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# Male Sterility Inducing Cytoplasm in Sorghum Classification, Genetics of Sterility and Fertility Restoration

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ABSTRACT: Sorghum shows immense morphological variability and adoption to varying habitats. This study was taken up using seed proteins to understand the phylogenetic relationship between <u>S. bicolor</u> and <u>S.</u> <u>halepense</u>, and to study the exact relationship between <u>S. purpureosericeum</u> parasorghum (2n=10) and Eusorghum. The seed protein pattern results of <u>S. bicolor</u> (2n=20) and <u>S. halepense</u> (2n=40) on SDS-PAGE confirmed the polygenetic relationship between them. The seed protein pattern of <u>S.</u> <u>purpureosericeum</u> (2n=10) showed its earlier evolutionary relationship with the other two species.

Practical exploitation of hybrid vigour in sorghum was made possible with the discovery of Cytoplasmic Male Sterile (CMS) lines. In 1954 Stephens and Holland reported for the first time that male sterility is conditioned by the interaction between nuclear genes and cytoplasmic factors. Later, many workers reported several male sterile lines (Quinby *et. al.*, 1958, Quinby and Schertz, 1970 and Quinby, 1971) for the production of  $F_1$  hybrids. The first commercial hybrids were produced in 1956 on some strains of combine kafir (Quinby *et. al.*, 1958). Shortly thereafter, a number of male sterile lines were developed and utilized in the hybrid seed production.

All the above reports on male sterile lines are based on milo cytoplasm only. The use of milo cytoplasm restricts the nuclear diversity. In recent years cytoplasmic uniformity has been recognized as a potential danger to stability of crop production. So alternate cytoplasmic systems are needed to avoid disease and environmental hazards, and to add nuclear diversity by using new parental combinations (Borikar *et. al.*, 1987).

Several alternative cytoplasms have been reported in sorghum from USA and India, and work has been reviewed by Schertz and Pring (1982),

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Rao et. al., (1984) and Worstell et. al., (1984). Study of the genetic relationship between diverse cytoplasmic system is important to understand sterility, fertility response of each cytoplasm with breeding lines and enables the use of alternative cytoplasm.

Still there is no definite classification of diverse male sterility producing cytoplasm. So this paper will give an idea about the diverse cytoplasm available, their present state of classification, genetics of sterility and fertility restoration.

### Classification of male sterility inducing cytoplasm in the USA

Stephens and Holland (1954) assigned the symbol  $A_1$  to milo cytoplasm.

Report on alternative cytoplasm:

Authority		Line	Source
1.	Schertz (1977)	A <sub>2</sub>	IS 12662 C
2.	Quinby (1980)	A <sub>3</sub>	IS 1112 C
3.	Worstell et. al., (1984)	A <sub>4</sub>	IS 7920 C

In the USA cytoplasm from diverse sources in sorghum are classified into four groups on the basis of fertility restoration, viz,  $A_1$ ,  $A_2$ ,  $A_3$  and  $A_4$ (Worstell *et. al.*, 1984). They classified the cytoplasm based on the studies on seed set, anther and pollen characteristics, differences in fertility response and related characteristics among the cytoplasm of male sterile female parents.

Quinby (1985) indicated the possibility of additional cytoplasm, other than the reported four groups.

### Alternative cytoplasm in India

Sterility caused by S-cytoplasm was reported by Mittal et. al., (1958). Rao (1961) detected another S-cytoplasm source from India and converted M 35-1 (Indian winter sorghum) and IS 3691 (Yellow hegari) into male steriles. Additional S-cytoplasm sources were also found in S. durra,  $G_2$ , VZM-1, VZM-2 by Hussaini and Rao (1964). At Raichur (India) another indigenous male sterile M 31 - 2 A is known to owe its origin to induced mutations. Nagur and Menon (1974) also found S-cytoplasm sources in Indian sorghums and distinguished them into four types  $S_1$  to  $S_4$  based on fertility restoration.

Tripathi *et. al.*, (1980) reported that cytoplasmic male steriles reported from India (Rao, 1961; Hussaini and Rao, 1964; Nagur and Menon, 1974) have a different restoration system compared to the milo - kafir system. It was therefore, inferred that these sterility inducing cytoplasms may be different.

Tripathi et. al., (1980) classified the cytoplasmic sources into three groups based on  $F_1$  studies of pollen sterility, pollen shedding and seed set.

1.	CK 60 A	}	Milo cytoplasm
	Nagpur-A	}	based on milo
2.	M.35-1A		$A_2$ (tentative)
	M.31-2A	}	
3.	VZM-2A	}	$A_3$ (tentative)
	G <sub>I</sub> A .}		

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Rao et. al., 1984 confirmed the above classification based on pollen fertility and seed set in  $F_1$  hybrids.

Gangakishan and Borikar (1989) studied the genetic analysis of cytoplasmic male sterility systems in sorghum to understand the genetic relationship between diverse cytoplasmic male sterility systems based on fertility restoration exhibited in  $F_1$  hybrid. They evaluated 25 A x B crosses and another set of 171 A x R crosses<sup>1</sup>. Fertility restoration in crosses was assessed by studying pollen sterility under microscopy and seed setting in selfed ear heads.

