (Murthy 1986; Murthy and Gangadhar 1990). Other studies on A_1 cytoplasm have concluded that 1 or 2 genes (Qian 1990) or even 1 to 3 genes (Lonkar and Borikar 1994) are involved in controlling fertility restoration. Murthy (1986) reported that at least three genes control the fertility restoration of A_2 cytoplasm. In another study, F_2 progenies with A_2 cytoplasm showed a 9:7 ratio indicating that two complementary genes (both *Msc*$_1$ and *Msc*$_2$) are necessary for fertility restoration in A_2 (Murthy and Gangadhar 1990). Lonkar and Borikar (1994) indicated that 2 to 4 genes are necessary, but three genes were more optimal for the fertility restoration in A_2 cytoplasm in backcross generations. Research at ICRISAT showed that the frequency of recovery of fertile plants were least on A_3 than A_4 and A_2 and A_1 indicating that more number of genes are involved in controlling fertility restoration on A_3 than the other systems (Reddy and Prasad Rao 1992). El'konin et al. (1996) concluded that the fertility restoration in sorghum is controlled by an interaction of two complementary dominant genes in 9E cytoplasm. These studies clearly suggest the involvement of at least two genes for fertility restoration on A_1 and three on A_2 cytoplasm.

CMS diversity assessment

The *milo* CMS system has been extensively used in developing the hybrids for commercial cultivation in America, China, Australia and India. Nearly all the hybrids released so far, and widely grown have *milo* (A_1) cytoplasm (Reddy and Stenhouse 1994). Cytoplasm diversity can be assessed through restoration pattern in testcrosses and anther morphology (classical method) and through biotechnological tools (molecular markers).

Classical method. Schertz and Pring (1982) summarized various cytoplasm sources with respect to restoration pattern of 42 lines from India, 24 from USA, and one from Africa. Some of the cytoplasms were similar in reaction considering their restoration pattern. For example, Schertz and Pring (1982) indicated that cytoplasms of G_1 (G_1-S, ms G_1, G_1-G, G_1A) are analogous to IS 1112C of USA. A more comprehensive classification of cytoplasm sources is provided in Table 13.

Over the years, many of these cytoplasm sources were either lost or not widely available. The most commonly available ones include: A_1 (*milo* source), A_2 (IS 12662C or TAM 428), and A_3 (IS 1112C) of USA origin, A_4 (Guntur, VZM and Maldandi) of Indian origin, and 9E (a selection made in 9E) from Ghana. These cytoplasms were grouped on the basis of fertility restoration patterns. Reddy and

Stenhouse (1994) reported the identification of minimum differential testers for A_1 to A_4 cytoplasms as:

TAM 428B (A_2) gives fertile F_1s only on A_1 cytoplasm,

IS 84B (A_4-Maldandi) gives fertile F_1s on A_1 and A_2 cytoplasms,

IS 5767R (A_4-Maldandi) gives fertile F_1s on all cytoplasms, except A_3, and

CK 60B (A_1) gives male-sterile F_1s on all cytoplasms.

Based on pollen development and anther morphology, these A_1 to A_4 (Guntur, VZM, Maldandi) and 9E cytoplasms were further subdivided into two distinct groups: (i) those with small anthers but without fertile pollen which degenerates during microsporogenesis (A_1, A_2, A_5 and A_6) and (ii) those with large non-dehiscent anthers that may contain some viable pollen (A_3, A_4 and 9E) (Schertz et al. 1997). A_1 to A_4 CMS cytoplasms are being maintained at Patancheru (Andhra Pradesh, India) by ICRISAT (Fig. 29).

The lack of differential restoration patterns, however, does not provide conclusive evidence that the CMS sources involved are necessarily similar as it is possible that the pollinator parents used in developing the testcrosses were not adequate in number and diverse enough to pick up the CMS differences. It is also important in such field studies that testcrosses to be evaluated are made on isonuclear A-lines to ensure that genotypic differences of the female parents are not confounded with their cytoplasmic differences in determining fertility restoration of testcrosses.

