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Genetic and Cytoplasmic-Nuclear Male Sterility in Sorghum*

Belum V. S. Reddy and S. Ramesh
Global Theme—Crop Improvement,
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),
Patancheru 502 324, Andhra Pradesh, India

Rodomiro Ortiz
International Institute of Tropical Agriculture (IITA),
Eastern and Southern Africa Regional Center (ESARC),
Plot 7, Bandali Rise, Bugolobi,
Kampala, Uganda

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I. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench, Poaceae] is the fifth most important cereal crop in the world after wheat, rice, maize, and barley. It is cultivated in the semi-arid tropics in 86 countries (FAO 1998). de Wet and his colleagues suggested that the cultivated sorghum had a diverse origin and probably arose from *S. verticilliflorum*, which is found in sorghum cultivated areas (House 1985) and that the domestication began around 3000 BCE (Doggett 1965). The cultivated sorghum *taxa* have been classified into five basic races (bicolor, caudatum, durra, guinea, and kafir), and 10 hybrid races (e.g., bicolor-caudatum) by Harlan and de Wet (1972).

The inflorescence of sorghum is a panicle consisting of racemes with one or several spikelets, which are either sessile or pedicellate. The sessile florets are bisexual, while the pedicellate are staminate and may rarely have a rudimentary ovary. The seed or caryopsis is usually the product of self-fertilization in sessile florets. However, there is 5 to 15% of out-crossing depending on the wind direction, nature of genotype, and humidity (House 1985). Discovery of genetic male sterility (GMS) and cytoplasmic-nuclear male sterility (CMS) facilitated the application of recurrent selection procedures and hybrid cultivar development methods, respectively, in sorghum improvement. In the following sections, the discovery, inheritance, and utilization of GMS and CMS in sorghum improvement are discussed.

II. GENETIC MALE STERILITY (GMS)

Genetic male sterility (GMS) in sorghum is imparted by (1) absence or degeneration of pollen grains, (2) lack of viable pollen, or (3) indehiscent anthers. In the first two situations, the anthers are small, scaly, and whitish, while the indehiscent anthers look normal, yellowish, and

plumpy (Reddy 1997a). Thus, genetic male sterility in sorghum is expressed in many ways.

Several sources of genetic male sterility have been reported from both India and the United States, and in all cases it was shown that a recessive allele in homozygous condition designated with a series of alleles such as ms_1 to ms_7 and all confer male sterility (Table 6.1). El'konin (2000) reported a dominant GMS mutation induced in sorghum tissue culture.

The goal of most population improvement programs across the globe is to accumulate favorable alleles for the traits of interest, while maintaining as much genetic diversity as possible. The recurrent selection methods used for such purposes require extensive hybridization. Because sorghum is a self-pollinated crop, there was relatively little effort in population improvement in sorghum. However, the discovery of GMS and the advantage of various mating systems and reciprocal recurrent selection methods in exploiting additive (A) and A × A and other epistatic genetic variation (Comstock and Robinson 1952; Eberhardt 1972), led many breeders to adopt population improvement methods (Maunder 1972; Doggett 1972) in the 1960s. In sorghum, both ms_3 and ms_7 alleles induced male sterility have been extensively used in population improvement, as they are stable across locations and seasons (Reddy and Stenhouse 1994; Murthy and Rao 1997).

The Ethiopian cultivar "Melka-mash" ensued from a population improvement program (Murthy and Rao 1997). In Nigeria, the national sorghum breeding program developed six random mating populations between 1963 and 1978 using ms_7 gene (Obilana and El-Rouby 1980). Only three of these (B composite, Y composite, and YZC composite) were used

Table 6.1. Genetic male sterility genes, their designated symbols and mechanism of sterility. Source: Adapted from Rooney (2000).

Gene symbol	Mechanism	Reference
ms_1	Normal pollen is dominant over aborted or empty pollen cells	Ayyangar and Ponnaiya (1937)
ms_2	" "	Stephens (1937)
ms_3	" "	Webster (1965)
ms_4	Empty pollen cells	Ayyangar (1942)
ms_5	Aborted pollen	Barabas (1962)
ms_6	Micro anthers without pollen	Barabas (1962)
ms_7	Empty pollen cells	Andrews and Webster (1971)
al	Antherless stamens	Karper and Stephens (1936)

as base populations for grain yield improvement, while a fourth, MSRC (modified *Striga* resistant composite), was used in recurrent selection for *Striga* resistance. After three cycles of mass selection in these populations, a gain of 38% and 40.4% for grain yield were observed in B and Y composites, respectively (Lukhele and Obilana 1980; Obilana and El-Rouby 1980). In Southern Africa, the four random mating populations developed jointly by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the National Agricultural Research Systems (NARS) using the ms_7 gene provided broad genetic-based gene pools from which NARS of the Southern African Development Community (SADC) could breed improved lines and new cultivars using recurrent selection (Obilana 1989).

Two random mating broad genetic-based sudan grass populations (NP34 and NP35) and two grain sorghum populations (NP36 and NP37) developed co-operatively by the United States Department of Agriculture–Agricultural Research Services (USDA–ARS) and the Nebraska Agricultural Research Division with the ms_3 gene were released in 1989 (Gorz et al. 1990a; Gorz et al. 1990b). NP34 is a good source of agronomically desirable R-lines and hydrocyanic acid potential (HCN-P), whereas NP35 is a good source of low-dhurrin. Both NP36 and NP37 were selected for reduced dhurrin content and they are valuable sources for A- and R-lines of grain sorghum. NP37 also possesses the brown *mid-rib-6* gene and therefore may be useful in producing sorghum-sudangrass or forage sorghum hybrids with brown mid-rib trait. Although population improvement programs are not the most common in sorghum breeding, they are an important source of genetic variation and improved traits (Rooney and Smith 2000). In GMS-facilitated population improvement programs, the breeder must take care to ensure that the alleles that cause male sterility are not eliminated.

Sorghum improvement at ICRISAT (Patancheru, Andhra Pradesh, India) was initiated in 1972 with population improvement using male sterility induced by the ms_3 gene following recurrent selection procedures to breed for wider adaptability. By 1980, the emphasis was shifted to specific adaptation and several specific disease and pest-resistant gene pools with ms_3/ms_7 male-sterile genes following half-sib/s_1/s_2 testing procedures. In the 1990s trait-specific gene pools improvement using ms_3 and ms_7 genes through simple mass selection alternated with recombination methods became the cornerstone of developing diverse breeding materials (Reddy et al. 2003). A total of 19 populations were developed at ICRISAT using ms_3 and ms_7 genes into which 501 diverse germplasm accessions were introgressed (Table 6.2). At ICRISAT, both ms_3 and ms_7 alleles are being maintained in different bulks.

Table 6.2. Details of the sorghum breeding populations developed and maintained by ICRISAT at Patancheru (Andhra Pradesh, India).

