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# 'Molecular markers in Male Sterility: Step Towards Crop Improvement'

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## Abstract

The molecular markers are used to characterize genic male sterility (GMS) lines in plants. The molecular characterization found to develop unique marker and polymorphism among GMS lines. The molecular phylogeny reveals the near and distant lines. In GMS, most breeding lines are restorers, so it is easy to combine any elite line to produce strong heterosis. The observations will assist in cotton crop breeding programme to select and combine inter GMS lines to obtain high vigor combinations.

## 1. Introduction:

The great phenotypic and genomic diversity available in the gene pool can be made use by developing a genetic marker system (Mace et al, 2008) that will assist breeders in efficient crop improvement. Marker in general term referred as an object used to indicate a position, place, or route, or a thing serving as a standard of comparison or as an indication of what may be expected (Mandaliya et al, 2013). A genetic marker is expressed or unexpressed, gene or DNA sequence from a known or unknown location on a chromosome that can be used to identify individuals or species. The genetic marker system can be categorized into three generations and each one of them has advantages and limitations. The first generation markers were based on morphological markers for genetic and breeding research. However, it showed several difficulties such as expression of dominance or epistatic interactions, pleiotropic effects, and incomplete penetrance and expressivity (Sargent et al, 2004). The second generations of genetic markers were based on isozymes, and it became popular during 1970s and early 1980s to analyze the gene product (Royo and Itoiz, 2004). Despite their great advantages, isozyme markers are very limited in number and often are not polymorphic among closely-related genotypes (Foolad et al, 1993). The third generations of genetic markers are the molecular markers, which work based on

genotypic traits and not on phenotypic traits (Mehetre et al, 2004). Molecular markers are broadly classified into three classes (Mandaliya et al, 2010a-d, 2011): 1.1 Hybridization-based molecular markers (e.g., RFLP); 1.2 PCR-based molecular markers (e.g., AP-PCR, CAPS, STS, RAPD, SCAR, AFLP, SSAP, SSR, ISSR, EST); and 1.3 sequencing-based molecular markers (e.g., SNP).

## 2. Molecular markers in GMS breeding:

Heterosis is an important way to improve yield and quality for many crops. Hybrids often exhibit heterosis, more commonly known as hybrid vigor, whereby hybrid progeny exhibit superior growth characteristics relative to either of the parental lines. Hybrid cultivars have been developed in many crops such as rice, maize, sorghum, sunflower, oil seed rape, and cotton (Eckardt, 2006). The utilization of crop hybrids has become one of the trends for increasing the yield potential (Virmani and Edwards, 1983). Thus, the exploitation and utilization of heterosis is one of the ways for increase in yield potential, enhancement in abiotic and biotic resistances and overall improvement in crop product.

In the production of hybrid seeds, several systems can be used to eliminate self-pollination of female lines including: (i) application of chemical hybridization agents, (ii) mechanical removal of anthers or male flowers, or (iii) use of cytoplasmic or nuclear-encoded male sterility (Zhou et al, 2005). Cytoplasmic-nuclear male-sterility is an important biological tool, which has been used by plant breeders to increase yields in cross-pollinated cereals and vegetables by commercial exploitation of the phenomenon of hybrid vigor (Saxena et al, 2005). Cytoplasmic male sterility (CMS), a condition under which a plant is unable to produce functional pollen, is widespread among higher plants (Schnable and Wise, 1998). CMS has been widely used in plant breeding for the production of F1 hybrids, such as maize, sorghum, onions (*Allium cepa* L.), sugar beet, sunflower (*Helianthus annuus* L.), carrot (*Daucus carota* L.), canola, and rice (Wang et al, 2010).

Hybrid cultivars have been successfully used to increase double-zero rapeseed production worldwide. Male sterility has been applied to this crop as an effective and economical pollination control system. The advantage of using CMS system is that it can generate a complete male sterile population economically. However, it usually takes years to develop A and R lines since most CMS systems have stringent restoring-maintaining relationships (Yi et al, 2006). There are several drawbacks to using the CMS system. The use of specific maintainer and restorer lines restricts exploitation of many elite lines. The complicated breeding procedures, longer breeding period of CMS lines and costly maintenance of the parental system are the other limitations (Lakshmi Praba and Thangaraj, 2005).

In contrast, GMS has many advantages. Firstly, GMS involves only two lines and is transferred feasibly among parental lines, which may result in a shortened breeding cycle. Secondly, for GMS, most breeding lines are restorers, so it is easy to combine any elite lines to produce strong heterosis. Thirdly, GMS does not have the negative Cytoplasmic effect on yield as CMS might do (Yi et al, 2006).

