

Constraints of hybrid seed production in upland and cultivated diploid cottons : Will different male sterility systems rescue? A review

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ABSTRACT : Cotton fibre is an important raw material for the textile industry. Upland cottons (*Gossypium hirsutum* L.) account for over 90 per cent of lint production because of their high productivity and it is corner stone of the textile industries worldwide. In addition, in India Asiatic cottons (*G. arboreum* and *G. herbaceum*) are known as *desi* were grown on about 98 per cent area around 1947 and the *G. hirsutum* on just around 2. Presently, the situation is now exactly the reverse. The diploids and their hybrids are cultivated on very less (0.50 lakh ha) area in which *desi* cotton hybrid contributes only 1 per cent in production.

However, now a day even short and coarse staple of diploid cotton is in great demand, particularly in fabrics like denim and upholstery, filling and medical purpose as well as fetches attractive price also. So, why not promote *desi* cottons, which are resistant to drought, water logging, diseases and pests especially cotton leaf curl disease, a dread disease of *G. hirsutum*, well adapted to the climatic aberrations, suitable under rainfed conditions, wider adaptability and low cost of management? Thus *desi* cotton is to the rescue of Indian cotton growers.

However, they are inherently low yielders and hence need to improve their genetic yield potential. Heterosis breeding (developing superior hybrids) is a good approach in these directions. The first ever success story of heterosis breeding in tetraploid cotton encouraged cotton breeders to explore the possibility of similar attempts in diploid cotton, that resulted in released hybrids viz., G.Cot.DH7 and G.Cot.DH9, which have not covered sizable area, due to the problem of seed production, the high cost of conventional hybrid seed, which are limiting factors for poor/ marginal farmers to grow hybrids. Hence, the systems of male sterilities are of great significance in practical, as it avoids laborious process of emasculation and it can add in production of hybrid seed. However, with the availability of genetic male sterility (GMS), photoperiod-sensitive genic male sterility (PGMS), thermo sensitive genic male sterility (TGMS) and environmental male sterilities (EGMS) lines in *G. arboreum* seed production cost can be reduced with increased purity. Therefore, these sterilities were thought to be a best, economical and alternative method for hybrid seed production technique in cotton and especially in diploid. Merits and demerits of these sterilities are reviewed and discussed.

A remarkable heterosis for growth and yield reported in upland and diploid GMS based hybrids. Based on the earlier reviews on different aspects viz., genetic effects of heterosis, inheritance of GMS, cytological aspects of microsporogenesis breakdown, physiological and biochemical indices associated with GMS, environmental effects on expression of GMS, development of GMS and their utilization in producing hybrid seeds, practical problems of their utilization etc., future line of research needs etc. are proposed.

Key words : *Gossypium*, environmental effect, hybrid seed production, environmental male sterilities (EGMS), hybrid cotton, genetic male sterility (GMS), photoperiod sensitive genic male sterility (PGMS), thermo sensitive genic male sterility (TGMS), microsporogenesis breakdown, molecular and biochemical bases sterilities

Cottons are not only a world's leading textile fiber and oilseed crop, but also a crop that is of significance for foil energy and bioenergy production. Out of the 50 *Gossypium* species, four, including two *G. hirsutum* and *G. barbadense*

allotetraploids and two *G. herbaceum* and *G. arboreum* diploids are cultivated. Upland cotton (*G. hirsutum*), known for, long staple cotton, or Mexican cotton, produces over 90 per cent of the world's cotton; *G. barbadense*, (Sea Island,

American Pima, or Egyptian cotton) also known for extra long staple (ELS) cotton, contributes 8 per cent of the world's cotton; and *G. herbaceum*, also known as Levant cotton, and *G. arboreum*, also known as Tree cotton, together provide 2 per cent of the world's cotton (Zhang, *et al.*, 2008).

After the unpredicted success of F_1 hybrids with maize, several attempts were made to apply the same principle to other crops as well (Christidis, 1955). A remarkable heterosis reported in cotton (Loden and Richmond, 1958, Rao, 1968, Davis, 1978, Khadi, *et al.*, 1993, 2010; Sun *et al.*, 1994, Basu, 1995, Basu and Paroda, 1995, Meredith, 1999, Feng *et al.*, 1993, Wang and Li 2002, Xing *et al.*, 2002 and Khadi, 2011). Further, genetic effects of heterosis (Anonymous, 1990), cotton MS lines and inheritance of male sterility (Feng *et al.*, 1998), observation of microsporogenesis breakdown (Feng *et al.*, 1993), physiological and biochemical indices associated with male sterility (Feng *et al.*, 1993, Xing *et al.*, 2002), development of GMS and their utilization of CMS and nuclear male sterile (NMS) lines in producing hybrid seeds (Xing *et al.*, 2002) and the relation between GMS and the external environmental factors on expression NMS including its potential, practical and problems for their utilization (Davis, 1978, Xing *et al.*, 2002, Anonymous, 1990, Turner, 1959) etc., were discussed.

In spite, appreciable heterosis registered in cotton, its commercial use was stymied due to the fact that cross pollination cannot be controlled. Thus, unless a practical means of sterilizing the male organs can be found, it was thought impractical to make controlled crosses in cotton on commercial scale as well as to develop techniques applicable to large scale hybrid seed cotton production (Turner, 1959).