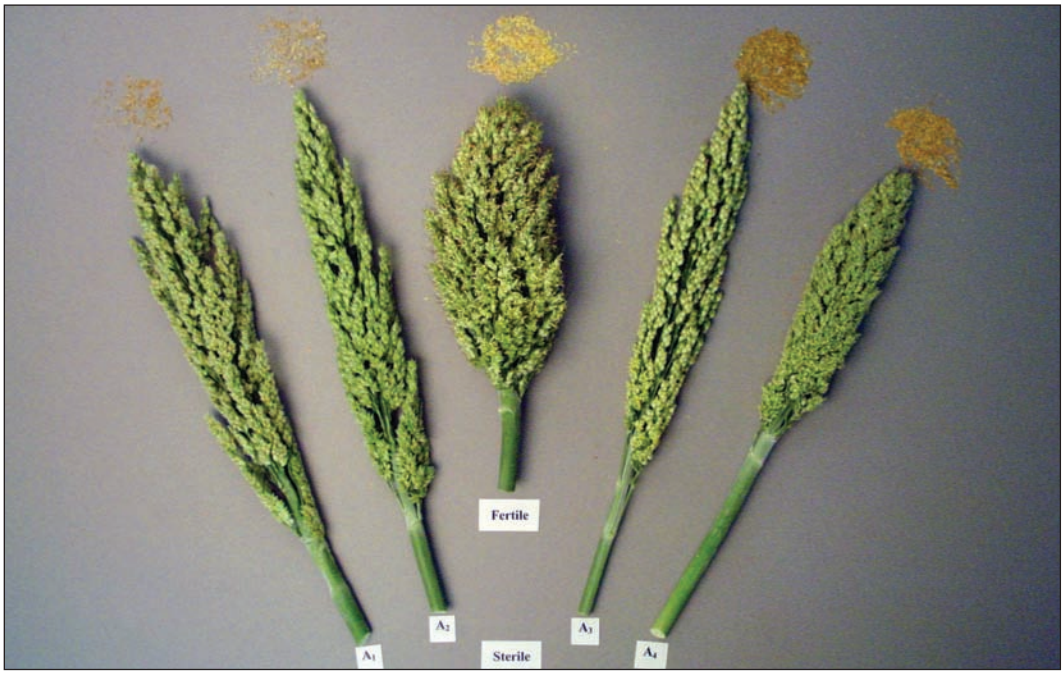

Fig. 29. Milo (A_1) and non-milo (A_2, A_3 & A_4) CMS systems in sorghum along with a fertile panicle.

Table 13. Sources of CMS systems in sorghum.

Cytoplasm	Source		
fertility group	Identity	Race[z]	Origin
A_1[y]	Milo	D	-
	IS 6771C	G-C	India
	IS 2266C	D	Sudan
	IS 6705C	G	Burkina Faso
	IS 7502C	G	Nigeria
	IS 3579C	C	Sudan
	IS 8232C	(K-C)-C	India
	IS 1116C	G	India
	IS 7007C	G	Sudan
A_2[z]	IS 12662C	G	Nigeria
	IS 2573C	C	Sudan
	IS 2816C	C	Zimbabwe
A_3[z]	IS 1112C	D- (DB)	India
	IS 12565C	C	Sudan
	IS 6882C	K-C	USA
A_4[z]	IS 7920C	G	Nigeria
9E[z]	IS 7218		Nigeria
	IS 112603C	G	Nigeria
A_5[z]	IS 7506C	B	Nigeria
A_6[z]	IS 1056C	D	India
	IS 2801C	D	Zimbabwe
	IS 3063C	D	Ethiopia

[z]D = *Durra*, G = *Guinea*, C = *Caudatum*, B = *Bicolor*, K = *Kafir*
[y]Type member for each fertility group
Source: Adapted from Schertz (1994).

Molecular markers. In conventional breeding, as indicated earlier, cytoplasms in various female parents are differentiated through the pattern of male sterility or restoration response in the testcrosses of various female lines. Other approaches to determine diversity among cytoplasms include restriction fragment length polymorphism (RFLP) as molecular markers (Schertz et al. 1997). Cytoplasmic factors associated with male sterility have been shown to be encoded by mitochondrial genome (Hanson and Conde 1985). As indicated earlier, using maize and pearl millet mitochondrial (mt) DNA specific probes, the comparison of RFLPs of mtDNA showed the difference between cytoplasms of A_1 to A_6 (Sivaramakrishnan et al. 1997). A_4 and 9E have been distinguished by RFLP analysis (Xu et al. 1995), and their cytoplasms were found to include an abnormal form of the mitochondrial gene

Cox 1 (Bailey-Serres et al. 1986a, Bailey-Serres et al. 1986b, Pring et al. 1995). Moreover, these cytoplasms also share several mtDNA RFLPs that distinguish them from all other Indian and US cytoplasms examined to-date, including polymorphism of the gene *atp9* (Schertz et al. 1997). Similarly, the restriction analysis of mtDNA from six Kansas State male-sterile lines using several endonucleases revealed two subgroups, with the patterns of KS 34, KS 38 and KS 39 corresponded to that of *milo*, as represented by CK 60 male-sterile; and KS 35, KS 36 and KS 37 differed both from *milo* and the other male-sterile lines (Schertz and Pring 1982).