But GMS system has its limitation of being difficult to derive a complete male sterile population. About 50% male fertile plants must be removed from the female lines during hybrid seed production. Development of a complete genic male sterile population with a temporary maintainer B-line of GMS was a breakthrough to overcome the previous limitation (Chen et al 1993, 1998). Consequently, several GMS-based hybrids have been released commercially worldwide (Yi et al, 2006).

There are several kinds of GMS system available as a possible alternative to CMS. The two-line breeding system using environmentally sensitive genic male sterility (EGMS) and chemical induction of male sterility (Ali et al, 1995). EGMS is composed of photosensitive genic male sterility (PGMS) based on variation in daylength and thermosensitive genic male sterility (TGMS) caused by high temperature. A PGMS line was found to be sterile under long daylength (>14 h) and fertile under less than 13 h of daylength. The PGMS system is more useful in hybrid rice production for subtropical countries such as China and Japan where daylength differences exist in a season (Lakshmi Praba and Thangaraj, 2005). Deploying the TGMS system for developing two-line hybrids has several advantages over the conventional CMS system. Male sterility expression in a TGMS line is heritable but regulated by temperature. At certain high temperatures occurring after panicle initiation, the TGMS line becomes completely male sterile and is used as the female parent for hybrid seed production. Under low temperature, the same male sterile individual regains its fertility and the seeds it sets can be used for hybrid rice production in the next growing season. Therefore, a hybrid production system based on TGMS can avoid using any maintainer line, which is required in the three-line system (Lakshmi Praba and Thangaraj, 2005).

There were sixteen different genes in tetraploid cottons (13 in *G. hirsutum* and 3 in *G. barbadense*) and two in *G. arboreum* have been identified for genetic male sterility. Sterility is conditioned by dominant alleles at five loci viz, MS4, MS7, MS10, MS11 and MS12 by recessive allele at other loci viz. ms1, ms2, ms3, ms13, ms14 (Dong A), ms15 (Lang A) and ms16 (81 A).Two male sterile phenotypes viz. m5ms6 and ms8ms9 are conditioned by duplicate recessive factors. The expression of male sterility varies greatly in extent and stability among the loci. Male sterility loci have been mapped. Both the dominant MS11 and the recessive ms8 have been mapped to chromosome 12. The recessive sterility factor ms3 and ms9 have been mapped to linkage group III of chromosome 16 and linkage group IX of chromosome 26 respectively. In diploid cotton, two genes have been identified for GMS from Akola and HAU, Hisar. At Akola, the male sterility was obtained from *anomalum x arboreum* crosses while at Hisar it was identified as a spontaneous mutant in *arboreum* variety DS 5 (Singh et al, 2002).

Use of molecular markers provides an accurate approach to encase the fertility and sterility traits in GMS. To the following molecular markers in GMS is described in two major points: (i) molecular markers for diversity and various traits, and (ii) nuclear makers for sterility and fertility.

***2.1 Molecular markers for diversity and various traits:***

Genetic male sterility in the *Gossypium arboreum* was found to be under the control of recessive gene (Geddam and Khadi, 2012). This GMS system has been used for diploid cotton hybrid breeding in India. They had characterized the Hisar GMS and SRT IGMS lines based on RAPD markers. They had converted RAPD marker specific to the male sterility into Putative SCAR marker which was first of its kind in diploid cotton (Geddam and Khadi, 2012). Sekhar and Khadi (2012) had worked on molecular analysis (EST-SSR) of thermosensitive genetic male sterility (TGMS) in cotton (*Gossypium arboreum* L). They had derived markers associated with TGMS traits.

Ma et al (2007) had worked upon genic male sterility (*Gossypium hirsutum* L.) using cDNA-AFLP analysis at different stages, i.e., sporogenous cell stage, pollen mother cell (PMC) stage, and pollen grain stage. They had identified seventeen differentially expressed fragments indicated that their corresponding genes may participate in the processes of signal transduction, transcription, energy metabolism, and plant cell wall development. They found that a sterility restorer factor-like gene, which only expressed in fertile anther and was notably homologous to T cytoplasm male sterility restorer factor 2 of maize (*Zea mays* L.).

Hu et al (2006) had studied PCR techniques in GMS lines. They had analysed MS2Bnap genomic DNA homologous to MS2 gene from *Arabidopsis thaliana* in two dominant digenic male sterile accessions of oilseed rape (*Brassica napus* L.). They have detected SNPs in male sterile and male fertile genomic DNA. They had discussed the probable role of SNP lead mutation in male sterility site in GMS lines.