India is pioneer in development and commercial cultivation of hybrids. The first intra specific [(*G. hirsutum*, (H) x *G. hirsutum*, (H)] commercial hybrid of the world 'Hybrid 4' was developed by using hand emasculation and pollination method by Dr. C. T. Patel, the 'Father'

of hybrid cotton during 1971 in India. Similarly, first inter specific [(*G. hirsutum*, (H) x *G. barbadense*, (B)] the world's first inter specific hybrid 'Varalaxmi' (Katarki, 1971) followed by 'Savitri' (Thombre and Ekbote, 1978) were also developed. The two events transformed the entire cotton scenario of India. H 4 became highly successful in central India giving more than twice the yield compared to the recent varieties G.67. The extent of heterosis observed in inter specific hybrid was 10 to 138 per cent and in *intra hirsutum* hybrid 7 to 50 per cent. In both cases, under highly favourable environments, 80 per cent to 187 per cent heterosis has been observed in India (Khadi, 2011). Hybrids have higher productivity; wider adaptability and high degree of resistance to biotic and abiotic stresses. Hybrids give 50 per cent higher yield than straight varieties. The wider adaptability of hybrids is due to their high buffering capacity to environmental fluctuations (Kairon and Singh, 1998).

Exploitation of heterosis is used to increase cotton yields in countries where a cheap labor force is available to make hand emasculation and crossing. About 60-70/day/ha labours are needed for emasculation and pollination work during peak flowering period while early and late stages of flowering, the labour requirement is less. Labour cost is the major expenditure in hybrid cotton seed production (Anonymous, 1990). The major limiting factor in using heterosis for hybrid cotton production is the lack of an efficient and dependable system for producing hybrid seed mainly due to the ineffectiveness of the male gametocide (Meredith and Brown, 1998), and the inconsistency of results from MS and restorer factors (Percy and Turcotte, 1991). In cotton producing countries, India and China have rapidly adopted hybrid cotton production systems and increased the yield.

Despite hand emasculation and pollination, hybrid seed production proved to be remunerative to the hybrid seed producers and provided, on an average, a net income of over \$

600/ha. The most significant impact of hybrid cottonseed production technology appeared in the form of employment generation of about a 25 million labour force (women being around 23 million) (Paroda, 2004). Since then heterosis has been exploited in several combination for economical use in H x H, H x B, *G. arboreum* (a) x (a) and *G. herbaceum* (h) x (a)³⁰. In view of economic importance of cotton in the world and to meet the increasing demand of cotton, and F₁ hybrid seed the use of CMS (cytoplasmic male sterility), GMS (genetic male sterile) and CGMS (cytoplasmic genetic male sterility) line approach may prove the best alternative method of hybrid seed production in cotton as this system can reduce the cost of hybrid seed production considerably less almost to half (Anonymous, 2011).

GMS is an inherited trait that prevents the production of functional pollen, but maintains female fertility. The basic contribution of GMS is that it provides a means of genetic emasculation which can be applied for the massive production of hybrids. In addition, it is mainly applied for the production of hybrid varieties and inter and intraspecific hybridization and back-crossing programmes for the introduction of genetic variation into crop varieties. Several schemes have been proposed for using GMS in hybrid breeding in different crops (Rao, *et al.*, 1990).

Genetic / nuclear male sterility genes in *G. hirsutum* and *G. barbadense* cottons

In *G. hirsutum* till 1988, 4 dominant MS materials and 3 recessive MS genes were reported (Huang, *et al.*, 1982). Later on total of 11 loci have been identified controlling GMS. Out of them 10 are in *G. hirsutum* and one in *G. barbadense* (Endrizzi, *et al.*, 1985, Turcotte, and Feaster, 1985). Four male sterile genes, namely *Ms₄* (Allison and Fisher, 1964), *Ms₇* (Weaver and Ashley, 1971) and *Ms₁₀* (Bowman and Weaver, 1979) are dominant and produce complete sterility while the remaining seven are recessive. Among the recessive genes, *ms₁* (Justus and Lienweber, 1960), *ms₂* (Richmond and Kohel, 1961), *ms₃*

(Justus, *et al.*, 1963), while *ms₅*, *ms₆* (Weaver, 1968), *ms₇* (Harvey, 1969) used this symbol, but it is actual *ms₂* (Weaver, 1971), as on the basis of test crosses it is proved it is a misnomer of *ms₂*, and is independent from (Weaver and Ashley, 1971), *ms₆*, *ms₈*, and *ms₉* indehiscent anthers (Rhyne, 1971) behave as duplicate recessive genes. In addition, *ms₁₄* (Dong A), (*ms₁₅* (Lang A), and *ms₁₆* (81 A) (Zhang and Pan, 1990) conditioning male-sterility have been identified in *G. hirsutum*. As compared with fertile sibs, the MS plants tend to have larger and tender leaves with deeper lobes and slight overall plant size reduction, especially during seedling stage (Zhang and Pan, 1990).

In *G. barbadense* GMS genes *viz.*, *MS₁₁* male sterile (Turcotte and Feaster, 1979) and a dominant male-sterile mutant, which is assigned the gene symbol *Ms₁₂*. Linkage tests between *Ms₁₂* and 23 marker genes were negative. Because dominant male steriles cannot be intercrossed, distinct loci designations are based on phenotypic differences, and gene designations have to be considered tentative (Turcotte and Feaster, 1985) and *ms₁₃* (Percy and Turcotte, 1991) were reported. The gene symbols *ms₍₁₄₎*, *ms₍₁₅₎* and *ms₍₁₆₎* respectively for Dong-A, Lang A and 81A instead of *msc₁*, *msc₃* and *msc₇* were proposed (Feng, *et al.*, 1993).

