

DEVELOPMENT OF HYBRIDS AND GENOME DIVERSIFICATION IN CMS LINES IN PEARL MILLET

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ABSTRACT

Pearl millet [Pennisetum glaucum (L.) R. Br.] is a C₄ species with a very high photosynthetic efficiency and dry matter production. It is an important course grain cereal and forage crop of the arid and semi arid tropics of the Indian sub-continent and several African regions. The protogynous flowering in pearl millet makes it a highly cross-pollinated crop with crossing rate of over 85 per cent. Pearl millet displays high degree of heterosis for grain yield, hence, providing hybrids as another cultivar option. The availability and knowledge of cytoplasmic-genetic male sterility (CMS), the development of CMS lines, and their maintainers and restorers, made it possible to produce the seed of commercial single-cross F₁ grain hybrids in India. Hybrid breeding received a major impetus when cytoplasmic male sterility was discovered and the male sterile (MS) lines Tift 23A and Tift 18A were released. The sterility inducing cytoplasm of Tift23A was designated as A₁. The difference in resistance based on Tift23 A cytoplasm indicated that the cytoplasm is not associated with downy mildew susceptibility. The breakdown in male sterility was also noted in Tift 23A by presence of pollen shedding plants. Later studies revealed that the susceptibility is due to interaction of both nuclear and cytoplasmic factors. Due to the susceptibility of the above hybrids to downy mildew, the need arose to widen the genetic base as well as cytoplasmic base of male sterility to induce high level of downy mildew resistance. Thereafter, many new cytoplasmic sources were identified and new cytoplasmic male sterile lines developed as well as many hybrids were released for cultivation.

KEY WORDS: *cytoplasmic genetic male sterility, diversification, hybrids, male sterility, pearl millet*

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a C₄ species with a very high photosynthetic efficiency and dry matter production. It is an important course grain cereal and forage crop of the arid and semi arid tropics of the Indian sub-continent and several African regions. Pearl millet is a short day plant adapted to hot

climates, more resistant to drought than sorghum

Pearl millet being a highly cross-pollinated crop with outcrossing rates being more than 85 per cent (Burton, 1983) and displaying a high degree of heterosis for grain and stover yields, attempts was made to exploit heterosis in the 1950s utilizing the protogynous nature of flowering of this

crop. The usual method at that time for production of hybrid seeds was growing the parental lines in mixture and allowing them to cross-pollinate (Gill, 1991). The resultant seed contained approximately 40 per cent hybrid seed when the two parental lines had synchronous flowering at about same time. These chance hybrids thus produced out yielded local varieties by 10 - 15 per cent. However, they could not become popular due to their limited yield advantage over OPVs, narrow range of adaptation and lack of seed production programmes.

The above mentioned limitations in the exploitation of heterosis were circumvented with the

discovery of cytoplasmic-nuclear male sterility and release of male-sterile lines Tift 23A and Tift 18A in early 1960s at Tifton, Georgia, USA. These lines were made available to Indian breeding programmes (Athwal, 1961 and 1966). The male-sterile line Tift 23A was extensively utilized, both at the Punjab Agricultural University and the Indian Agricultural Research Institute, because of its short stature, profuse tillering, uniform flowering and good combining ability [30]. This laid the foundation of pearl millet hybrid breeding in India. There have been three conspicuous phases in hybrid development in India (Table 1).

Table 1: The four phases of pearl millet improvement in India and their most distinguishing features.

Phase	Period	Number of hybrids released	Most distinguishing features of each phase
Pre-hybrid	1950 – 1965	-	Pre-hybrid phase, few open-pollinated varieties and mostly traditional cultivars largely grown
Hybrid phase I	1966 – 1980	17	Witnessed hybrid development in pearl millet, a few hybrids dominated cultivation, periodic downy mildew epidemics were common
Hybrid phase II	1981 – 1995	40	A large number of hybrids based on genetically diverse parental lines developed, downy mildew was largely contained
Hybrid phase III	1996 – 2012	81	A much larger number of highly diverse seed and pollinator parents used in hybrids, targeting niche adaptation in different zones

(Source: Yadav and Rai, 2013)