No.	Name of population	Gene	Original population	Traits/lines introgressed	Present cycle	Suggested use	Expected products
1	ICSP HT^z	*ms*$_3$	US/B C6	Sudangrass lines, early maturing lines, tillering lines, good grain characteristics, sweet stalk lines, DM, AN, LB, resistant lines, tall maturity & tillering	C_4	Tillering ability with high biomass & sweetness	Cvs. & restorers
2	ICSP LG^z population (rainy season)	*ms*$_3$	US/B C6	Large grain lines, early maturing lines, short height, shoot pest populations, large grain lines	C_2	Large grain & high yielding	Cvs. & restorers
3	ICSP LG^z population (post-rainy season)	*ms*$_3$	US/B C6	M 35-1, SPV 1359, M 35-1-19, M 35-1-36, NTJ 2 & others	C_1	Large grain & high yielding	Cvs. & restorers
4	ICSP B^z (rainy season)	*ms*$_3$	US/B C6	QL3, 286B, DM, midge resistant lines, SF & SB resistant lines, large grain durra lines, M 35-1, stay-green B-lines, SF B-lines	C_3	Source for new cytoplasmic male-sterile lines	Large grain, dwarf B-lines for rainy season
5	ICSP B^z (post-rainy season)	*ms*$_3$	US/B C6	OL3, 296B, DM, midge resistant, SF & SB resistant lines, large grain durra lines, M 35-1, stay-green B-lines, SF B-lines	C_0	Source for new cytoplasmic male-sterile lines	Large grain, dwarf B-lines for rainy season
6	Fast Lane R^y	*ms*$_3$	Nebraska, USA	White pearly grain, short height, photosensitivity	C_3	High yielding rainy season	Cvs. & restorers
7	Fast Lane B^y	*Ms*$_3$	Nebraska, USA	White pearly grain, short height, photosensitivity	C_3	High yielding rainy season	Maintainer lines

(*continued*)

Table 6.2. *Continued*

No.	Name of population	Gene	Original population	Traits/lines introgressed	Present cycle	Suggested use	Expected products
8	US/R^y	Ms_3	Purdue & Nebraska, USA	Grain yield & quality, agronomic desirability, resistance to leaf diseases, charcoal rot & molds, & pests (shoot fly, SB & midge), late maturity, white pearly grain, photo-insensitivity, short height	C_6	High yielding rainy season	Cvs. & restorers
9	US/B^y	ms_3	Purdue & Nebraska, USA	Grain yield & quality, agronomic desirability, resistance to leaf diseases, charcoal rot & molds, & pests (shoot fly, SB & midge), late maturity, white pearly grain, short height, photo-insensitivity	C_6	High yielding rainy season	Cvs. & B-lines
10	Serere elite^y	ms_3	EA Pop lines	White pearly grain, short height, photo-insensitivity, good grain quality & grain color	C_1	High yielding rainy season	Red/brown Cvs. & restorers
11	Tropical^y conv.	ms_3	Puerto Rico & Serere R/S Pop	White pearly grain, short height, photo-insensitivity, good grain quality & grain color	C_3	High yielding rainy season	Cvs. & restorers
12	Good grain^y	ms_3	Good grain, grain pop & red flinty pop	GPR 370 & other Indian bred lines	C_1	High yielding, short stature, early, with good grain quality	Post-rainy season, photo-sensitive
13	West African^y early	ms_7	WABC & Bulk Y, Nigeria	Grain yield & quality, agronomic desirability, resistance to leaf diseases, charcoal rot & molds, & pests (SF, SB & midge), photoperiod sensitive & pest resistant lines	C_4	Early seeding vigor	Post-rainy cvs. & restorers

14	Indian diallel[y]	ms_3 & ms_7	Diallel crosses world collection	High yielding tan bred lines from different programs including Indian program	C_0	A broad cyto-plasmic base for male-sterile lines & restorers	Rainy season cvs. & restorers
15	Indian[y] synthetic	ms_3 & ms_7	Indian lines	CK 60B, pioneer lines, 2Kx from East Africa	C_1	A broad cyto-plasmic base for male-sterile lines & restorers	Rainy season cvs. & restorers
16	RS/B[y]	ms_3	Developed by Doggett at Serere	White pearly grain, elimination of photo-sensitivity, stabilization of plant height (reduced), grain color, grain yield & quality, agronomic desirability, resistance to leaf diseases, charcoal rot & molds, & pests (SF, SB & midge)	C_6	A broad cyto-plasmic base for male-sterile lines & restorers	Rainy season cvs. & maintainers
17	RS/R[y]	ms_3	Developed by Doggett at Serere	White pearly grain, elimination of photo-sensitivity, stabilization of plant height (reduced), grain color grain yield & quality, agronomic desirability, resistance to leaf diseases, charcoal rot, & molds, & pests (SF, SB & midge), also, resistance to *Striga*, grain mold, shoot fly, SB & midge	C_6	A broad cyto-plasmic base for male-sterile lines & restorers	Rainy season cvs. & restorers
18	ICSP 2B/R[y] MFR	ms_3	RS/B, RS/R, US/B, US/R	Improved grain yield, agronomic desir-ability, resistance to SF, SB, midge, good seedling emergence	C_1	High yielding restorers	Post-rainy season B-lines/R-lines
19	Grain mold[y] population	ms_3	US/B C6	High yielding cultivars, mold resistant parents	C_1	Resistance to GM	Cvs. & restorers

[z]Mass selection with recombination used

[y]Half-sib, S_1 or S_2 methods with recombination used

DM = downy mildew; AN = anthracnose; LB = leaf blight; SF = shoot fly;
SB = stem borer; GM = grain mold

III. CYTOPLASMIC-NUCLEAR MALE STERILITY

Cytoplasmic-nuclear male sterility (CMS) has been used extensively to exploit heterosis in hybrids development on a large scale for commercial cultivation since the 1960s. In the pre-hybrid era of the early 1960s, the average sorghum productivity (t ha^{-1}) was 0.49 in India, 0.66 in China, 0.76 in sub-Saharan Africa, 1.48 in Australia, and 2.8 in the USA. In North and Central America, where commercial hybrids were exploited, there was a 40% increase in productivity from the early 1960s to the early 1990s. A similar trend was noticed globally. The productivity increases were 47% in China and 50% in India from the early 1960s to the early 1990s. However, it remained static at 0.79 t ha^{-1} in Africa from the 1960s to the early 1990s (FAO 1960–1996), and this may be attributed to non-exploitation of hybrids for commercial cultivation in Africa.