Over view of linkage of MS genes with others indicated, *ms₃* gene is located on chromosome 16 at 20 units apart from *R₁* (Red plant) on the far side of *cl* (Cluster bolls) and probably near to *ygl* (yellow green foliage) (Kammacher, *et al.*, 1967), leaf abnormality was found linked with GMS (Quisenberry, and Kohel, 1968), particularly with *ms₇* (Weaver, 1969), *ms₃* genes was linked with *R₁*, and *DW* (dirty white fibre) were located on chromosome 16. Since *ms₃* and *ygl* are linked with recombination value 6.9 per cent, the *ygl* can be used as a marker for identification of MS plants (Leport, 1970). Genes *MS₄*, *MS₇* are non-allelic to *Msp*. *Msp* is linked with '*gl₁*' (glandless) and '*N₁*' (naked seed) with 9.38 ± 0.98 per cent and 13.95 ± 1.89 per cent recombination, respectively (Turcotte and

Feaster, 1979) while ms_3 was located in linkage group III (Justus, *et al.*, 19963), whereas ms_8 and ms_9 are in the V, IX (Rhyne, 1971). As compared with fertile sibs, the MS plants tend to have larger and tender leaves with deeper lobes and slight overall plant size reduction, especially during seedling stage (Zhang and Pan, 1990).

Inheritance of Ms /ms genes GMS in *G. hirsutum* 81A controlled by one pair of recessive gene and was always associated with virescence due to pleiotropy as male sterility and virescence showed no recombination. However, very close linkage could not be ruled out. Allelic tests indicated that virescence observed in 81A is non allelic to all virescence genes identified earlier in upland cotton and its male-sterility is non allelic to msc_1 , msc_2 and msc_3 , hence proposed msc_7 gene for the control of GMS in 81A (Zhang and Pan, 1990). Six GMS genes, msc_1 , msc_2 , msc_3 , Msc_4 , Msc_5 and msc_6 have been identified and were tested (Huang, *et al.*, 1982). The male sterility in 'Dong A', 'Shi A' and 'Lang A' was under the control of the three recessive genes msc_1 , msc_2 and msc_3 in order (Huang, *et al.*, 1982). It could make the frequencies of both sterile plant and sterile flower of the subsequent generations of cotton plant with a pair of msc_1 genes maintain 100 per cent sterility (Huang, *et al.*, 1982).

Breeding GMS lines : The methodology of developing nuclear male-sterile line and its usage in cotton were reviewed (Khadi, *et al.*, 2010, and Anonymous, 1990). GMS system involving ms_5ms_5 ms_6ms_6 (Weaver, 1968), found in 'Gregg' MS, is the only stable source, utilized in India¹⁷ (Plate I, Figs. 1-4). All *G. hirsutum* genotypes which carry Ms_5 or Ms_6 , (or both genes) are restorers. Any *G. hirsutum* line can be converted into GMS system by repeated back crossing with alternate selfing and selection (Anonymous, 1990).

Ms "Dong A" was derived from complete MS plant was found in a plot of a cultivated variety of *G. hirsutum* L., Dong ting 1, in 1972. The male-sterile character in this MS line was conditioned by a pair of homozygous recessive alleles at a

single locus. In 1978, a full maintainer line MB of cotton NMS line "Dong A" was developed (Huang, *et al.*, 1982). The sib-mating between the MS plants and the MF plants from same line produced F_1 progenies with each 50 per cent MS and MF plants which could be used as A and B line, respectively to produce hybrid seeds in cotton (Anonymous, 1990 and Huang, *et al.*, 1982). In addition to the one pair of recessive major genes for male sterility, a polygenic system for the pollen spreading anther character was also found involved. When a dominant major gene for fertility was present, the action of the polygenic system was concealed and GMS was inherited as a qualitative character. But when the major genes were homozygous recessive, the polygenic system manifested modifying effects on GMS plants, so that there was some pollen spreading or partial fertility. Additive, dominance and additive x dominance epistasis were all very significant for pollen spreading; additive and additive x dominance were positive and dominance was negative. This type of genetic model is referred to as the major gene polygene interaction model (Mao, *et al.*, 1995). By using a score of 'pollen spreading index' of pollen spreading sterile plants and their F_1 , a NMS line M-B 159 with high fertility was developed. The maintainer line had pollen spreading index of 76 per cent and the sterile plant rate and sterile degree of its F_1 were 100 per cent (Huang, *et al.*, 1982).

The maintainer line Mb could make the sterile plant rate and sterile degree in the progeny of the GMS cotton "Dong A" reach 100 per cent and 96-100 per cent, respectively. Propagating sterile line with the MB and then breeding F_1 hybrids was named as "two step method" or MMS method (The method of producing hybrids, based on the GMS AB line and with the specialized maintainer line MB and R line). The practical model of this method was reported⁵⁹ and compared with "three line method" of CMS (CMS, The method of producing hybrids, based on the CMS A, maintainer B, and restorer R lines and "two line method" of GMS (The method

of producing hybrids, based on the GMS A B A and B lines) in the propagation of sterile line, seed yield and quality of hybrids. The results indicated that the effect of MMS method was evident in raising yield and quality of hybrid seeds (Huang, *et al.*, 1982).

Using msc_1 and msc_2 , three NMS lines 473A, Kang A and 1355A have the merits of high yield, disease resistance and high fibre strength have been selected and they have been used as both A and B line in the seed production of hybrid cotton. For the present NMS lines, a complete maintaining line M line has been selected from a material in which there probably are modifying genes or gene interaction. It could make the frequencies of both sterile plant and sterile flower of the subsequent generations of cotton plant with a pair of msc_1 genes maintain 100 per cent seeds (Huang, *et al.*, 1982). The new recessive NMS line M A developed along with its maintainer for which both *G. hirsutum* and *G. barbadense* varieties were found restorers (Huang, *et al.*, 1982). Different methods including selection from mutants, artificial induction, genetic improvement etc., used to develop 17 kinds of NMS lines. The sterile plant rate of NMS was enhanced per generation that reached 65 per cent after 3rd generation of selfing (Hou, *et al.*, 2002). Male sterility of a MS material, derived storm proof variety, was controlled by two recessive genes (ms_5ms_6) that had no apparent deleterious effect on yield. The F_1 hybrids between these female parents had superior hybrid vigor. It was suggested that Its F_2 seeds may be utilized on a commercial scale, since only one out of 16 plants was MS.