Molecular characterization of CMS systems

As a consequence of the 1970 epidemic of southern corn leaf blight CMS-T cytoplasm is no longer widely used in commercial maize hybrids (Kishan and Borikar 1988, Wise et al. 1999). Therefore diversification of cytoplasm base of the cultivars is important. It is relatively easy to study diversity through the molecular markers. Now, it is well known that changes in the mitochondrial genome are responsible for male sterility (Sivaramakrishnan et al. 1997). Mitochondrial DNA of CMS line, IS 1112C in sorghum exhibits several unusual configurations—two forms of the *atp6* open reading frames (*orf* 107 and *orf* 265) and chloroplast DNA insertions (Pring et al. 1998). RNA editing of transcripts of mitochondrial *atp6* was strongly reduced in anthers of A3Tx 398 male-sterile line of sorghum, suggesting that loss of *atp6* RNA editing contributes or causes CMS (Howad and Kempken 1997). The incompatibility in nuclear cytoplasmic interactions leading to aberrant microgametogenesis in sorghum may be explained in terms of incompatible subunits being synthesized by the mitochondria and nucleus for a multi-subunit complex of the mitochondrial membrane such as ATP synthase (Sane et al. 1994). Further, aberrant microgametogenesis in sorghum CMS line IS 1112C occur very late in pollen maturation and restoration of pollen fertility is conferred by two genes designated as Rf3 and Rf4. Rf3 is tightly linked to Rf4 or represents a single gene that regulates a transcriptional processing activity that cleaves transcripts of *orf* 107, a chimeric mitochondrial open reading frame specific to IS 1112C and results in fertility restoration (Tang et al. 1998, Pring et al. 1998, Tang et al. 1999).

Factors influencing the use of CMS systems

Although numerous CMS sources have been found, all have not been commercially useful. There are various factors that determine CMS options. These include stability of male sterility, effect of male sterile cytoplasm on agronomic traits, restorer gene frequency in germplasm and the availability of commercially viable heterosis.

Stability of CMS systems

Instability of male sterility in A-lines increases the problem of rouging of pollen shedders in seed production plots, which results in increased seed production cost. Such an unstable CMS system also reduces breeding efficiency as the backcross progenies found fully sterile initially may not be necessarily so during the subsequent generations, leading to their rejection. Stability of male sterility also has a direct bearing on the cost and quality of hybrid seed production. Ideally, a commercial male sterile line should neither shed pollen nor should set seed when selfed, regardless of the location and the season. This however is seldom possible. For instance, several A-lines based on A_1 CMS systems in sorghum have been extensively used to breed hybrids, which are planted in millions of hectares in India alone. Yet, most of these A-lines produce, albeit low frequency (less than 1%) of pollen shedders, depending on the environment (Reddy et al. 2003). Thus, stability of male sterility across environments is an important criterion in the utilization of CMS systems for commercial production of hybrids. Several workers reported the role of temperature on the expression of male sterility and restoration in sorghum (Downes and Marshall 1971, Li et al. 1981). It affects some cytoplasms more than others (Schertz et al. 1997). The work at ICRISAT showed that restoration is poor when night temperature falls below 10°C, just before flowering, during postrainy season, and that the male sterility in CMS lines breaks down when the day temperature rises above 42°C, before flowering (Reddy and Stenhouse 1994). This evidently increases the need to screen the CMS lines in areas where the temperature rises above 42°C before flowering for the absence of seed setting under bag to ensure stability of male sterility. The hybrids need to be screened in areas where night temperatures are low (below 10°C) for seed setting under bags to identify stable fertility restorers.