A. Origin of CMS Systems

CMS is a physiological abnormality, resulting from a disharmonious interaction between the cytoplasmic factors (now widely identified as mitochondrial genes) and nuclear genes leading to the production of degenerated or non-viable pollen grains or non-dehiscent anthers with or without functional pollen grains. Understandably, this disharmonious interaction is likely to be more pronounced in populations incorporating divergent sources of cytoplasm and nuclear genes (Reddy et al. 2003). Sorghum is no exception to this. For example, the A_1 CMS source in sorghum was identified in the F2 population of cross Double Dwarf Yellow Sooner Milo × Texas Blackhull kafir by Stephens and Holland (1954), in which the milo inbred belongs to the durra race from the Sudan and Ethiopia border (Duncan et al. 1991), and the kafir inbred from Eastern Africa (House 1985). In the F2 generation, 25% of male-sterile plants were observed from this cross if milo was the female parent. The male-sterile segregants from this cross produce male-sterile hybrids if crossed with the kafir parents and fully fertile hybrids if crossed with the milo parent. Thus, it was recognized that kafir could be used as a maintainer source of cytoplasmic-genetic male sterility. Since the progeny received the cytoplasm from the female, it was hypothesized that the milo parent had a male sterility-inducing cytoplasm and dominant nuclear genes for the restoration of pollen fertility, whereas the Combine kafir parent contained a normal (fertile) cytoplasm but recessive nuclear genes for fertility restoration. All progenies of the milo × kafir cross contained milo (sterility-inducing) cytoplasm, but individuals 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 thus designated as A-lines and their maintainer lines as B-lines.

B. Induction of CMS Systems

In Russia, L. A. El'konin and his colleagues used tissue culture to induce male sterility and fertility restoration. A new CMS source, called *Atc 1*, different from milo cytoplasm was developed from callus cultures of milo 145. When *Atc 1* mutants were crossed with milo, restorers resulted in sterile hybrids (El'konin 1995). El'konin et al. (1995) also showed a partial restoration of male fertility in 3 of the 20 regenerants from panicle fragments of a CMS F_2 plant of the cross A_1 Savatovskoya-3 × 3752 callus culture. Moreover, the induced fertility was retained even after eight generations of selfing. It is not known, however, whether the induced CMS or fertility restoration is different from non-milo CMS systems.

C. Inheritance of Fertility Restoration

The inheritance of fertility restoration is dependent on the specific combinations of cytoplasms and nuclei. Fertility restoration is controlled by a single gene in some combinations (e.g., A_1 cytoplasm) but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm (Schertz 1994).

Analysis of F_2 segregating progenies with A_1 cytoplasm revealed that a single gene was responsible for fertility restoration of A_1 male-sterile cytoplasm (Murthy 1986; Murthy and Gangadhar 1990). Other research on A_1 cytoplasm concluded that one or two genes (Qian 1990) or even 1 to 3 genes (Lonkar and Borikar 1994) are involved in fertility restoration. However, at least three genes control the fertility restoration of A_2 cytoplasm (Murthy 1986). 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 needed 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 was lower for A_3 than A_2, A_4, and A_1 cytoplasm, which indicated that more genes are involved in fertility restoration on A_3 than in 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. In their study, the tester line, KVV 114, was a restorer for 9(ET) × 398 and a maintainer for 9E milo 10, indicating that one or more dominant inhibitor genes in milo 10 suppressed the action of restorer gene of KVV 114. Further, a novel and unusual phenomenon of gradual restoration of male fertility was observed in subsequent backcross generations of A_4 and 9E cytoplasms in sorghum (El'konin et al. 1998). This research clearly suggests that at least two genes are needed for fertility restoration on A_1 and three on A_2 cytoplasm.

Fertility restoration in *T*-cytoplasm of maize is controlled by dominant alleles at two unlinked and complementary nuclear encoded genes (*Rf1* and *Rf2*) (Schnable and Wise 1994). However, additional restorer genes and duplicated loci complicate their analysis and identification in maize (Sisco 1991). Hence, it may be easier to find restorers on A_1 than A_2 CMS systems in sorghum owing to the number of genes involved in fertility restoration.

D. 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 that are widely grown have milo (A_1) cytoplasm (Moran and Rooney 2003; Reddy and Stenhouse 1994). Cytoplasmic diversity may be assessed through the restoration pattern in testcrosses and anther morphology (classical method) or by using molecular markers.

1. Classical Method. Schertz and Pring (1982) summarized various cytoplasm sources with respect to the restoration pattern of 42 lines from India, 24 from the USA, and one from Africa. Some of the cytoplasms were similar in reaction considering their restoration pattern. For example, Schertz and Pring (1982) and U. R. Murthy (1996, pers. commun.) indicated that cytoplasms of G_1 (G_1-S, ms G_1, G_1-G, G_1A) are analogous to IS 1112C of the USA. The more comprehensive classification of cytoplasm sources is provided in Table 6.3.

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 U.S. 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: (1) those with small anthers but without fertile pollen that degenerates during microsporogenesis (A_1, A_2, A_5, and A_6), and (2) those with large non-dehiscent anthers that may contain some viable

Table 6.3. Sources of cytoplasmic-nuclear male sterility in sorghum. Source: Adapted from Schertz (1994).

Cytoplasm fertility group[z]	Identity	Source	
		Race[y]	Origin
A_1	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	IS 1262C	G	Nigeria
	IS 2573C	C	Sudan
	IS 2816C	C	Zimbabwe
A_3	IS 1112C	D-(D-B)	India
	IS 12565C	C	Sudan
	IS 6882C	K-C	USA
A_4	IS 7920C	G	Nigeria
9E	IS 7218		Nigeria
	IS 112603C	G	Nigeria
A_5	IS 7506C	B	Nigeria
A_6	IS 1056C	D	India
	IS 2801C	D	Zimbabwe
	IS 3063C	D	Ethiopia

[z]Type member for each fertility group
[y]D = durra, G = guinea, C = caudatum, B = bicolor, K = kafir

pollen (A_3, A_4, and 9E) (Schertz et al. 1989). A_1 to A_4 CMS cytoplasms are being maintained at Patancheru (Andhra Pradesh, India) by ICRISAT.

The lack of differential restoration patterns, however, does not provide conclusive evidence that the CMS sources involved are necessarily similar. 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.

2. Molecular Markers. Conventional breeding 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 the use of 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 the mitochondrial genome (Hanson and Conde 1985). Using maize and pearl millet mitochondrial (mt) DNA specific probes, RFLP of mtDNA showed the difference between A_1 to A_6 cytoplasms (Sivaramakrishnan et al. 1997). A_4 and 9E were distinguished by RFLP analysis (Xu et al. 1995), and their cytoplasms included 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 RFLP 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 of six male-sterile lines from Kansas State University using several endonucleases revealed two subgroups, with the patterns of KS 34, KS 38 and KS 39 corresponding to that of milo, as represented by CK 60 male-sterile; and KS 35, KS 36, and KS 37 being distinct both from milo and the other male-sterile lines (Schertz and Pring 1982).