In pre-hybrid phase (1950–1965), pearl millet improvement largely concentrated, as described above, on the enhancement of yield in locally adapted materials using mass selection and progeny testing, and a good number of OPVs were developed and released. The introduction of landraces from African countries as

well as selection within them also yielded a few useful OPVs for Indian conditions. The average rate of improvement in pearl millet productivity during this phase was only 5.2 kg/ha/year. Utilization of cytoplasmic-nuclear male-sterile line Tift 23A in the breeding programmes marked the beginning of new phase in

pearl millet improvement in India. Tift 23A was widely utilized in hybrid development using pollinators bred in India. As a result, five hybrids (HB 1, HB 2, HB 3, HB 4 and HB 5) based on this line were released between 1965 and 1969. There also existed limited variation in pollinator parents of hybrids (Dave, 1987). Cultivation of few hybrids with narrow genetic base on large scale led to downy mildew (DM) epidemics offsetting impressive achievements made in hybrid development in the mid 1970s. Thus, there was only modest increase (6 kg/ha/year) in pearl millet productivity during 1966–1980. The recurring problem of DM epidemics in pearl millet hybrids till 1980 led to strengthening of research on the diversification of the genetic base of male-sterile lines (seed parents) of hybrids. As a result, a large number of genetically diverse male-sterile lines were developed and utilized in hybrid breeding during 1981 -1995 (Yadav *et al.*, 2012). Consequently, DM was largely contained and the productivity during this period increased at twice the rate compared to that during the previous phase. Current phase (1996 onwards) of hybrid development has put a much greater emphasis on genetic diversification of both seed and pollinator parents. The high productivity with niche adaptation and greater degree of tolerance to diseases are currently being targeted. As a result, rate of improvement in grain productivity has further increased to 24 kg/ha/year. In the meantime, it was clearly shown that hybrids have 25 -30 per cent grain yield advantage over improved OPVs. Thus, considering the grain yield advantage of hybrids over OPVs, and the potential for yield gains and production stability (including stemming of any DM epidemics), there was a rapid move away from

population improvement and OPV development towards hybrid development.

Pollination in pearl millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a highly cross pollinated crop. Its floral biology is unique among major crop species in that the hermaphrodite flowers are protogynous with the fully emerged and unpollinated stigma normally remaining receptive for 3-4 days. Such a situation makes both crossing (without emasculation) and selfing operations convenient in the breeding of pearl millet. The style starts protruding two to three days after the emergence of the panicle. The stylar branches protrude first from the florets in the upper middle region of the panicle and then proceed both upwards and down wards. In bisexual flowers, the stigmas emerge faster than the anthers and stigma receive pollen from inflorescence of the other plants. The time required for complete emergence of varies from 2 to 3 days.

Additional characteristics that make this crop an ideal organism for breeding methodology studies are:

1. Tillering response to spacing and input management, leading to normally 5-6 tillers per plant under and input management,
2. High seed number, up to 2000 seeds per panicle,
3. Low seed rate, 3-4 kg ha,
4. Short life cycle, normally 75-85 days, and
5. Excellent regenerating ability, and
6. Transplanting establishment.

Genetics of heterosis

High heterosis for grain yield has been reported in pearl millet by various workers. Ugale *et al* (1989) reported the performance of a large number of hybrids based on CMS lines under a wide range of ecological conditions in India. These hybrids

were found to be superior to the parental lines as well as open-pollinated varieties, and some of the best hybrids out yielded the control varieties by margins ranging from 75 to 200 per cent. Virk (1988) indicated average heterosis of 40 per cent for grain yield in 377 studies. The heterotic expression has shown some relation with combining ability, since several average $\times$ average combinations have been observed to show heterosis due to complementation of dispersed genes. For positive heterosis, the dominance component [h] must be positive and greater than the additive component [d] when epistasis is negligible. In addition, heterosis requires predominantly unidirectional dominance, so that [h] is not reduced through internal cancellation.