Hirsche et al (2009) had worked upon gene promoter based studies leading to GMS. They assayed interspecies compatibility of the anther specific cell wall invertase promoters to members of the two different plant families Solanaceae (*Nicotiana tabacum*) and Brassicaceae (*Arabidopsis thaliana*). This had lead to generating male sterile plants. Yang et al (2007) had characterized and identified the candidate gene of rice thermo-sensitive genic male sterile gene. Similarly, Chang et al (2006) had identified Taigu genic male sterile wheat genes.

***2.2 Nuclear makers for sterility and fertility:***

Male-sterility is a useful plant characteristic for the production of hybrids in cotton. Five dominant and seven recessive genes conditioning male-sterility have been identified in allotetraploid *Gossypium* since Justus and Leinweber (1960) first found a heritable partial male-sterile line in Upland cotton L. (Endrizzi et al, 1985; Turcotte and Feaster, 1985). The recessive duplicate factors ms5ms6 are used in India to facilitate crossing in the production of F1 seeds (Weaver, 1968). Meyer (1975) also reported a cytoplasmic genetic male-sterile line using *G. hirsutum* L. nuclear genome in *G. harknessii* Brandegee cytoplasm. Unfortunately, this cytoplasm may have some detrimental effects to F1 hybrid yield (Weaver, 1986). In P.R. China, hybrid cotton based on the genetic male-sterile line Dong-A (*Gossypium hirsutum*) is grown on

about 40,000 hectares every year from 1984. However, a seed line consists of a mixed population of fertile and sterile plants (1:1), and the segregation and elimination of fertile plants can take place only after the first flowers bloom. This has largely restricted the use of Dong-A male sterility (Zhang and Pan, 1990).

In India, 18 loci have been identified in upland cotton, three in Egyptian cotton (*G. barbadense*) and two in Asiatic cotton (*G. arboreum*) respectively for genetic male sterility. Male sterility in cotton was found to be governed by both recessive and dominant genes. However the cases of recessive male sterility are higher than the dominant male sterility. In the Asiatic cotton the two loci reported for genic male sterility so far are under the control of single recessive gene designated as ams1 (Singh and Kumar,1993) and arms (Meshram et al, 1994). Several genic male sterile genotypes of diploid cotton were developed in India by transferring the ams1 and arms1 genes and the genotypes were classified into two major groups viz., Hisar Source GMS lines and Akola Source GMS lines. These lines have been successfully used in the heterosis breeding and it led to the release of the world first GMS based diploid cotton hybrid AAH 1 (Hisar Source) in 1999 and several other hybrids viz., AKDH 7 (Akola,source), G.cot MDH 11 (Hisar source) and CICR 2 (Hisar source) for commercial cultivation in different cotton zones of India (Geddam and Khadi, 2012).

Peng et al (2010) had done fine mapping of the recessive genic male-sterile gene (Bnms1) in *Brassica napus* L. (XianS line) based on SSR. Yi et al (2006) had done fine mapping of the recessive genic male-sterile gene (Bnms1) in *Brassica napus* L. (S45 AB line) based on AFLP. Zhen et al (2007) had done fine mapping of the recessive genic male sterility gene (Bnms3) in *Brassica napus* L. (77365AB line) based on AFLP and SCAR. Zhang et al (2011) had done fine mapping of a male sterility gene MS-cd1 in *Brassica oleracea* (79-399-3 line) based on RAPD and cDNA AFLP.

PCR-based selection of plants carrying the genic male sterility gene allele at the seedling stage should greatly enhance selection efficiency in backcross breeding. This will also help in the breeding of temporary maintainer B-line with great accuracy in early generations (Yi et al, 2006). The several authors expected to develop more useful markers (including SNP markers) by comparatively sequencing the monomorphic PCR products between the fertile and sterile plants (He et al, 2008; Zhang et al, 2011, Chariya et al, 2013).

## 3. Molecular characterization of GMS lines:

Xue et al (1999) had regenerated plants from protoplasts of photoperiod sensitive genic male sterile rice (*Oryza sativa* L.). They had compared different light and temperature conditions on growth of pollen and female fertility of diploid protoplast-derived clones. They had derived a promising PGMS (photoperiod sensitive genic male sterile) protoplast clone. Zhang et al (2006) had reported the relationship

between phytohormone and male sterility in plants. They had worked upon TGMS in wheat and suggested that the fertility alternation of TGMS wheat was closely related to levels of phytohormones in the anthers, and changes of endogenous hormone levels were among the important factors responsible for the fertility alternation of TGMS wheat. Kalaiyarasi and Vaidyanathan (2006) shown a novel breeding approach in quest of temperature sensitive genic male sterile lines among rice inter subspecies derivatives. Their investigation had revealed that the TGMS system may utilized as an innovative tool for developing inter subspecies two line hybrids with strong heterosis in rice.