IV. MOLECULAR CHARACTERIZATION OF CYTOPLASMS

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). Cytoplasmic diversification in the new cultivars is therefore important. It is relatively easy to assess diversity with molecular markers. Changes in the mitochondrial genome are known to be responsible for male sterility in sorghum (Pring et al. 1993; Sivaramakrishnan et al. 1997; Tang et al. 1996a), e.g. mitochon-

drial DNA of CMS line IS 1112C showed unusual configurations. Although initially a cell type-specific loss of *atp6* RNA editing was reported to occur (Howad and Kempken 1997), e.g. in anthers of an A3T × 398 male-sterile sorghum line, more recent research did not confirm this finding (Pring and Tang 2001). It seems that a transcript processing internal to a mitochondrial open reading frame may be correlated with fertility restoration in male-sterile sorghum (Tang et al. 1996b).

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 occurs very late in pollen maturation and the restoration of pollen fertility is conferred by two genes (*Rf3* and *Rf4*). *Rf3*, which is tightly linked to *Mmt1* that confers transcript processing on orf25, represents the transcript processing activity, or it is tightly linked to the processing activity, for orf107. This chimeric mitochondrial open reading frame is specific to IS 1112C and results in fertility restoration (Tang et al. 1998; Pring et al. 1998; Tang et al. 1999).

In maize, some genes, especially ATPase, are disrupted by genetic changes, which produce otherwise normal plants, and cause pollen sterility. The hybrids derived from such sterile plants using the pollen of restorer plants are fertile; e.g., *Rf1* affects the expression of maize mitochondrial T-urf13 and encodes the 13kDa sterility protein URF13. Considering the importance of mitochondrial genome in male sterility, CMS lines have been studied in a number of crops. Pedigree and RFLP-based analyses indicate that seven independent *rf2* alleles for *Rf2* locus produce a functional product necessary for pollen fertility restoration in Texas cytoplasm (CMS-T). Molecular markers flanking the *rf1* and *rf2* loci were used to decipher segregating patterns in progenies (Schnable and Wise 1994).

V. DNA POLYMORPHISM AND MAPPING RESTORER GENES

Fertile plants from *S. versicolor*, *S. almum*, *S. halepense*, and *Sorghastrum nutans* (yellow Indian grass) each possess a 3.8 kb DNA fragment, which differed from CMS lines containing A_1, A_2, and A_3 cytoplasms with a 3.7 kb DNA fragment. A 165bp deletion located in the middle of the RNA polymerase β-subunit, which was encoded by the gene *rpo*C2, occurred in the CMS lines (Chen et al. 1993). A_1 and A_2 cytoplasms produced similar patterns with *Hind III* restriction enzyme, while restriction

fragments ensuing from *EcoRI* and *Pst I* showed identical patterns in A_1, A_2, A_3 and A_4 cytoplasms (Thin et al. 1993).

Genetic similarity and co-ancestry coefficient in sorghum suggested that RFLP might help to quantify the degree of relatedness in sorghum germplasm (Ahnert et al. 1996). A total of 276 out of 326 patterns of RFLP bands were common to both R- and B-lines, whereas 32 and 18 bands were unique to R- and B-lines, respectively. Cluster analysis further revealed that R-lines could be in two main groups (fertile and zera-zera), while B-lines lie within different sub-clusters.

RFLP and expression pattern of mitochondrial genes indicated that the cytoplasms classified tentatively as Indian A_4 types were distinct from the American A_4 and A_1 types. Although the geographical origin of cytoplasms was identical to each other, they are distinguished from each other based on RFLP analyses for *atp 6*, *atp 9*, and *rrn 18*. The three A_4 cytoplasms also differed from their maintainers in the location of *nad* 3, *rps* 12, and *atp* A. The differences in the pattern of expression of *atp* A between all the CMS and their respective maintainers was also observed (Sane et al. 1996). The molecular differences observed within the A_4 cytoplasmic group also provide an explanation for the inconsistency in fertility restoration behavior with a definite set of testers (Sivaramakrishnan et al. 1997).

In maize, RFLP analyses were used to localize the restorer genes for CMS-C and *Rf4*, demonstrating that a single dominant restorer gene for CMS-C was in chromosome 8, approximately 2 cM from the RFLP marker locus *NPI 114A* (Sisco 1991). The *Rf3* allele of the nuclear gene *rf3* gametophytically restores male fertility with the S-type of CMS. The *rf3* locus is on the long arm of maize chromosome two (2L). Using 2L RFLP and three-point mapping analysis, it was shown that the *rf3* locus is located at 4.3 cM distal to the *whp* locus, and 6.4 cM proximal to the *bnl*17.14 locus. This information was used in combination with RFLP on two additional maize chromosomes to show that *Rf3*/*rf3* CMS-S plants may aberrantly transmit the non-restoring allele, *rf3*, through the male gametophytes (Kamps and Chase 1997). Recently the A_1 and A_3 restorer genes *Rf1* and *Rf4*, respectively, were mapped in sorghum (Klein et al. 2000; Wen et al. 2002).

VI. FACTORS INFLUENCING CMS SYSTEMS USE

Although numerous CMS sources have been found, all are not 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.

A. Stability of CMS Systems

The instability of male sterility in A-lines increases the roguing of pollen shedders in seed production plots, which results in increased seed production cost. Such an unstable CMS system also reduces breeding efficiency, as backcross progenies that may be fully sterile initially may not remain as such in subsequent generations, leading to their rejection. Male sterility stability influences also 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, which appears to be seldom feasible. For example, several A-lines based on A1 CMS systems in sorghum are extensively used to breed hybrids, which are planted in millions of hectares in India alone. Most of these A-lines, however, produce a low frequency (< 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), which affects some cytoplasms more than others (Schertz et al. 1997). Restoration may be poor when night temperature falls below 10°C just before flowering during the post-rainy season. Also, the male sterility in CMS lines breaks down when the day temperature rises above 42°C before flowering (Reddy and Stenhouse 1994). This finding evidently increases the need to screen the CMS lines for the absence of seed setting under bag to ensure stability of male sterility in areas where the temperature rises above 42°C before flowering. 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.

While comparing seed setting in A_1, A_2, A_3, and A_4 male-sterile lines upon selfing during the summer (> 42°C) at Bhavanisagar (Tamil Nadu, India), Reddy and Stenhouse (1994, 1996) reported that A_1 was more stable for maintaining male sterility than others, whereas A_3 stability was greater than that of A_2 and A_4, and that of A_2 better than that of A_4. The tapetum was intact and pollen was sterile in A_2 male-sterile lines in winter (< 10°C), while partial or complete degeneration of tapetum and pollen grains were fertile in summer (> 42°C), indicating the unstable nature of male sterility in the A_2 CMS system (Devi and Murthy 1993). At Patancheru, the low temperature-induced female sterility in A_1 CMS female lines was similar to the line 296A, which indicated that sterility may be reduced significantly by using their non-parental single cross F_1 male-sterile lines (Reddy 1992). In the sorghum breeding program at ICRISAT, the frequency of maintainer lines observed in A_1 (Table 6.4)

Table 6.4. Maintainers and restoration frequency in sorghum A_1 cytoplasm in rainy and post-rainy seasons at Patancheru (Andhra Pradesh, India).