Conventional hybrids

Before the discovery of male sterility, only chance hybrids were developed. Protogyny and the time lag between stigma emergence and anther dehiscence facilitated effective cross pollination without emasculation. Crossing is affected by covering the female flower with glassine paper before the emergence of stigma. The appropriate stage to cover inflorescence is about one third of the inflorescence is out of flag leaf sheath. When all stigmas have emerged, pollen may be collected from the male parent by bagging and dusting the pollen on the female parent. However, this type of hybrid was not so popular due to its non-uniformity in phenotypic appearance.

Cytoplasmic genetic male sterility

The usual method of developing chance hybrids by taking advantage of the natural crossing was not successful in India in obtaining yield and uniformity of the desired level to make such hybrids a viable

alternative to local land race varieties. Besides this approach did not become a commercial proposition owing to the lack of a method to produce pure hybrid seed. Also, their adaptation was narrow and gains were not as much as expected. The availability and knowledge of cytoplasmic-genetic male sterility (CMS), the development of CMS lines, and their maintainers and restorers, made it possible to produce the seed of commercial single-cross F_1 grain hybrids in India (Athwal, 1966). Such hybrids have substantial grain yield advantage over popular open pollinated varieties OPVs). The occurrence of male sterility in *Pennisetum* was first cited by Kajjari and Patil (1956). The first commercial use of CMS millet was based on developments at Tifton, Georgia (Burton, 1958). The source of this sterility was observed in the two selected F_2 s of the cross 556 $\times$ Tift 23. The sterile mechanism was found to result from a break down of microsporogenesis after anaphase II and before first pollen division. Tift 23 with excellent fodder quality and combining ability was converted in to sterile by repeated back crossing to Gahi, the male sterile line Tift23A had been developed. Tift 23A is an excellent seed parent possessing the following features.

1. Stable male sterility
2. High tillering capacity
3. Early maturing
4. Excellent fodder quality
5. Wide adaptation and
6. Dominant red nod

Hybrid breeding received a major impetus when cytoplasmic male sterility was discovered in Georgia, USA, in 1958 and the male sterile (MS) lines Tift 23A and Tift 18A were released. The former was made available to breeding programmes in India and Africa. It showed poor

adaptation in Africa and shed pollen. The limited numbers of hybrids based on this line were completely destroyed by downy mildew (*Sclerospora graminicola*) and further hybrid breeding was discontinued. Tift 23A was, however, extensively utilized in India and five hybrids from HB 1 to HB 5 based on this line were released for commercial cultivation from 1965 to 1969 (Dave, 1987). Production increased from 3.5 to 8.0 million tons during this period.

Sterility and hybrid seed production

Both male and female sterility has been found to occur in Pearl millet. The sterility is caused by a number of factors.

1. Meiotic disturbances such as desynapsis
2. Segmental interchange such as translocation
3. Other abnormalities such as stickiness of chromosomes and agglutination of chromosomes
4. Incompatibility between genomes in inter specific crosses
5. Female sterility due to absence of pistil or portions of the seed forming organs
6. Cytogenetic male sterility caused by sterile cytoplasm, sterile nuclear genes and interaction between cytoplasm and nuclear genes

Among the above mentioned sterility, male sterility due interaction of cytoplasm and sterility inducing genes of nucleus is of greater interest in view of its application in hybrid breeding.

Genetics of male sterility

The inheritance studies were carried out by various authors and some of the findings were given below:

- The cytoplasmic male sterility results from the interaction of a specific recessive gene, ms, in

the homozygous condition with sterility inducing factors in the cytoplasm (Burton and Athwal, 1967).

- Different single genes in recessive form are responsible for male sterility; in different cytoplasmic systems (Burton and Athwal, 1967).
- The possible role of modifiers and environmental factors in the maintenance of male sterility were also suggested (Burton and Athwal, 1967).
- Siebert (1982) reported that the fertility restoration is governed by two dominant genes and a modifier for the A₁ system and two dominant factors for the A₂ system.
- Possible involvement of multiple major genes and modifier in determining male sterility has also been suggested.

Diversity of CMS source

The sterility inducing cytoplasm of Tift23A was designated as A₁. However, intensive cultivation of hybrids based on a single MS line resulted into cytoplasmic and genetic homogeneity leading to a downy mildew epidemic in the early 1970s. Production fell to 3.3 million tons. The cause for the susceptibility of these hybrids to downy mildew was associated with male sterility inducing cytoplasmic factor.