An account of the sterility mechanism such as morphology, cytomorphology, physiology, biochemistry and molecular biology of nuclear MS (NMS) line “Dong A” was summarized and discussed the problems (Zhang *et al.*, 1990), application (Zhang *et al.*, 1990) and its development prospect (Zhang *et al.*, 1990) of NMS in cotton.

Cytological basis of GMS : Cytological

observations regarding pollen abortion in GMS 81A (Zhang and Pan, 1990); 473A (derivative line of Dong A) (Zhang and Pan, 1990), TM 1 fertile plants (Zhang and Pan, 1990) 1355A GMS “Dong A” (Liu and Nie, 1994), MA in comparison with “Dong A”.

Female and male sterility in F_2 was due to asynapsis during megasporogenesis and microsporogenesis, respectively controlled by as_1 and as_2 , are proposed for this character (Weaver and Ashley, 1971). Cytological observations of microsporogenesis breakdown were used to differentiate a new dominant male sterile character in upland cotton, *G. hirsutum* L., from the two known dominant male steriles, Ms_4 and Ms_7 . Microsporogenesis breakdown in Ms_{10} occurred in pre meiotic stage except for a few degenerating sporogeneous cells which distinguished from Ms_4 (Bowman, *et al.*, 1978). Microsporogenesis breakdown in pre meiotic stage in Ms_4 (Allison and Fisher, 1964)

In the Ms_7 genotype breakdown occurs during either premeiotic or early stages of meiosis or at the onset of pollen wall formation, while the pollen in the new male sterile aborted only after the entire pollen wall, both intine or exine, had been fully developed (Bowman and Weaver, 1979). Thus, breakdown was consistently post meiotic although a few sporogenous cells may first degenerate. Compared to normal fertile pollen grains, the sterile grains had a significantly thicker and more intensely staining intine. They also possessed plugs at germ pore regions that failed to stain with toluidine blue. Histo chemistry revealed these plugs were composed of hemicellulose or some complex polysaccharide. Histo chemical studies also disclosed a unique layer of insoluble carbohydrate located between the intine and exine walls in the sterile microspores. Cytoplasm disintegrated progressively during pollen development resulting in sterile pollen grains that were conspicuously shriveled and vacuolated at maturity. The Ms_{10} gene differed from Ms_4 and Ms_7 , in microsporogenesis breakdown (Bowman and

Weaver, 1979).

Cytological observations indicated $2n = 52$ chromosome number (Zhang and Pan, 1990), the normal meiosis and followed by a regular tetrad formation and separation of microspores and their degeneration during the development of the pollen wall. Occurrence of pollen abortion was observed mainly in the late uninuclear stage (Zhang and Pan, 1990). Cytomixis of pollen mother cells (PMCs) in the stages of meiosis (Zhang and Pan, 1990) was found, in addition to abnormalities such as lag behind univalent in Metaphase I and Anaphase I and II (Zhang and Pan, 1990), unequal distribution of chromosomes and production of monad, dyad, triad and polyad (Zhang and Pan, 1990) etc., were observed. In a few locules, some microspores developed were vacuolated uninuclear or early binucleate pollen. The developing pollen grains were shriveled and shrunken and lost their contents (Zhang and Pan, 1990) and finally disintegration and shrinkage of microspore cytoplasm (Soddi, 1995) even in a few locule, some microspores developed were, vacuolated, uninuclear or early binucleate pollen. Though some residues of nucleus and karyotheca were observed in some instances after the pollen wall differentiation (Zhang and Pan, 1990). The wall of aborted pollen was incompletely developed and they had either few small spines cent protuberances on the pollen wall but without aperture or without exine spine (Khadi, *et al.*, 1994). Many kinds of abnormal changes happened in the tapetum be due to the abortion of pollens (Khadi, *et al.*, 1994). On the contrary, normal behavior of the tapetal cells and middle layer did not appeared to be the cause of MS (Zhang and Pan, 1990). In some instances degeneration of dyad and tetrad occur or microspores degenerate during development of pollen wall in the Ms_7 (Bowman and Weaver, 1979). The supply/inability of developing microspore to absorb the nutrients might be the reason for the pollen abortion in the MS line because of early disintegration of tapetum (Kajjidoni, *et al.*, 2009) (Plate I, Figs.5-6).

Pollen abortion in "Dong A" happened during all the stages of pollen development. It began at the PMC stage, the 5th day after budding, but most pollen aborted at the prophase I of meiosis, the 7th to 8th day after budding. Numerous different sized and abnormal pollens were spread from the tetrads of 10 day development. Uninuclear (Khadi, *et al.*, 1994) and few binuclear pollens aborted in succession during 15 to 20 days after budding, The stage and appearance of the abortion of the MS line "MA" are basically similar to those of "Dong A", but one day earlier.

The observations on abortion of pollens in comparison with "Dong A" at different development stage using scanning electron microscope with paraffin dissection revealed abnormalities like too more callus settlings outside the PMCs and very slow melting of settlings (Khadi, *et al.*, 1994). In Ms_4 examination of mature rudimentary anthers showed no development of sporogenous tissue indicating the meiosis was abnormal or absent, thus preventing the development of pollen. No chromatin material found other than present in tapetal cells (Allison and Fisher, 1964). Pollen abortion in 81A occurred at uni-nucleate stage. Even though bi-nucleate pollen observed, they were smaller and could not participated in fertilization (Zhang and Pan, 1990).