Season (2002)	A_1 line	Total tested	Frequency	
			Maintainers	Restorers
Rainy	ICSA 56	75	0.71	0.29
	ICSA 84	66	0.83	0.17
	ICSA 101	87	0.84	0.16
	CK 60 A	49	0.55	0.45
	Total	277	0.75	0.25
Post-rainy	ICSA 1	39	0.62	0.38
	ICSA 9	39	0.87	0.13
	ICSA 101	200	0.95	0.06
	ICSA 88005	21	0.90	0.10
	Total	299	0.89	0.11

and A_2 CMS systems (Table 6.5) was higher in the post-rainy season (< 10°C) than in the rainy season (Reddy et al. 2003). However, Indian researchers have reported higher fertility restoration in the A_2 CMS system in the post-rainy season than in the rainy season (U.R. Murthy, pers. comm.). Thus, stability of the expression of male sterility may vary with the temperature as well as type of cytoplasm. Research involving the same CMS lines in both seasons may provide a better understanding of the stability of different CMS systems in different seasons.

Table 6.5. Maintainers and restoration frequency in sorghum A_2 cytoplasm in rainy and post-rainy seasons at Patancheru (Andhra Pradesh, India).

Season	A_2 line	Total tested	Frequency	
			Maintainers	Restorers
Rainy 1999	MR 750	130	0.62	0.38
	ICSA 94003	140	0.63	0.37
	Total	270	0.62	0.38
Post-rainy 1999	MR 750	19	0.47	0.53
	ICSA 88004	110	0.45	0.55
	ICSA 94001	20	0.85	0.15
Post-rainy 2000	ICSA 38	133	0.97	0.03
	ICSA 743	72	1.00	0.00
	ICSA 88001	34	0.85	0.15
	Total	388	0.79	0.21

B. Effect of CMS Systems on Economic Traits

The observed frequency of segregation for tall and dwarf plants in crosses of two dwarf isocytoplasmic lines carrying A_1 cytoplasm and two tall tropical landraces (IS 2317 and IS 35613) confirmed that height was controlled by four recessive non-linked genes (Murthy 1986). However, in crosses between 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 an effect of the A_2 cytoplasm on plant height.

A comparative assessment of five pairs of sorghum iso-nuclear A_1 and A_2 CMS lines in Mexico revealed that CMS did not have any effect on days to flowering (Table 6.6) (Williams-Alanis and Rodriguez-Herrera 1992). Similarly, considerable variation was observed at the ICRISAT sorghum breeding program between the available male-sterile lines and maintainer lines in the A_1 CMS system for flowering. In the early group, a few A-lines tended to be late by a day or two, but in the medium and late maturity groups, A-lines tended to be significantly late in flowering, and there was a tendency of increased delay in flowering in A-lines with the increased maturity period (Table 6.7). B-lines had more open panicles than those from A-lines. Rodriguez-Herrera et al. (1993) also reported a delay in flowering of A- lines (A_2) compared to their maintainer (B) counterparts.

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) (Sharma et al. 1994; Sharma 2001). At Patancheru, the maintainer lines (B) flowered early by one or two days and had 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

Table 6.6. Days to anthesis in sorghum iso-nuclear CMS lines A_1 and A_2 at three planting dates in Mexico. Source: Williams-Alanis and Rodriguez-Herrera (1992).

	20 February		8 March		23 March	
Lines	A_1	A_2	A_1	A_2	A_1	A_2
LRB-1104A	84	83	80	79	76	77
LRB-1102A	83	82	80	76	74	75
LRB-1110A	84	85	79	82	78	76
LRB-1106A	79	82	78	80	73	76
E-15A	87	86	81	79	76	78
Means	83	83	79	79	75	76
Significance	NS		NS		NS	

Table 6.7. Frequency of sorghum male sterile and maintainer lines differing in days to 50% flowering at Patancheru (Andhra Pradesh, India).

Difference in days (A-B)	Early maturity group (< 67 days)	Medium maturity group (67–74 days)	Late maturity group (>74 days)
−2	0.00	0.02	0.04
−1	0.00	0.08	0.08
0	0.74	0.41	0.31
1	0.24	0.40	0.35
2	0.03	0.09	0.19
3	0.00	0.00	0.04
Total number tested	34	108	26

et al. 2003). This finding has significance in developing shoot fly and stem borer resistant hybrids.

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), and Guelatao (drought), during the 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) (Table 6.8). However, no significant differences were

Table 6.8. Economic traits as influenced by iso-nuclear A_1 and A_2 sorghum CMS lines evaluated in four locations in Tamaulipas (Mexico). Source: Rodriguez-Herrera et al. (1993).

Location Isogenic lines	Grain yield[z] (t ha^{-1})	Days to flowering[z]	Plant height[z] (cm)	Panicle length[z] (cm)	Panicle exertion[z] (cm)
Rio Bravo					
A_1	NS	82 ab	NS	30 ab	NS
A_2	NS	84 a	NS	33 a	NS
El Tapon					
A_1	2.3 b	75 b	137 b	NS	15 ab
A_2	2.1 b	78 a	135 b	NS	11 b
El Canelo					
A_1	1.3 b	83 a	NS	27 b	NS
A_2	1.5 a	83 a	NS	30 a	NS
Guelatao					
A_1	0.34 a	82 ab	NS	NS	NS
A_2	0.25 b	83 a	NS	NS	NS

[z]Means followed by same letter at each location are not significantly different; NS = non-significant.

Table 6.9. Grain yield and agronomic characteristics of sorghum isonuclear hybrids (in A_1 and A_2 CMS backgrounds) evaluated in ten environments in Northern Mexico. Source: Williams-Alanis et al. (1993).

Characteristics	A_1	A_2	CV (%)
Grain yield (kg ha^{-1})	4195 a[z]	4210 a	23
Days to flowering	76 b	77 a	4
Plant height (m)	1.4 a	1.4 a	7
Panicle length (cm)	30 a	30 a	15
Panicle exertion (cm)	17 a	17 a	33

[z]Means in rows followed by same letter are not significantly different

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 line-based hybrids evaluated in 10 environments in Northern Mexico during the fall–winter season of 1990, 1991, and 1993 under irrigated and dry conditions, Williams-Alanis et al. (1993) reported an absence of significant differences between A_1 and A_2 CMS lines-based hybrids for grain yield, plant height, panicle length, and panicle exertion (Table 6.9).

Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines with common three dual restorers at Patancheru during the post-rainy season of 2001 and the rainy season of 2002 indicated an 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 (Tables 6.10 and 6.11). Although hybrids based on

Table 6.10. Effect of A_1 and A_2 CMS systems on mean performance for grain yield and other economic traits in sorghum during post-rainy season of 2001 at Patancheru (Andhra Pradesh, India).