The molecular marker technique has been widely used for characterization of cotton, maize, and *Brassica napus* and several other species (Ma et al, 2007; Bhatt et al, 2011; Girnari et al, 2011; Ramavat et al, 2010; Ramanuj et al, 2010). Dong et al (2000) worked upon molecular analysis of TGMS in rice. They had performed molecular mapping of a rice gene conditioning thermo sensitive genic male sterility using AFLP, RFLP and SSR techniques. Viraktamath and Virmani (2001) studied the expression of thermosensitive genic male sterility in rice under varying temperature situations. Ying et al (2003) worked upon the molecular markers (AFLP and STSs) for genic male sterility in Chinese cabbage. This molecular marker found to useful for marker assisted selection of male sterile plants among segregating populations. Chen et al (2009) worked upon genetic and histological characterization of a novel recessive genic male sterile line of *Brassica napus* derived from a cross with *Capsella bursa-pastoris*. They had used AFLP markers for characterization and discussed possible mechanisms for the production of the male sterile hybrid and its potential in breeding.

### *3.1 Percent polymorphism of GMS lines:*

Geddam and Khadi (2012) had used RAPD marker system to characterize the Hisar GMS and SRT 1 GMS lines. They had used 60 random decamer primers, among which 34 had generated 60.73 per cent polymorphism between male sterile and male fertile plant. They found certain primers OPAB3, OPAB4, OPAB5, OPAB19, OPH20, OPI2, OPI3 and OPI7 shown notable differences in the amplicon profile of male sterile and their fertile counterparts. Mandaliya et al (2015) had generated RAPD (25.88%), ISSR (13.45%) and SRAP (9%) polymorphism in GMS lines. They found RAPD primers had shown highest polymorphism in GMS lines. Total 49 primers out of 74 primers in combine analysis had generated 19.45 % polymorphism among GMS.

### *3.2 Heterozygosity (H) and polymorphic information content (PIC) value of GMS lines:*

Ritschel et al (2004) has estimated PIC and H value for melon (*Cucumis melo* L.) based on microsatellite markers. PIC value was ranging from 0.28 to 0.65 and h value varying from 0.45 to 0.70. Mandaliya et al (2015) had used 49 primers together and found that H value ranged from 0.000 to 0.500, PIC value ranged from 0.000 to 0.375

with mean of 0.166. This indicated that a small set of selected markers should be sufficient to solve questions regarding genotype identity.

***3.3 Unique markers for GMS lines:***

Geddam and Khadi (2012) had found that primer OPI3 amplified male sterile specific fragment of 486 bp. They observed that this RAPD markers associated with male sterility may facilitate the utilization of the GMS system in hybrid breeding in the Asiatic cotton. There were several other reports on various markers studied in GMS lines. Wang et al (2004) had worked upon RAPD analysis of GMS in rice (*Oryza sativa* L.). They had developed a genetic marker linked to a new thermo-sensitive male sterile gene in rice. Hong et al (2006) had worked up on AFLP and SCAR markers that linked to the suppressor gene (*Rf*) of a dominant genetic male sterility in rapeseed (*Brassica napus* L.). The available SCAR markers of the *Rf* gene will greatly facilitate future breeding programs using dominant GMS to produce hybrid varieties.

Hayashi et al (2011) had developed SCAR and CAPS markers linked to a recessive male sterility gene in lettuce (*Lactuca sativa* L.). Their markers studies could be useful to screen a large population in a short time to find out sterile individuals and will greatly accelerate the cloning of the genetic male sterility gene. Ke et al (2004) had worked upon AFLP markers techniques and they had linked AFLP markers to recessive genic male sterility (RGMS) in rapeseed (*Brassica napus* L.) and converted it to SCAR markers for marker-aided selection. Mandaliya et al (2015) used SRAP, RAPD and ISSR markers for successful development of unique markers to discriminate each GMS lines. The molecular markers were proven to develop unique markers in GMS lines.

***3.4 UPGMA relationship of GMS lines:***

Geddam and Khadi (2012) had calculated pair wise similarity coefficient value for four near isogenic GMS lines (Hisar GMS and SRT 1 GMS). They found that overall similarity indices ranged from 0.77 to 0.85. Least similarity (0.77) had observed between fertile and sterile plant of genotype SRT 1 and between sterile plants of genotype Hisar and fertile plant of SRT 1 genotype. Whereas, highest similarity (0.85) had observed between fertile plants of genotypes Hisar and fertile plant of genotype SRT 1. They found that the dendrogram revealed two distinct clusters, all fertile plants made independent cluster, similarly, all sterile plants made another independent cluster. Mandaliya et al (2015) found highest similarity between G-203 and G-205 (0.8350) and their UPGMA analysis also revealed same that shown that G-203 and G-205 lines had most near lines and G-217 had been most diverted from other all GMS lines.

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