The difference in resistance based on Tift23 A cytoplasm indicated that the cytoplasm is not associated

with downy mildew susceptibility. The breakdown in male sterility was also noted in Tift 23A by presence of pollen shedding plants. Later studies revealed that the susceptibility is due to interaction of both nuclear and cytoplasmic factors. Due to the susceptibility of the above hybrids to downy mildew, the need arose to widen the genetic base as well as cytoplasmic base of male sterility to induce high level of downy mildew resistance.

The work on these aspects was carried out in many centers and improved Tift23A lines were developed for commercial utilization in hybrid development. Thereafter, a series of downy mildew resistant hybrids were released from time to time, but the majority of them succumbed to this disease to the lack of diversity and inadequate resistance to downy mildew in MS lines rather than to cytoplasmic susceptibility. However, much greater efforts are now being made to breed for downy mildew resistance and the MS lines currently being used are highly resistant. Consequently, the life span of single-cross hybrids produced in recent years is longer than for those produced in the 1970s. PHB 10 and PHB 13 were the first downy mildew resistant hybrids released in India (Gille *et al.*, 1975). Various breeding approaches were

adopted to improve Tift23A and many improved lines were constituted. Thereafter, a series of downy mildew resistant hybrids were released from time to time (Dave, 1987), but the majority of them succumbed to this disease to the lack of diversity and inadequate resistance to downy mildew in MS lines rather than to cytoplasmic susceptibility (Yadav *et al.*, 1993). However, much greater efforts are now being made to breed for downy mildew resistance and the MS lines currently being used are highly resistant (Rai and Singh, 1987). Consequently, the life span of single-cross hybrids produced in recent years is longer than for those produced in the 1970s.

Two other sources of cytoplasm contributing sterility were identified in L 66A and L67A by Athwal (1961) having different from Tift 23 A and designated as A₂ and A₃, respectively. The cytoplasmic source viz., A₂, A₃, A₄ and A₅ has been characterized by isonuclear studies (Appa Rao *et al.*, 1996) and using restriction fragment length polymorphism (RFLP) analysis of mt DNA of several A lines (Smith and Chowdhury, 1989 and Sivaramakrishnan *et al.* (1991). The male sterile cytoplasm characters and male sterility lines developed using different cytoplasmic sources are furnished below.

Different sources of cytoplasm characterized in pearl millet

Type of cytoplasm	Reference	Remarks
A ₂	Athwal (1961)	Late maturing type selected from genetic stock of IP189
A ₃	Athwal (1962)	Population of natural cross of stock possessing pearly amber grains in Ludhiana
A ₄	Hanna (1989)	Selected from accession of <i>P. glaucum</i> sub.sp. <i>monodii</i> from Senegal, Have more stable male sterility

A ₅	Rai (1995)	Source from large seeded gene pool. More stable male sterility. Best for producing seed parents of forage hybrids. Any inbred is a potential maintainer. Restorers are hard to find.
A _{egg}	Sujata <i>et al.</i> (1994)	Derived from ICRISAT early gene pool. More stable. Restorers occur in materials of diverse origin
Unclassified source	Appadurai <i>et al.</i> (1982)	Derived from PT 819 from Bellary. More stable