Trait	A_1	A_2	Difference	Significance
Days to 50% flowering	69.20	69.37	–0.17	NS
Plant height (m)	2.11	2.10	0.01	NS
Grain yield (t ha^{-1})	7.01	6.80	0.21	NS
Plant agronomic performance[z]	2.41	2.65	–0.24	*
Lodging resistance[y]	1.98	2.04	0.06	NS
Aphid resistance[x]	3.11	3.09	0.09	NS
Seed set under open pollination	92.04	91.20	0.84	*
Seed set upon selfing	84.63	78.80	5.83	*

* and NS indicate significant at $P = 0.05$ and non-significant

[z]1–5 scale where 1 = good and 5 = poor

[y]1–5 scale where 1 = less than 10% plants lodged and 5 = more than 80% plants lodged

[x]1–5 scale where 1 = leaf free from aphids damage and 5 = more than 60% leaf area damaged

Table 6.11. Effect of A_1 and A_2 CMS systems on mean performance for sorghum grain yield and other economic traits in the rainy season of 2002 at Patancheru (Andhra Pradesh, India).

Trait	A_1	A_2	Difference	Significance
Days to 50% flowering	68.44	68.59	–0.15	NS
Plant height (m)	2.58	2.55	0.05	NS
Plant agronomic performance[z]	1.65	1.78	–0.13	*
Grain yield (t ha^{-1})	5.71	5.72	0.01	NS

*and NS indicate significant at $P = 0.05$ and non-significant
[z]1–5 scale where 1 = good and 5 = poor

A_2 cytoplasm showed superior plant agronomic 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 iso-nuclear 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 50% flowering, plant height, dry matter of forage yield, *in vitro* dry matter digestibility and protein content (Table 6.12). 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 the Texas Agricultural Experimental Station farm located near College Station (Texas) during 1998 and 1999, Moran and Rooney (2003) 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 (Table 6.13). However, grain yield in A_3 cytoplasmic background was significantly reduced as compared with A_1

Table 6.12. Effect of A_1 and A_3 cytoplasms on forage traits in sorghum-sudan grass hybrids evaluated during 1990 and 1991 at Univ. of Nebraska. Source: Pedersen and Toy (1997).

Cytoplasm source	Days to 50% flowering	Plant height (m)		Dry matter yield (t ha^{-1})		In vitro dry matter digestibility yield (t ha^{-1})		Crude protein (%)	
		Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
A_1	72	1.77	2.16	3.52	5.85	637	602	12.2	9.4
A_3	72	1.79	2.13	3.50	5.97	635	607	12.3	9.5
Significance[z]	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]NS indicates non-significant

Table 6.13. Effect of A_1, A_2, and A_3 cytoplasms on average grain yield, days to anthesis, and plant height in iso-nuclear grain sorghum hybrids evaluated during 1998 and 1999 at Weslaco and the Texas Agricultural Experimental Station farm. Source: Moran and Rooney (2003).

Trait	Cytoplasm source		
	A_1	A_2	A_3
Average grain yield (t ha^{-1})	5.01 a[z]	4.92 a	4.74 b
Days to anthesis	70 a	70 a	71 b
Plant height (m)	1.44 a	1.43 a	1.42 b

[z]Means followed by same letter are not significantly different

and A_2 cytoplasm-based hybrids. Although the 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.

C. 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 3,507 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. Both results suggest that the restorer frequency is highest on A_1, and lowest on A_3 CMS system. Hence, considering the restoration frequency, A_1 CMS system provides the widest possible choice in selecting restorers.

D. Cytoplasm Effects on Heterosis for Economic Traits

Even if all the requirements are met in a CMS system, the existence of economically viable heterosis ultimately determines the use of a CMS system. In sorghum, as indicated earlier, the A_1 cytoplasm is more stable than other alternative cytoplasms. The frequency of restorer genes for 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 standard heterosis with A_1 CMS system for grain yield ranged from 18 to 31% in the rainy season, and from 19 to 29% in the post-rainy season in the years 1999

and 2000. The heterobeltiosis (or highest parent heterosis) estimates for the same period ranged from 15 to 26% in the rainy season and 1.5 to 11% in the post-rainy season (Reddy et al. 2003). Siddiq et al. (1993) reported that heterobeltiosis was 38% for grain yield in the rainy season. Similar studies with alternate CMS systems are limited. Senthil et al. (1998) also reported that the A_1 CMS system produced a higher number of heterotic combinations than the 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 post-rainy season study (Kishan and Borikar 1989b). The favorable and higher frequency of mid-parent heterosis observed in the landrace-based hybrids for several of the post-rainy season traits such as grain and fodder yields in the Advanced Hybrid Trials (AHT) and Landrace-based Advanced Hybrids and Parents Trials (LRAHPT) at Patancheru and Nandyal (India) during the post-rainy season (Table 6.14) indicates the potential for exploitation in the field for post-rainy season adaptation. Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines and three dual common restorers at Patancheru during the post-rainy season of 2001 and the rainy season of 2002 indicated an absence of significant differences between A_1 and A_2 CMS systems for mean heterosis (%) for any of the traits (Tables 6.15 and 6.16).

Table 6.14. Standard heterosis of landrace-based sorghum hybrids over M 35-1 (check) in landrace advanced hybrid trials at two locations during post-rainy season at Patancheru (Andhra Pradesh, India).

			Hybrids showing standard heterosis (%)		Range of standard heterosis (%)	
Trial	Location	Year	Grain yield	Fodder yield	Grain yield	Fodder yield
Advanced Hybrid Trial (AHT)	ICRISAT, Patancheru, India	1993	79	45	3.8–49.2	0.4–40
Landrace Advanced Hybrids and Parents Trial (LRAHPT)	Nandyal, India	1995	100	97	23.2–81.3	0.3–118

Table 6.15. Effect of A_1 and A_2 CMS systems on mean heterosis for grain yield and other economic traits in sorghum during post-rainy season of 2001 at Patancheru (Andhra Pradesh, India).

Trait	Mean heterosis (%)			Significance[w]
	A_1	A_2	Difference	
Days to 50% flowering	−6.67	−6.45	0.22	NS
Plant height (cm)	47.18	46.61	−0.58	NS
Grain yield (t ha^{-1})	31.78	27.87	−3.90	NS
Plant agronomic performance[z]	−1.62	7.88	9.51	NS
Lodging resistance[y]	62.57	67.93	5.37	NS
Aphid resistance[x]	−6.22	−6.34	−0.12	NS
Seed set under open pollination	4.05	3.11	−0.93	NS
Seed set upon selfing	9.04	1.99	−7.05	NS

[z]1–5 scale where 1 = good and 5 = poor
[y]1–5 scale where 1 = < 10% plants lodged and 5 = > 80% plants lodged
[x]1–5 scale where 1 = leaf free from aphids damage and 5 = > 60% leaf area damaged
[w]NS indicates non-significant

It may be advantageous to use the A_2 CMS system among the alternate cytoplasms available after considering the restoration frequency, development of high-yielding male-sterile lines, and hybrid performance when using A_1 restorers. However, the A_2 CMS system is not popular, as the anthers in A_2 male-steriles, unlike the A_1 male-steriles, mimic the fertile or maintainer lines and lead to complex monitoring of the purity of hybrid seed production. Extensive research is underway at ICRISAT and among Indian researchers for the development of A_2 cytoplasm-based hybrids. Based on A_2 CMS systems, the hybrid 'Zinza No. 2' was released in China for commercial cultivation. This hybrid is now grown

Table 6.16. Effect of A_1 and A_2 CMS systems on mean heterosis for grain yield and other economic traits in sorghum during rainy season of 2002 at Patachenru (Andhra Pradesh, India).