On comparison of A_1, A_2, A_3 and A_4 male-sterile lines for seed setting upon selfing during summer (when temperature goes beyond 42°C) at Bhavanisagar, Tamil Nadu, India, it was found that A_1 is more stable in maintaining male sterility than A_2, A_3 and A_4 (Maldandi); A_3 than A_2 and A_4; and A_2 than A_4 (Reddy and Stenhouse 1994 and 1996). Tapetum was intact and pollen was sterile in A_2 male-sterile lines in winter (below 10°C temperature), while there was partial or complete degeneration of tapetum and pollen grains were fertile in summer (beyond 42ºC temperature), indicating the unstable nature of male sterility in A_2 CMS system (Devi and Murthy 1993). In the sorghum breeding program at ICRISAT, the frequency of maintainer lines observed in A_1 and A_2 CMS systems was higher in postrainy season (below 10°C temperature) than in rainy season (Reddy et al. 2003). However, Indian researchers have reported higher fertility restoration in A_2 CMS system in postrainy season than in rainy season (Murty UR, personal communication). Thus, stability of the expression of male sterility varies with the temperature as well as type of

cytoplasm. Research involving same CMS lines in both the seasons would give a clear picture of the stability of different CMS systems in different seasons.

Effect of CMS systems on economic traits

The observed frequency of segregation for tall and dwarfs confirmed the known theory that height is controlled by four recessive non-linked genes in crosses of two dwarf isocytoplasmic lines carrying A_1 cytoplasm and two tall tropical landraces (IS 2317 and IS 35613) (Murthy 1986). While in the crosses of dwarf isocytoplasmic lines of A_2 cytoplasm and two landraces, the segregation pattern of dwarf and tall deviated significantly from the four gene theory indicating the effect of A_2 cytoplasm on plant height.

Considerable variation was observed at ICRISAT sorghum breeding program between the available male-sterile lines and maintainer lines in A_1 CMS system for flowering. In the early group, a few A-lines tend to be late by a day or two but not of much significance. But in the medium and late maturity groups, A-lines tend to be significantly late in flowering and there is a tendency of increased delay in flowering in A-lines with the increased maturity period. B-lines have more open panicles than those of their A-lines.

Sharma et al. (1994) and Sharma (2001) reported that the spikelet damage and adult emergence of midges was significantly lower on midge resistant B-lines (PM 7061 and PM 7068) than their corresponding A-lines, and *vice versa* in the midge susceptible parental lines (296A and ICSA 42). At Patancheru, the maintainer lines (B) flower early by one or two days and has more open panicles than those of their A-lines. Further, A_1 cytoplasm was more susceptible to shoot fly than the maintainer line cytoplasm, while the reverse was true for stem borer resistance (Reddy et al. 2003). This finding has significance in developing shoot fly and stem borer resistant hybrids. In a different study using five pairs of sorghum isonuclear A_1 and A_2 CMS lines in Mexico, it was revealed that CMS system did not have any effect on days to flowering (Williams-Alanis and Rodriguez-Herrera 1992).

Evaluation of five pairs of sorghum isonuclear A_1 and A_2 CMS lines in four locations of Tamaulipas, Mexico, viz., Rio Bravo (irrigated), El Tapo (drought), El canelo (drought), Guelatao (drought), during fall summer season of 1992 indicated significant differences between A_1 and A_2 CMS lines for grain yield only in drought conditions (Rodriguez-Herrera et al. 1993). However, no significant differences were found between A_1 and A_2 CMS lines for plant height, panicle length and panicle exertion. In yet another study using 32 isonuclear A_1 and A_2 CMS lines-based hybrids evaluated in ten environments in Northern Mexico during fall winter season of 1990, 1991 and 1993 under irrigated and dry conditions, Williams-Alanis et al.

(1993) reported absence of significant differences between A_1 and A_2 CMS lines-based hybrids for grain yield, plant height, panicle length and panicle exertion.

Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines with three common dual restorers at Patancheru during postrainy season of 2001 and rainy season of 2002 indicated absence of significant differences between A_1 and A_2 CMS systems for mean performance for traits such as days to 50% flowering, plant height and grain yield, lodging resistance and aphid resistance. Although hybrids based on A_2 cytoplasm showed agronomically superior performance, A_1 based hybrids excelled in seed set under open pollination as well as selfing. A comparative evaluation of A_1 and A_3 cytoplasm-based isonuclear sorghum-Sudan grass hybrids at the University of Nebraska field laboratory, Ithaca during 1990 and 1991 by Pedersen and Toy (1997) revealed that cytoplasm had no effect on days to 50% flowering, plant height, dry matter of forage yield, *in vitro* dry matter digestibility and protein content. However, while fertility restoration was equivalent in A_1- and A_3-based hybrids, it was significantly lower in a few A_3-based hybrids. Recently, by evaluating a set of 12 isonuclear hybrids each in A_1, A_2 and A_3 cytoplasmic background at Weslaco and Texas Agricultural Experimental Station farm located near college station, TX during 1998 and 1999, Moran and Rooney (2003) have reported that A_1, A_2 and A_3 cytoplasms had no effects on plant height and had minimal practical effect on days to anthesis. However, grain yield in A_3 cytoplasmic background was significantly reduced as compared with A_1 and A_2 cytoplasm-based hybrids. Although specific reason for the reduced yield of A_3 hybrids is not known, seed set data indicated that it was not associated with fertility restoration.