Promising male sterile lines and hybrids developed for commercial use

CMS Line	Distinguishing features	Reference/ Origin	Hybrid(s)
Tift 23A	Medium height, narrow leaves, good tillering, good combiner	Burton (1965a)	HB 1 to HB 5
126D2A	Moderately short, fairly resistant to downy mildew	GAU (Jamnagar)	GHB 1399
L111A	Medium tall, mid late, 4-6 ear bearing tillers, thick and long panicles with small bristles	Athwal <i>et al.</i> (1976)	PHB 10, PHB 14, PHB 47
5071A	Medium tall, induced downy mildew resistant mutant of Tift 23 A	Pokhriyal <i>et al.</i> (1976)	NHB 3, NHB 5
5141 A	Tall, medium narrow leaves purple nodal hairs, very good combiner	Pokhriyal <i>et al.</i> (1976)	BJ 104, BK 560, BD 763, HHB 45, GHB 27, GHB 32
5054 A	Early, thin stem, medium tall, narrow leaves	Pokhriyal <i>et al.</i> (1976)	CJ 104, CM 46, GHB 30
81 A (ICMA 1)	d ₂ dwarf, 3 to 4 effective tillers, fairly resistant to downy mildew resistant mutant from Tift 23 BD	Anand Kumar and Andrews (1984)	ICMH 451, HHB 50, HHB 60
834 A	Medium tall, early flowering 2 to 3 tillers, derived from Serer 10 A from Uganda	ICRISAT, Patencheru	ICMH 501
842 A	Large-seeded, early and dwarf male-sterile line	KSU, USA	HHB 68
843 A	Large –seeded early and dwarf male-sterile line	KSU, USA	HHB 67

MS2	A derivative of S10A and S10 B from Uganda	MAHYCO, Jalna	MBH 110, MBH 118, MBH 136
PT 732 A	Photosensitive, dwarf, an Indian inbred incorporating PT 819 cytoplasm	Appadurai <i>et al.</i> (1982)	PNBH 1, CoHCu8
Ms4	A-line from S10 A from Uganda via ICRISAT	MAHYCO, Jalna	MBH 149
Pb 405A ₃	Induced downy mildew resistant mutant from L110B	PAU, Ludhiana	PHB 108

ICMA 88004	Large seeded, yellowish seedling foliage	ICRISAT, Patancheru	ICMH 356
ICMA 89111	High synchronous tillering	ICRISAT, Patancheru	HHB 94

Conversion of male sterile lines

The male sterility will be a desirable phenomenon in seed parent if it has restorer pollen parent. The conversion of the desirable parents in to male sterile seed parent is achieved by repeated back crossing. The following parents can be converted in to male sterile seed parent. They are

- a) Inbred seed parent
- b) F_1 seed parent and
- c) Population seed parents.

a) Inbred seed parent

In the case of inbred seed parent type a maintainer line, which has normal pollen but does not restore fertility can be converted into a inbred male sterile parent by several back crossing.

b) F_1 seed parents

F_1 seed parents are used for producing three way hybrids. It is produced by back crossing A line with B lines of unrelated A line. Complete male sterility of the F_1 is essential for breeding three way cross hybrids. The advantages of using F_1 as seed parents are:

1. Higher seed yield of the F_1 s as compared to inbred seed parent.
2. Late maturing promising inbred lines can be used by crossing them with early B lines and crossed with early pollinators to produce early hybrids.
3. The dominant nature of downy mildew resistant can be exploited to increase the longevity of promising but susceptible male sterile

inbred lines through their use in producing F_1 seed parents.

c) Population seed parent

In population as parents the heterogeneous population is converted into male sterile to overcome the problem of adaptation and diseases resistance. Work is already underway at ICRISAT, using Nigerian Dwarf composite (NCD 2) as recurrent parent. Seed parents are mainly improved for downy mildew resistance. This could be achieved either through improvement of B lines or exploiting residual variability present in the original population.

Pollen parents and its improvement

Dominant nuclear gene(s) capable of restoring pollen fertility of a CMS lines must be present or incorporated into the agronomically superior line that is to be used as the male or pollen parent in seed inbreds capable of restoring fertility to certain CMS lines, especially those using the A_1 cytoplasm, have been developed in pearl millet. These lines are developed by selfing and selection. Pollinators can also be developed from open-pollinated populations or F_2 populations from crosses between desirable genotypes. To ensure selection of segregates possessing fertility restoration alleles, it may be desirable to carry out test crosses in early generations (S_2/F_3 using an A-line as a tester. When selected lines are fairly uniform (usually at about S_4 or S_5 generation or equivalent), they are crossed with male-sterile lines (A-lines). The F_1 hybrids thus produced are studied for combining ability for

grain yield and agronomic traits. Lines producing hybrids with good fertility restoration are carried forward.