Trait	Mean heterosis (%)			Significance[y]
	A_1	A_2	Difference	
Days to 50% flowering	−3.27	−3.071	0.20	NS
Plant height (m)	42.46	41.23	−1.23	NS
Plant agronomic performance[z]	0.72	10.26	9.55	NS
Grain yield (t ha^{-1})	43.79	44.49	0.70	NS

[z]1–5 scale where 1 = good and 5 = poor
[y]NS indicates non-significant

in an area of 200,000 ha, accounting for one sixth of total sorghum area in China (Liu Qing Shan et al. 2000).

VII. DIVERSIFICATION OF CMS SYSTEMS

Most of the sorghum hybrids worldwide depend on the A_1 CMS system, which may lead to cytoplasm uniformity among hybrids for this crop (Moran and Rooney 2003; Reddy and Stenhouse 1994). This uniformity may increase the risk of outbreak or quick transmission of cytoplasmically inherited susceptibility to pests or diseases, as reported in maize with *Helminthosporium* leaf blight, which was linked to *T*-cytoplasm (Kishan and Borikar 1988) and pearl millet downy mildew disease epidemic (C. T. Hash 1998, pers. commun.). There are no reports of such an occurrence of cytoplasm-linked diseases or pests in sorghum. However, cytoplasmic diversity should be kept in this crop to avoid such hazards that may arise in future.

In addition to causing uniformity of cytoplasm in the hybrids, the use of milo (A_1) cytoplasm-based hybrids restricts the nuclear diversity (Schertz and Pring 1982). With the introduction of additional cytoplasmic-nuclear male sterility systems, new parental combinations should be possible. Hence, new and alternate cytoplasms have been sought with additional nuclear diversity, and are studied in several countries (Rao 1972; Nagur and Menon 1974; Tripathi 1979; Quinby 1981; Schertz and Pring 1982; Rao et al. 1984), based on restoration patterns in testcrosses made with common pollinators.

The frequency of maintainer genes in a diverse range of improved populations and breeding lines has a direct bearing on the success of genetic diversification of A-lines. Conversely, the frequency of restorers for several established CMS sources directly influences the use of such diversified male steriles in grain hybrid development. In addition, as already discussed in previous sections, the factors influencing the use of alternate CMS systems that include stability of their male sterility, their effect on economic traits, and extent of heterosis for economic traits, should be analyzed to make a rational judgment about CMS diversification. Often such analyses are not carried out, leaving new useful CMS systems unused or underused.

A. Germplasm Base and Traits

Although the need to diversify the cytoplasm base of seed parents has been recognized, research at ICRISAT showed that the kafir-based crosses with CK 60B produced higher frequency of B-lines than the

caudatum-based B-lines with A_1 CMS, which essentially derives its male sterility maintainer genes from kafir. This finding, coupled with the reduced frequency of restoration and the difficulties in distinguishing the fertiles from male steriles among alternate CMS systems at field level, forced many programs to depend mostly on the A_1 CMS system for the production of hybrids (Moran and Rooney 2003). There are four major national programs (USA, India, China, and Australia) and an international program (ICRISAT) engaged in diversifying the base of the male steriles and hybrids. All the national programs depend mostly on kafir-milo based male-sterile lines. The U.S. program diversified its kafir-milo (durra) male-sterile system by crossing with other kafir or bicolor lines in red- or brown-grain color background up to the mid-1980s (Schertz et al. 1997). The U.S.-based program influenced the sorghum breeding programs in China and Australia. Later on, the U.S.-based program further diversified its female parent base by crossing the kafir-bicolor B-lines with kauras (durra-caudatum) and other caudatum lines of African origin (Duncan et al. 1991; Schertz et al. 1997). This approach led to the development of a series of white-grain color male-sterile lines (e.g., Tx 623A, Tx 624A). The Indian program introduced CK 60A (milo-kafir) from the USA in the early 1960s, and developed 2219A (kafir-shallu), 2077A, 3675A, and 3677A among other kafir types in the late 1960s. Later on, male steriles were diversified by further selection and identification of maintainers in the crosses of the kafir B-lines with Indian durras, which are usually restorers on the milo-kafir system (Rao 1972). The most significant heterotic female line developed from such a program is 296A. However, this line was reported to be temperature-sensitive and seed production based on this line, therefore, became difficult due to low temperature-induced female sterility. The national programs diversified their male-sterile line base mostly for grain yield or the yield components such as grain number, grain size, etc. (Rao 1972; Duncan et al. 1991). Little effort was directed toward breeding male-sterile lines for resistance to abiotic and biotic stresses during the 1970s and 1980s (J. W. Stenhouse, pers. comm.).

During late 1970s, ICRISAT began the development of high-yielding male-sterile lines on the A_1 CMS system in white-grain background by extensively using kafir-caudatum-guinea crosses, and selecting white-grain male-sterile lines for grain yield. This program resulted in the development of a series of male steriles such as ICSA 1 to ICSA 110 and ICSA 88001 to ICSA 94013. In the late 1980s, ICRISAT made a major shift in its emphasis on the improvement of A_1 CMS male-sterile lines for resistance to various abiotic and biotic yield constraints. Considering the high correlation between *per se* performance of lines and hybrids (Rao 1972), ICRISAT followed simultaneous selection, test-crossing,

Table 6.17. Trait-specific resistant male sterile lines developed by ICRISAT sorghum breeding program at Patancheru (Andhra Pradesh, India).

Trait	Lines
Downy mildew	ICSA 201 to ICSA 259
Anthracnose	ICSA 260 to ICSA 295
Leaf blight	ICSA 296 to ICSA 328
Rust	ICSA 329 to ICSA 350
Grain mold	ICSA 351 to ICSA 408
Shoot fly	ICSA 409 to ICSA 463
Stem borer	ICSA 464 to ICSA 474
	ICSA 475 to ICSA 487 in post-rainy season
Midge	ICSA 488 to ICSA 545
Head bug	ICSA 546 to ICSA 565 in rainy season
Striga	ICSA 566 to ICSA 599
Acid soils	ICSA 600 to ICSA 614
Early maturity	ICSA 615 to ICSA 637
Bold grain	ICSA 638 to ICSA 670
Tillering	ICSA 671 to ICSA 674
Stay green	ICSA 675 to ICSA 68

and backcrossing methods to convert the maintainer lines selected for resistance to individual stresses and grain yield, under specific screenings in trait-specific breeding populations (Reddy et al. 1992). As a result, several trait-specific resistant male-sterile lines were developed (Table 6.17) Each ICRISAT breeding population involved different source materials, namely guinea for grain mold, durra for shoot fly, stem borer, and *Striga*, and caudatum-guinea for stay-green lines.