Biochemical bases of GMS : Analysis of the contents *e.g.*, carbohydrates, free amino acids, IAA, GA_3 and ABA in anthers of different stages of 'Dong A' 'Kang A 1' and 'MA' recessive GMS lines reveled less starch accumulation and abnormal contents of 4 free amino acids in sterile anthers. The sterile anthers had higher contents of aspartic acid and lower contents of proline, arginine and phenylalanine than that of fertile anthers. Markedly higher ABA content, lower IAA and GA_3 contents than the fertile anthers were also detected in all four anther development stages. Abnormal contents of carbohydrates, free amino acids and phytohormones may be

associated with the pollen abortion. The electrophoretic profile of seed protein was unique and distinct for each genotype more by their quantitative than qualitative difference. A total of eight bands of different staining intensities were observed with relative mobility (Rm) values ranging from 0.30 to 0.90. Two bands were found to be more common in AKH 07 R, PKV - HY 5, CAK 053 A, AKH 545 R, CAHH 185 and CAK 071 A genotype under study. A minimum of three and a maximum of four bands were observed. The quantitative and qualitative variation in the banding pattern helped in distinguishing all the genotypes from one another (Koshatwar, *et al.*, 2010).

Seventeen differentially expressed fragments were identified by cDNA amplified fragment length polymorphism (cDNA-AFLP) analysis between sterile and fertile plants of *ms5ms6* double recessive GMS of *G. hirsutum* at sporogenous cell, PMC, and pollen grain stages. 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.), was identified which demonstrated the credibility of the result of cDNA-AFLP (Ma, *et al.*, 2007). Markers highly linked to *ms₁₅* and *ms₅* genes on chromosome 12 (Linkage group V) were reported (Chen, *et al.*, 2009). A male sterile mutant by T-DNA insertion was crossed with a wild type upland cotton (*G. hirsutum* L.) cultivar Coker312. The segregation ratio of male sterile to male fertile was 16:1 in generation F₁. The results of morphological observation, kanamycin and herbicide resistance assay, PCR identification and corresponding genetic analysis showed that the male sterile was co segregated with the T-DNA insertion. This male sterile mutant was considered as dominant heterozygous mutation caused by the T-DNA insertion, which would lay the basis for cloning the male sterile related genes of upland cotton by T-DNA tagging. The *ms5ms6* source was analysed by RAPD using a set of 340 primers. Primers *viz.*, OPB4, OPC5, OPE17,

OPG7, OPL2, OPF2, OPK20, OPL 16, OPL9, OPM7, OPL 19 were polymorphic. The primer OPL19 has shown high and consistent reproducibility, marked with the presence of the ~500bp polymorphic amplicon detected in the sterile plant having correlation with the phenotypes of individual plants. Thus marker assisted selection with genetic male sterility system, offers clear advantages (Kopulwar, *et al.*, 2007) overcoming the problem of identification of male sterile and fertile plants in seed production plot (Kopulwar, *et al.*, 2007).

A differentially expressed fragment, which was similar to plant ADP ribosylation factor between "Dong A" male sterile (*ms₅ms₆*) and its maintainer line was differentiated using cDNA-AFLP differentially expressed genes in sterile (*ms₅ms₆A*) and maintainer (*ms₅ms₆B*) during anther development (Hou, *et al.*, 2002). Involvement of many genes in various aspects of anther development and reverse gene expression pattern was observed in GMS mutant, that indicated diverse gene regulation pathways are involved in GMS mutant anthers (Wei, *et al.*, 2013).

Effect of environmental factors on expression of GMS : Based on analysis of the mean day temperature and the relative humidity at the meiosis of PMC and uninuclear stage of microspore development, it was revealed that the mean day temperature play a key role than the relative humidity in the development of PMC and uninuclear microspores NMS lines *viz.*, Jinan, Pingdu, Laixi and Yantai while the fertility of MA and MB derived from the "Dong A" MS line was different under various climatic conditions. Further even though MA and MB were MS lines in one region, they reverted to fertile in some degree in other regions. While such change was not observed in 59A and 62A (Zhang, *et al.*, 1990). New TGMS line 'Kang A1' was sterile in low temperature and fertile in high temperature. The critical temperature of sterility transformation was 27°C and the sensitive time was 7-13 days before blooming. Commercial varieties

Damian 1 or 67—69 (Huang, *et al.*, 1988).

Fertility of full maintainer line mb of cotton and NMS line “Dong A” found changed in different climatic locations (Liu, *et al.*, 1994). Under the different climatic condition, the full maintainer line MB NMS “Dong A” reached about 76per cent in pollen spreading. Similarly the fertility of MB disappeared, just the same as MA.

Performance of GMS based hybrids :

Evaluation of GMS based F_1 cotton hybrids proved their superiority in seed cotton yield over the best varietal/conventional hybrid check under cultivation (Khadi, 2011) and as a result hybrids CPH 2 and CPH 4 were released for commercial cultivation (Santhanam, *et al.*, 1972; Srinivasan, *et al.*, 1972, a; Srinivasan and Gururajan, 1976; 1978, a and b). The inheritance of GMS of some *G. hirsutum* genotypes by crossing with Gregg revealed that some genotypes had homozygous dominant alleles either at one or two locus/loci (Srinivasan, *et al.*, 1972, b). The GMS system has an advantage over CMS where large number of males can be tested with GMS as female parents as almost all of them give fertile F_1 s whereas limited number of crosses can be made with CMS as females due to limited number of restorers are available at present. Most importantly, conversion of male genotypes is not required in GMS system while it is a must in case of CMS system which is time consuming and tedious process. The genetic background, local adaptability and genetic diversity of both the parents in a cross may also be responsible for the superiority of GMS hybrids over CMS (Bhale, and Bhat, 1990). Overall performance of GMS hybrids was better than CMS over the years (Bhale, and Bhat, 1998) might be because of large number of cross combinations that could be tested in GMS system unlike to CMS where limited number of crosses could be made and became available for testing due to the availability of only a few restorers. In addition when, F_2 hybrids are to be grown commercially, double recessive GMS types are better than single recessive GMS types as

only 6.25per cent plants are sterile in double recessive type while 25per cent plants are sterile in single recessive type in F_2 generation (Padaki, *et al.*, 1980 and Zhan, 1993).