Pollen parent may be an inbred or a top cross pollinator which is capable of restoring fertility. The inbreds are developed by selfing and selection. The specific characters of the pollinators are:

1. Good general combining ability,
2. High tillering,
3. Good panicle size,
4. Good grain quality,
5. Medium to short height,
6. Resistant to diseases and pests,
7. Abundant pollen production, and
8. And adequate level of photo period sensitivity.

Top cross hybrid

Top cross hybrid (TCH) cultivars are bred by crossing an inbred male-sterile line with an open-pollinated variety. It may be referred to as an inbred variety, a line x variety or, where CMS line is used, as a CMS line x variety cross. TCH as cultivars are a recent alternative to SCHs in pearl millet and a TCH (ICMH 88088) has come into commercial cultivation on a limited scale in India. The advantages of TCH:

- A top cross pollinator does not require many generations of selections as an inbred pollinator.
- The greater spread of flowering in the top cross pollinator allows them to nick well with a wider range of male-sterile lines than is possible for most inbred pollinators.
- There is greater potential for effective selection in top cross pollinators for

specific characters (e.g., disease resistance) than in inbred pollinators.

- Top cross pollinators are more vigorous than inbred pollinators and are also high-yielding. Thus, their seed multiplication is less expensive than that of inbred pollinators.
- Seed production of TCHs is more economical than single-cross hybrids, since pollinator rows can be fewer in number, should it be required.
- The disease resistance of top cross pollinator, and hence of TCH, can be expected, on theoretical grounds, to be more durable than that of inbred pollinators. Variable pollinators produce variable top cross hybrids. These hybrids can be less susceptible to diseases such as downy mildew, provided they carry different genes for disease resistance.
- TCHs provide a potential compromise between high yield levels and their stability, and therefore, might prove superior to single-cross hybrids in the harsher areas of the semi-arid regions.

Three-way hybrids

Three-way hybrids (TWHs) are an extension of single/cross hybrids that utilize a male sterile F_1 hybrid as seed parent instead of an inbred line. The significance of an F_1 seed parent in developing TWHs depends on

- (i) The yield superiority of the F_1 seed parent over

- the best available CMS lines;
- (ii) The relative yield and yield stability of TWHs compared to the best available single-cross and top cross hybrids; and
- (iii) The relative phenotypic variability in TWHs as compared to single-cross and top cross hybrids.

Recently, a private company in India commercialized a TWH called GK 1011, which is as good as SCH called GK 1004.

Problems in exploitation of hybrids

- The major problem limiting hybrid development is its susceptibility to downy mildew after little year release of hybrids.
- The Hybrid becomes susceptible to downy mildew after few years of cultivation due its narrow genetic base. Hence the necessities for wide cytoplasmic sources as well improvement of parental and seed parents were realized.
- The changes in hybrid as well as male sterile sources were realized as a result of break down of male sterility and susceptibility to downy mildew resistance.
- Because of narrow genetic base, the hybrids do not have flexibility to adjust to varying nature of Semi-arid tropical climates.

- Because of difficulties in seed production i.e., maintenance of A, B and R lines in isolation the small seed producers are hesitant to take up hybrid seed production.
- Crop failure due to drought and small difference in yield over OPVs in marginal lands.
- Consumer preference for local land races

CONCLUSION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a C₄ species had very high photosynthetic efficiency and dry matter production. The protogynous flowering in pearl millet makes it a highly cross-pollinated crop with crossing rate of over 85 per cent. The availability and knowledge of cytoplasmic-genetic male sterility (CMS), the development of CMS lines, and their maintainers and restorers, made it possible to produce the seed of commercial single-cross F₁ grain hybrids in India. Hybrid breeding received a major impetus when cytoplasmic male sterility was discovered and the male sterile (MS) lines Tift 23A and Tift 18A were released. The sterility inducing cytoplasm of Tfit23A was designated as A₁. The breakdown in male sterility was also noted in Tift 23A by presence of pollen shedding plants. Later studies revealed that the susceptibility is due to interaction of both nuclear and cytoplasmic factors. Due to the susceptibility of the above hybrids to downy mildew, the need arouse to widen the genetic base as well as cytoplasmic base of male sterility to induce high level of downy mildew resistance. Thereafter, many new cytoplasmic sources were identified

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