Restorer gene frequency

The availability of restorers determines the extent of the use of various CMS systems in hybrid seed production. Scheuring and Miller (1978) reported a frequency of 0.62 restorers and 0.23 maintainers on milo (A_1) cytoplasm in the world collection of 3507 sorghum accessions. The work carried out at ICRISAT showed a restoration frequency of 0.9 on A_1, 0.5 on A_2, 0.1 on A_3, and 0.3 on A_4, when 48 germplasm lines were test crossed onto A_1, A_2, A_3 and A_4 CMS systems (Reddy et al. 2003). Senthil et al. (1998) found that the frequency of restoration was 0.15 on A_1, 0.04 on A_2, 0.01 on A_3 and 0.03 on A_4 CMS systems. This suggests that the restorer frequency is high on A_1 and low on A_3 system. Hence, considering the restoration frequency, A_1 CMS system provides the widest possible choice in selecting restorers.

Cytoplasm effects on heterosis for economic traits

Even if all the requirements are met in a CMS system, the existence of commercially viable heterosis ultimately determines the use of a CMS system. In sorghum, as indicated earlier, A_1 cytoplasm is more stable than other alternative cytoplasms, and restorer frequency with A_1 CMS is higher than with others. The heterosis estimates reported for grain yield using A_1 CMS system vary. For example, results from the Indian National Program Testing showed that the superiority over check with A_1 CMS system for grain yield ranged from 18 to 31% in the rainy season, and from 19 to 29% in the postrainy season in the years 1999 and 2000. The heterobeltiosis estimates for the same period ranged from 15 to 26% in the rainy season and 1.5 to 11% in the postrainy season (Reddy et al. 2003). Siddiq et al. (1993) reported that heterobeltiosis was 38% for grain yield in rainy season. Similar studies with alternate CMS systems are limited. Senthil et al. (1998) also reported that the A_1 CMS system produced higher number of heterotic combinations than A_2, A_3 or A_4 system. Kishan and Borikar (1989a) observed that A_2-based hybrids had larger grains and higher yields than A_1- and A_4-based hybrids. Based on testing of 15 hybrids derived from three isonuclear male-sterile lines and five common restorers, the A_4-based hybrids were inferior to others for grain yield in the rainy season. However, another report indicated that A_4-based hybrids had higher grain yield and larger grain size than A_1 hybrids during the postrainy season study (Kishan and Borikar 1989b).

Keeping in view the importance of diversifying cytoplasms in sorghum hybrids, ICRISAT, Patancheru has developed as many as 46 A_2 based CMS lines in different racial backgrounds; 17 A_3 based CMS lines and 12 A_4 based CMS lines. Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines with three common dual restorers at ICRISAT-Patancheru during postrainy season of 2001 and rainy season of 2002 indicated absence of significant differences between A_1 and A_2 CMS systems for mean heterosis (%) for any of the traits indicating that A_2 cytoplasm can be used in commercial hybrid seed production.

Efforts at NRCS, Hyderabad in the diversification of CMS lines resulted in the development of nine A_2 based CMS lines with high yield and grain mold resistance (GMR) and a unique hybrid SPH 1225 based on A_2 cytoplasm promising for grain yield and fodder yield in both irrigated and deep soil conditions in the postrainy season.

Considering the restoration frequency, development of high yielding male-sterile lines using A_2 restorers, and hybrid performance, it is advantageous to use A_2 CMS system among the alternate cytoplasms available. Based on A_2 CMS systems, the hybrid, Zinza No.2, was released in China for commercial cultivation. This hybrid is

now grown in an area of 200,000 ha accounting for one sixth of the total sorghum area in China (Liu Qing Shan et al. 2000). Extensive research is underway at ICRISAT and the Indian national program for the development of A_2 cytoplasm based hybrids.

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