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 F₂ 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 (A₁, A₂, A₃, A₄, A₅ and A₆) (Table 13) were discovered.

Inheritance of fertility restoration in CMS systems

The inheritance of fertility restoration is dependent on the specific combinations of cytoplasmics and nuclear genes. Fertility restoration is controlled by a single gene in
some combinations (e.g., A₁) but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm (Schertz 1994).

Segregating progenies with A₁ cytoplasm in F₂ generation showed that a single gene was responsible for fertility restoration of A₁ male-sterile cytoplasm (Murthy 1986; Murthy and Gangadhar 1990). Other studies on A₁ 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 A₂ cytoplasm. In another study, F₂ progenies with A₂ cytoplasm showed a 9:7 ratio indicating that two complementary genes (both Msc₁ and Msc₂) are necessary for fertility restoration in A₂ (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 A₂ cytoplasm in backcross generations. Research at ICRISAT showed that the frequency of recovery of fertile plants were least on A₃ than A₁ and A₂, and A₄ indicating that more number of genes are involved in controlling fertility restoration on A₃ 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 A₁ and three on A₂ 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 (A₁) 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 G₁ (G₁-S, ms G₁, G₁-G, G₁A) 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: A₁ (milo source), A₂ (IS 12662C or TAM 428), and A₃ (IS 1112C) of USA origin, A₄ (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 A₁ to A₄ cytoplasms as:

- TAM 428B (A₂) gives fertile F₁s only on A₁ cytoplasm,
- IS 84B (A₃-Maldandi) gives fertile F₁s on A₁ and A₂ cytoplasms,
- IS 5767R (A₄-Maldandi) gives fertile F₁s on all cytoplasms, except A₃, and
- CK 60B (A₅) gives male-sterile F₁s on all cytoplasms.

Based on pollen development and anther morphology, these A₁ to A₄ (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 (A₁, A₂, A₃, and A₆) and (ii) those with large non-dehiscent anthers that may contain some viable pollen (A₃, A₄, and 9E) (Schertz et al. 1997). A₁ to A₄ 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 (A₁) and non-milo (A₂, A₃ & A₄) CMS systems in sorghum along with a fertile panicle.*
Table 13. Sources of CMS systems in sorghum.

<table>
<thead>
<tr>
<th>Cytoplasm fertility group</th>
<th>Identity</th>
<th>Race</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1^y</td>
<td>Milo</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>IS 6771C</td>
<td>G-C</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>IS 2266C</td>
<td>D</td>
<td>Sudan</td>
<td></td>
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<tr>
<td>IS 6705C</td>
<td>G</td>
<td>Burkina Faso</td>
<td></td>
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<tr>
<td>IS 7502C</td>
<td>G</td>
<td>Nigeria</td>
<td></td>
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<tr>
<td>IS 3579C</td>
<td>C</td>
<td>Sudan</td>
<td></td>
</tr>
<tr>
<td>IS 8232C</td>
<td>(K-C)-C</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>IS 1116C</td>
<td>G</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>IS 7007C</td>
<td>G</td>
<td>Sudan</td>
<td></td>
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<tr>
<td>A_2^z</td>
<td>IS 12662C</td>
<td>G</td>
<td>Nigeria</td>
</tr>
<tr>
<td>IS 2573C</td>
<td>C</td>
<td>Sudan</td>
<td></td>
</tr>
<tr>
<td>IS 2816C</td>
<td>C</td>
<td>Zimbabwe</td>
<td></td>
</tr>
<tr>
<td>A_3^z</td>
<td>IS 1112C</td>
<td>D-(DB)</td>
<td>India</td>
</tr>
<tr>
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<td>C</td>
<td>Sudan</td>
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</tr>
<tr>
<td>IS 6882C</td>
<td>K-C</td>
<td>USA</td>
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<td>A_4^z</td>
<td>IS 7920C</td>
<td>G</td>
<td>Nigeria</td>
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<td>9E^z</td>
<td>IS 7218</td>
<td>G</td>
<td>Nigeria</td>
</tr>
<tr>
<td>IS 112603C</td>
<td>G</td>
<td>Nigeria</td>
<td></td>
</tr>
<tr>
<td>A_5^z</td>
<td>IS 7506C</td>
<td>B</td>
<td>Nigeria</td>
</tr>
<tr>
<td>A_6^z</td>
<td>IS 1056C</td>
<td>D</td>
<td>India</td>
</tr>
<tr>
<td>IS 2801C</td>
<td>D</td>
<td>Zimbabwe</td>
<td></td>
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<tr>
<td>IS 3063C</td>
<td>D</td>
<td>Ethiopia</td>
<td></td>
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^D = Durra, G = Guinea, C = Caudatum, B = Bicolor, K = Kafir
^T 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.
Moreover, these cytoplasts also share several mtDNA RFLPs that distinguish them from all other Indian and US cytoplasts 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 \textit{milo}, as represented by CK 60 male-sterile; and KS 35, KS 36 and KS 37 differed both from \textit{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 (\textit{orf} 107 and \textit{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 is 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 \textit{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 A₁ 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 A₁, A₂, A₃ and A₄ 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 A₁ is more stable in maintaining male sterility than A₂, A₃ and A₄ (Maldandi); A₃ than A₂ and A₄; and A₂ than A₄ (Reddy and Stenhouse 1994 and 1996). Tapetum was intact and pollen was sterile in A₂ 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 42°C temperature), indicating the unstable nature of male sterility in A₂ CMS system (Devi and Murthy 1993). In the sorghum breeding program at ICRISAT, the frequency of maintainer lines observed in A₁ and A₂ 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 A₂ 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 A₁ and A₂ 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 A₁ and A₂ isonuclear CMS lines with three common dual restorers at Patancheru during post-rainy season of 2001 and rainy season of 2002 indicated absence of significant differences between A₁ and A₂ 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 A₂ cytoplasm showed agronomically superior performance, A₁ based hybrids excelled in seed set under open pollination as well as selfing. A comparative evaluation of A₁ and A₃ 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 A₁- and A₃-based hybrids, it was significantly lower in a few A₃-based hybrids. Recently, by evaluating a set of 12 isonuclear hybrids each in A₁, A₂ and A₃ 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 A₁, A₂ and A₃ cytoplasms had no effects on plant height and had minimal practical effect on days to anthesis. However, grain yield in A₃ cytoplasmic background was significantly reduced as compared with A₁ and A₂ cytoplasm-based hybrids. Although specific reason for the reduced yield of A₃ 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 (A₁) cytoplasm in the world collection of 3507 sorghum accessions. The work carried out at ICRISAT showed a restoration frequency of 0.9 on A₁, 0.5 on A₂, 0.1 on A₃, and 0.3 on A₄, when 48 germplasm lines were test crossed onto A₁, A₂, A₃ and A₄ CMS systems (Reddy et al. 2003). Senthil et al. (1998) found that the frequency of restoration was 0.15 on A₁, 0.04 on A₂, 0.01 on A₃ and 0.03 on A₄ CMS systems. This suggests that the restorer frequency is high on A₁ and low on A₃ system. Hence, considering the restoration frequency, A₁ 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 $A_3$-based hybrids were inferior to others for grain yield in the rainy season. However, another report indicated that $A_2$-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 A₂ cytoplasm based hybrids.

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