In India efforts were made to develop In *G. hirsutum*, GMS lines developed in India LRA 5166, SRT 1, DGMS 1, HGMS 2, GAK 32A, SHGMS 9, DGMS2, SHGMS 5 (Sing, *et al.*, 2002) and hybrids developed utilizing these and other lines were tested for yield and fibre properties along with check released GMS based CPH 2 and CPH 4 and conventional H 4, Dhan Laxmi, LHH 144 (Bhale, and Bhat, 1990, 1998; Nirania, *et al.*, 2004a, b and Tuteja *et al.*, 2005).

The GMS hybrids were superior to CMS hybrids NIGMSH 11 and NIGMSH 6 (Bhale, and Bhat, 1990), superior to H 4 (Bhale, and Bhat, 1998); NIGMSH 0-13, 0-159, 0-165, 0-26 and 0-91 superior to H 4 (Bhale, and Bhat, 1990), for 50per cent economic heterosis over H 4, NIGMSH 220, 222, 228, 239, 245, 248, 262 and 266 (Santhanam, *et al.*, 1972; Srinivasan, *et al.*, 1972; Srinivasan and Gururajan 1976, 1978), CSHG 18516 x PHP 7 (Tuteja, *et al.*, 2005), and CSHG 12517 x PHP 7 and GMS 15 (Tuteja, *et al.*, 2005), CSHH 198 and GMS 15 (GMS 4 x 002 NAH) (Tuteja, *et al.*, 2011, a). Thus, the cross combinations of GMS 4 x F 1861, and GMS 4 x LH 2076 (Tuteja, *et al.*, 2011, b), IAN579 x A72-15 and SA278 x G6030 (Nirania, *et al.*, 2004).

From the literature reviewed so far, it appeared that in China *G. hirsutum* Dong A (ms_{14}), Lang A (ms_{15}), and 81 A (ms_{16}) (Zhang *et al.*, 1992), 81A (msc_7) controlled by one pair of recessive gene and was always associated with virescence due to pleiotropy (Zhang and Pan, 1990), Dong A (msc_1), Shi A (msc_2) and Lang A (msc_3) (Huang, *et al.*, 1982). 70416A (ms_5ms_6 double allele). Dong A (Huang, *et al.*, 1982) M-B 159 (Huang, *et al.*, 1982) 473A (msc_1), Kang A (msc_2) and 1355A (msc_3) (Huang, *et al.*, 1982), M-A derived from cross “Dong A” x MB (B-line) (Huang, *et al.*, 1982). Mian A₁ and Zhongkang A based on ms_5ms_6 double recessive (Xing, *et al.*, 2002). Different male sterility genes utilized for developing cotton

hybrids in China were, ms_{14} and ms_5ms_6 and GMS lines 473A—a derivative from Dong-A × Shon 1-170 (Huang, *et al.*, 1982), Dongton 1 (Huang, *et al.*, 1982), Jinan, Pingdu, Laixi and Yantai (Hao, *et al.*, 2002).

GMS based promising cotton hybrids viz., Chuanza 3, Chuanza 4 and Zajiaozhao (Huang, *et al.*, 1982) Chuan hybrid cotton 15 (Xing, *et al.*, 2002). ‘Zhongmiansuo 38’ and ‘Nannong 6’ were developed⁷³ and the GMS based hybrids 473A × Kiangen 203, 473A × 67—69, 473A × Heisan 1 (Huang, *et al.*, 1982) reported potential for commercial cultivation.

Extent of heterosis in GMS based hybrids Significant heterosis for yield and fibre quality parameters in GMS based hybrids was observed as compared to conventional hybrid (Bhale and Bhat, 1998; Ano, 1976). Heterosis for seed cotton yield (Huang, *et al.*, 1982; Bhale and Bhat, 1990; Tuteja, *et al.*, 2005; Tuteja, *et al.*, 2011, b; Yue, *et al.*, 2007; Khadi, *et al.*, 2003; Ano, 1976; Tuteja, *et al.*, 2011, a; lint yield (Huang, *et al.*, 1982, 1988); lint weight /boll (Patel, *et al.*, 2000); early flowering Ano, 1976, bolls/plant (Tuteja, *et al.*, 2005; Tuteja, *et al.*, 2011a), boll weight (Tuteja, *et al.*, 2005; Tuteja, *et al.*, 2011a), monopods/plant (Tuteja, *et al.*, 2005; Tuteja, *et al.*, 2011a) sympodia/ plant (Tuteja, *et al.*, 2005), plant height (Tuteja, *et al.*, 2005), lint index (Tuteja, *et al.*, 2005; Patel, *et al.*, 2000), seed index (Tuteja, *et al.*, 2005), Tuteja, *et al.*, 2011, a) Patel, *et al.*, 2000; Tuteja, *et al.*, 2011, a,; ginning percentage (Tuteja, *et al.*, 2011a and b), GOT (Tuteja, *et al.*, 2005), Patel, *et al.*, 2000; Tuteja, *et al.*, 2011, a).

Heterosis for fibre properties of GMS hybrids observed (Huang, *et al.*, 1982, 1988; Yue, *et al.*, 2007; Ano, 1976), for quality traits like 2.5 per cent span length Tuteja, *et al.*, 2011, a and b, Tuteja, *et al.*, 2011, a, and for fibre strength (Tuteja, *et al.*, 2011, a and c), bundle strength, maturity coefficient, micronaire value (Tuteja, *et al.*, 2011, b).