In exotic lines, the degree of sterility increased from  $A_1 \rightarrow A_2 \rightarrow A_4$ 

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<sup>&</sup>lt;sup>1</sup> The male sterile lines are A<sub>1</sub>Tx 398, A<sub>2</sub>Tx 398, A<sub>3</sub>Tx 398, A<sub>4</sub>Tx 398 from USA. CK 60 A, M.31-2A, M.35-1A, VZM 2A, G<sub>1</sub>A from India, of diverse source.

→  $A_3$ , and consequently, fertility restoration also became increasingly difficult in the same order. In Indian lines magnitude of sterility increased from M.31-2A and M.35-1A → VZM 2A →  $G_1A$ .

When exotic and Indian male steriles were considered together, the degree of sterility increased from  $A_1 \rightarrow A_2 \rightarrow A_4 \rightarrow M.31-2A$ ,  $M.35-1A \rightarrow A_3$  and VZM  $2A \rightarrow G_1A$ , and consequently, fertility restoration became increasingly difficult in the same order. The following grouping was suggested by Ganga Kishan and Borikar (1989).

A <sub>1</sub> Tx 398, CK 60A	- A <sub>1</sub>
A <sub>2</sub> Tx 398	- A <sub>2</sub>
$A_3$ Tx 398 and VZM 3 A	- A <sub>3</sub>
A₄ Tx 398	- A4
M.31-2A, M.35-1A	- A,
G <sub>i</sub> A	- A,
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From the above studies, the correspondence of male sterile lines of  $A_1$ ,  $A_2$ ,  $A_3$  cytoplasm of Indian classification (Rao *et. al.*, 1984) of Indian with the USA  $A_1$ ,  $A_2$ ,  $A_3$ ,  $A_4$  cytoplasm is established by Ganga Krishan and Borikar (1989).

Senthil et. al., (1994) studied diverse cytoplasmic male sterile lines and classified  $G_1$  male sterile line in between  $A_2$  and  $A_3$  cytoplasmic group. This result is confirmed by the anther protein studies (Senthil et. al., 1993).

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#### Classification by mitochondrial and chloroplast DNA restriction analysis

Ten groups of cytoplasm were differentiated by restriction endonuclease analysis of mitochondrial DNA (mt DNA). These groups were again differentiated by use of Hind III, Eco RI and Bam HI on chloroplast DNA (cp DNA). Some lines differentiated by mt DNA analysis were not differentiated by cp DNA analysis. So, the above ten groups of cytoplasm were made into 3 groups based on the cp DNA restriction endonuclease study (Pring *et. al.*, 1982).

Conde (1982) studied six cytoplasmic male sterile lines in sorghum (KS 34 through K 39), which have cytoplasm from sources other than the milo group. These were tested for fertility expressions in  $F_1$  hybrid produced with nine lines, and their organelle DNA's were examined by RFLP

analysis. Three of KS lines had cytoplasm indistinguishable from milo cytoplasm. The remaining three lines differed from milo, both in fertility response and their mt DNA restriction pattern. Cp DNA restriction patterns of all six KS lines were indistinguishable from that of milo cp DNA. The results indicate a relationship between mt DNA and genetic behaviour of the male sterile cytoplasm.

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Zengjian Chen *et. al.*, (1990) stated that restriction endonuclease patterns of cp DNA were consistently distinguishable between fertile and male sterile lines of sorghum, whereas there was no difference in restriction patterns of cp DNA among male sterile  $(A_1)$  lines. He suggested that cp DNA may contribute to the male sterility of  $A_1$  lines that are used in hybrid sorghum production.

## Genetics of sterility and fertility restoration in CMS system

- Male sterility is conditioned by a pair of recessive Fr<sub>1</sub> genes interacting with milo type(s) cytoplasm. Fertility is restored by one dominant Fr<sub>1</sub> gene. Modifier or weak restorer genes reduce the expression of normal restorers and produce plants having various degrees of pollen sterility (Maunder and Pickett, 1959).
- 2. Male sterility is controlled by two pairs of recessive genes, fertility being restored by one or both dominant genes, in hetero (or) homozygous condition (Pi and Wu, 1963).
- 3. More than two pairs of recessive genes or association with S-cytoplasm condition male sterility (Stephens and Holland, 1954).
- 4. Three pairs of recessive genes cause male sterility; partial sterility is conditioned by one (or) more major genes, plus modifiers; complete fertility restoration is under a multigenic control, especially in sudan grass (Craigmiles, 1962). Fertility restoration depends upon the presence of at least a single dominant fertility restorer gene (Alam and Sandal, 1967). Thus fertility restoration occurred when one, two (or) three genes where present.
- 5. A single major gene and one (or) more modifiers with additive effect coordinate fertility restoration. Three dominant modifier genes can induce fertility in the absence of major restorer genes (Kidd, 1961).

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7. Two independently acting major dominant genes are necessary for full restoration of fertility (Miller and Pickett, 1964).

8. A four gene model for fertility restoration was suggested by Tripathi et. al., (1985). The four gene system is still inadequate to bring about fertility restoration on VZM and  $G_1$  steriles. So a fifth major gene needs to be explored for fertility restoration on VZM and G<sub>1</sub> steriles. From the above it is apparent that the genetic control of male sterility is diverse and complex in sorghum.

In some, a single pair of recessive genes causes male sterility, while in others, two, three or more Fr genes are necessary for male sterility induction. Like male sterility, genetic control of fertility restoration is equally diverse and is conditioned by one, two or more dominant Fr genes acting singly or in combination. Also the influence of environment on sterility fertility expression is very high. This eludes the precise determination of genetic control of male sterility and its restoration in this crop.

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