B. Other Cytoplasms

Breeding programs in the USA also developed a few high-yielding A_2 and A_3 CMS lines (Duncan et al. 1991). ICRISAT has converted some of the high-yielding A_1 CMS restorers into A_2 (ICSA 688 to ICSA 738), A_3 (ICSA 739 to ICSA 755), and A_4 (ICSA 756 to ICSA 767) CMS lines. Most of these lines were developed from kafir-durra-caudatum crosses.

C. Information Management and Knowledge Sharing through the ICRISAT Website

The development, characteristics, and pedigrees of the above male-sterile lines improved at ICRISAT are described fully in the ICRISAT web page, which can be accessed under crop at http://www.icrisat.org/text/research/grep/homepage.htm (Mahalakshmi et al. 2002). Infor-

mation about the performance of individual lines for traits of interest can be displayed, and further information on parents used in the breeding program can be obtained by clicking on the icon for the parent, which is linked to the germplasm passport data. Seeds of both breeding lines can be obtained by accepting the material transfer agreement on line and placing the request. Other information available in the sorghum web page includes gene bank germplasm and its score collection, crop diseases, pest, parasitic weeds, and plant nutritional disorders. The on-line system from the main crop page allows a query using the common or scientific name for a pest. There is also a two-module on-line learning system on sorghum that is based on sorghum practices ensuing from ICRISAT training manuals on breeding and seed technology in sorghum.

D. Effect of Genetic Background

Nuclear genetic background also has profound influence on male sterility. The fertility maintenance patterns differ from cross to cross, although the parents involved in such crosses by themselves are maintainers. Work at ICRISAT showed that the frequency of non-maintainers in B × B crosses ranged from 0 to 25%. For example, all the progenies were male-sterility maintainers in a cross of ICSB 554 × ICSB 79. On the other hand, in a cross like ICSB 583 × ICSB 64, nearly 20% of the progenies failed to maintain male sterility. The extent of the influence of genetic background thus determines the effectiveness of diversification of male-sterile lines. At ICRISAT, it is also observed that the maintainer gene frequency among the progenies of B × B crosses depends on the tester (male-sterile line) used, indicating the role of the "residual" cytoplasm factors accumulated in the converted male-sterile lines. It is useful to use, as much as possible, the original CMS source. For example, to diversify A_1 CMS lines, it may be desirable to convert to female the original male-sterile line CK 60A.

E. Seed Parents' Purity

Maintaining the purity in hybrid parents is important because impurity may arise due to mechanical admixture of the parents among themselves or with other lines. Impurity can also arise due to occurrence of pollen shedders in A-line, which depends on the CMS systems and the seed production season. The pollen shedding revertants may be due to mutation occurring either in cytoplasm or nuclear genes. The cytoplasm fertile revertants can be pulled out at flowering before they contribute to seed setting to maintain the purity of seed. If a nuclear gene mutation is the cause of reversion to male fertility, A-line seed purity can be

maintained by making plant-to-plant crosses between A- and B-line progenies for two successive generations.

VIII. HETEROSIS AND HYBRID DEVELOPMENT

Restorer and male-sterile lines should be as divergent as possible to gain maximum advantage in heterosis (Allard 1960). There is a considerable body of evidence to suggest that *per se* performance of the parents is highly correlated with hybrid performance (Rao and Rana 1982). Besides, other characters such as flowering behavior, pollen quantity (in R-lines), seed setting (in A-lines), and relative plant height of A- and R-lines also influence the hybrid seed production. A-lines with <50% of seed setting under open pollination are usually rejected. The restorers should be taller than male steriles usually by 0.1 to 0.8m, and possess good pollen shedding ability (Reddy 1997b).

Milo (A_1) cytoplasm male-sterile lines and restorer lines are usually based on kafir and caudatum races (Reddy and Prasada Rao 1993). In the Indian program, caudatum race (particularly IS 3541 from Sudan) has been thoroughly exploited not only for developing restorers but many of the released hybrids can be traced to caudatum source parents (AICSIP 1999).

Research was undertaken by ICRISAT at Patancheru involving a complete set of hybrids made by crossing five representative lines from each of the sorghum landraces guinea, bicolor, caudatum, durra, and kafir onto six common male-sterile lines to assess the relative magnitude of hybrid performance and heterosis in various landrace groups. Hybrids with landraces such as guinea followed by bicolor in the post-rainy season, and guinea followed by caudatum in the rainy season, were the highest yielders. However, the heterosis was greater with restorers of caudatum in the rainy season, and of guinea in the post-rainy season. Therefore, considering mean performance and heterosis for grain yield, further gains in sorghum hybrid yields can be realized by exploiting guinea race in restorer line development (Reddy and Prasada Rao 1993).

CMS has been used in sorghum population improvement to a limited extent. Dr. O. J. Webster at the University of Nebraska developed the first random mating population of sorghum using CMS in 1960. This population improvement was continued by Dr. Paul Nordquist at the same University (Murthy and Rao 1997). In Africa, Doggett and Jowett (1964) developed improved populations using the gene msc_1. In India, a random mating population was developed by Rao and his coworkers using an indigenous CMS source (Murthy and Rao 1997).

IX. CONCLUSION

The milo cytoplasm male-sterility system still remains the most widely used because the hybrids based on this cytoplasm produce sufficient heterosis (20–30%) over the best available pure lines in sorghum. In spite of A_2 cytoplasm being as promising as A_1 cytoplasm for either mean performance or heterosis for economic traits such as grain yield, days to 50% flowering, or plant height, the A_2 CMS system is not popular because the anthers in A_2 male-steriles, unlike the A_1 male-steriles, mimic the fertile or maintainer lines and lead to complex monitoring of the purity of hybrid seed production. Although diverse male-sterile cytoplasms are available, alternate sources are not useful for sorghum hybrids primarily because the frequency of restorer genes is low, and male steriles cannot be readily distinguished from male fertiles. There is therefore a need to search for a more useful form of male sterility, yet different from milo (A_1). Milo restorers need to be diversified in guinea background to further enhance the yield advantage in hybrid development. Restorer frequency is very low on other cytoplasms. Hence, there is a need to identify and breed for high-yielding non-milo cytoplasm restorers.

Limited work has been carried out on the inheritance of fertility restoration, but it has involved distinct genetic backgrounds. The use of isonuclear lines will provide a better means to determine the inheritance of fertility restoration. Similarly, the work on the identification of molecular markers will hasten the process of directed transfer of restorer genes. Although milo (A_1) male-sterile lines are diversified for resistance to biotic and abiotic stresses at ICRISAT, further breeding for grain yield potential and bold grain, and superior agronomic performance among them is needed.

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