The component traits, namely, sympodial branches, bolls/plant and boll weight showed significant positive association with seed cotton

yield as well as among themselves (Tuteja, *et al.*, 2005). Disease resistance (Huang, *et al.*, 1988), resistance to worm as the female parent resistance to pink worm and bollworm (Yue, *et al.*, 2007).

Yield and contributing traits exhibited superiority of conventional (euplasmic × euplasmic) hybrids over CMS based alloplasmic × euplasmic hybrids as well as and over GMS based euplasmic × euplasmic hybrids. However, for fibre quality traits the trend of performance was variable. Alien cytoplasm and nuclear genes did not exhibit deleterious effects for fibre quality related traits, even though a gain was reported in some of the CMS based alloplasmic × euplasmic hybrids for 2.5 per cent span length and uniformity ratio over the euplasmic × euplasmic hybrids. CMS based alloplasmic × euplasmic hybrids expressed their superiority over the GMS based hybrids for most of the fibre quality traits. The average performance of conventional euplasmic × euplasmic hybrids was unambiguously superior over the two types of hybrids for yield and its component traits (Tuteja, *et al.*, 2011 a and c).

GMS hybrids have certain advantages due to genetic makeup; hence, they are better than their conventional hybrid ‘H 4’ (Anonymous, 1990.).

Genotypes × environment interactions involved in various traits of GMS based hybrids Variances due to genotypes (G), environments (E) and G × E interaction were significant for all the traits studied, except G × E (linear) for micronaire value (Nirania, *et al.*, 2004). Both linear and non linear components were important for seed cotton yield, ginning outturn, 2.5 per cent span length, lint index, fibre fineness, maturity coefficient and bundle strength (Nirania, *et al.*, 2004). G × E (linear) was higher in magnitude than non-linear for ginning out turn, maturity coefficient and seed cotton yield (Nirania, *et al.*, 2004), whereas pooled deviation was higher for lint index, 2.5 per cent span length, micronaire and bundle strength (Tuteja, *et al.*, 2011 a). Non-significant bi and S-2

di values, and were more adaptive cross combinations observed were IAN579 x A72-15 and SA278 x G6030 (Nirania, *et al.*, 2004).

Gene action involved in various traits of GMS based hybrids : Importance of both the genetic variances for inheritance of all the characters (seed cotton yield, boll weight, boll number, mean halo length and ginning percentage). The estimates of average degree of dominance had also indicated additivity of genes for all the characters (Singh and Singh, 2006).

Combining ability of GMS based parents : FP₁, FP₂, FP₃ and FP₄ were the best general combiners for seed cotton yield while LRA 5166, IC 295 and 476-31-1 having high general combining ability (gca) effects were the best among the testers. IC 295 was the best tester for seed cotton yield, boll number and ginning percentage. In general, in the expression of high specific combining ability effects the crosses involved parents with high (H) x high (H), high (H) x medium (M) and high (H) x low (L) gca effects for all the characters (Singh and Singh, 2006).

A-72-15 has best gca for seed cotton followed by H 777 (Nirania, *et al.*, 2004) while HP Acala for maturity coefficient (Nirania, *et al.*, 2004) and GS-9 for 2.5 per cent span length (Singh and Singh, 2006). As regards GOT, fibre fineness and bundle strength Tamcot SP37H, NI83, and J2P7 respectively were best general combiners (Nirania, *et al.*, 2004). Parents showing high gca for yield generally poor combiners for quality (Nirania, *et al.*, 2004).

The new GMS AB line 'Kang A3' with *Bt* transgenic resistance to boll worm was developed by transferring *Bt* transgenic to GMS AB line Kang A2 wilt resistant line. Kang A3 had high resistance to pink worm (*Pectinophora gossypiella*) and nearly high resistance to bollworm (*Helicoverpa* sp.) (Yue, *et al.*, 2007) Induced pleiotropy for curved stigma and GMS in a bollworm tolerant *G. hirsutum* variety Abadhita, treated with 100 Gy of gamma rays followed by

0.2 per cent EMS for 10 hours. The flowers of MS mutant were found smaller with indehiscent and rudimentary anthers with short filaments and a characteristic feature of curved stigma, than its fertile isolate (Badigannavar, *et al.*, 2003).

Seed production : Major economic character of nineteen F₁s involving female parents 70416A (*ms₅ms₆* double allele) indicated that this line was ideal sterile parents. Hybrid seed production has not become possible in certain states due to labour shortage and high wage rates. GMS in hybrid seed production is not become feasible or practicable due to problem of mechanical pollen transfer. At Central Institute of Cotton Research, Nagpur, India, the parents of conventional hybrids H 4 and Varalaxmi were reconstituted on Gregg genetic background (Anonymous, 1990). In Gregg the cross boll setting was 54 per cent with elimination of emasculation. Thus, cost of hybrid seed was reduced to less than 50 per cent (Anonymous, 1990). The techniques and system of seed production of hybrid cotton by the method using the NMS line as both A and B line opened up a new way (Huang, *et al.*, 1988) and key achieve break through for the utilization of heterosis of cotton as well as alternative to manual task of emasculation. Although, GMS has been identified long back, its commercial application in hybrid seed production is not yet realized because identification of male sterile and male fertile plants before anthesis in female genotype has not been possible due to absence of genetic marker (Badigannavar, *et al.*, 2003).

GMS was successfully used in hybrid seed production in cotton (Weaver, 1979). Procedure for producing F₁ hybrid cotton seed utilizing GMS was standardized where cost of production was substantially reduced in comparison with conventional method (Srinivasan, and Gururajan, 1973, 1974; Bhale, 1986). According to Justus and Leinweber, (1960) partial male sterile lines can be used for hybrid seed production, if percentage of natural crossing is sufficient. In P.R. China, hybrid cotton based on the GMS line

Dong A is grown on about 40,000 ha every year from 1984 (Zhang and Pan, 1990). However, a seed line consists of a mixed population of fertile and sterile plants (1: 1) (Anonymous, 1990) 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).

Facing the current situation of high labor price and increasing labor strain, cotton hybrid seed production by utilizing nuclear male sterility is superior to by artificially removing pollen (Anonymous, 1990). In addition, according to our practice in hybrid cotton seed production, it is summarized that pollination with vial, postponed artificial emasculation is the suggested techniques for artificially removing pollen method, and sowing beforehand, moving pot in seedbed, and early weeding techniques for Nuclear Male Sterility utilization method. The development of 81A genetic male-sterile line associated with virescence trait could raise considerably the efficiency of the hybrid seed production in cotton (Zhang and Pan, 1990).

CGMS line using *G. hirsutum* nuclear genome in *G. harknessii* Brandege cytoplasm (Meyer, 1975) have some detrimental effects to F_1 hybrid yield (Weaver, 1986) and hence the recessive duplicate factors ms_5ms_6 are used in India to facilitate crossing in the production of F_1 seeds (Weaver, 1968). The seed production plots of GMS female contain 50 per cent fertile plants which need to be rouged out during flowering but before pollination (Khadi, 2011; Zhang and Pan, 1990) due to this reason GMS was considered non profitable mechanism for hybrid seed production (Khadi, 2011). Maintenance of GMS lines involves sib mating between $MS(ms_5ms_5ms_6ms_6)$ in 'Gregg' and fertile ($ms_5ms_5MS_6ms_6$ or $Ms_5ms_5ms_6ms_6$) plants (Khadi, 2011; Srinivasan and Gururajan 1973, 1974). GMS has been widely used in breeding programs for F_1 hybrid seed production in *G. hirsutum* because of its remarkable advantages like complete sterility, wide source of recovery, and ease of obtaining

high vigor combinations. Several sterile lines, e.g., Mian A₁ and Zhongkang A in China, based on ms_5ms_6 double-recessive sterile line were developed through cross breeding and back-crossing (Xing, et al., 2002) and using them as female parents, heterotic hybrids like 'Zhongmiansuo 38' and 'Nannong 6' were developed (Ma, et al., 2007).

Shoot tips from 8 to 10 day old seedlings and axillary buds from 35 to 40 and 55 to 60 days field grown plants of *G. hirsutum* CMS LH 900 and *G. arboreum* GMS DS 5 were aseptically cultured on different media, Explants taken from younger plants gave better response than older plants. Subsequent sub culturing lowered the mean rate of axillary bud proliferation. Further screening of genotypes and refinement of micro propagation techniques are required to increase the rate of in vitro multiplication up to the level where it can be used for commercial purposes (Girhotra et al., 2001).

Genetic male sterility in diploid / Asiatic – *desi* cottons (*G. arboreum* and *G. herbaceum*): The antiquity of cotton in the Indian subcontinent has been traced to the 4th millennium BC. The fabrics dated approximately 3000 BC, recovered from the *Mohenjo-daro* excavations in Sind (Pakistan), were identified to have originated from cotton plants, closely related to the *G. arboreum*. The diploid ($2n = 26$) species *G. arboreum* and *G. herbaceum* are indigenous in Asia and Africa. The cotton textiles of the *Harappan* civilization (2300-1750 BC) were produced by sophisticated textile craftsmanship. During the reign of Chandragupta Maurya (321-297 BC) the manufacture of cotton goods was reported to have reached a state of excellence. Kautilya in his *Artha-sastra* (economics) written during the second century BC has referred to the fine cotton goods of Vanga i.e., present Bangladesh.

The short and coarse fibre of Asiatic diploid cotton is suitable for filling and medical purpose. Keeping in view the shrinking area the

domestic requirement and export potential of coarse lint, enhancement of production and productivity these cottons become crucial. heterosis breeding is the sure way to meet this challenge (Pathak and Gill, 2011). The *desi G. arboreum* being resistant to abiotic and biotic stresses gets well adapted to the climatic aberrations and also well suited in resource limited environments are still preferred in the low rainfall areas because of suitability under rainfed conditions (Mokate and Shinde, 2004) because of low cost management (Mokate and Shinde, 2004). In addition they are highly resistant to major pests and diseases¹²⁰ especially cotton leaf curl disease, a dread disease of American cotton in the North India (Pathak and Gill, 2011; Mokate and Shinde, 2004).

In spite above merits, the diploid cottons (20 lakh ha) and their hybrids (0.5 lakh ha) are cultivated on very less area. Hybrid cotton in India covers 80 per cent of total cotton area and contribute about 90 per cent of the country's production, in which *desi* cotton hybrid contributes only 1 per cent (Sekhar and Khadi, 2010). Asiatic cotton (*G. arboreum*) are locally known as *desi* were grown on about 98 per cent area around 1947 and the American cotton (*G. hirsutum*) on just around 2. Presently, the situation is now exactly the reverse. However, now a days even short staple cotton is in great demand, particularly in fabrics like denim and upholstery. Also, it fetches price *on par* with the long-staple one. So, why not promote *desi* varieties, which had come to stay in India after developing resistance to indigenous conditions like drought, water logging and local pests? (Latha, 2012) and hence *desi* cotton is to the rescue of Indian cotton growers (Latha, 2012).

Diploid cottons are inherently low yielders. So, there is lot of scope to improve their genetic potential for yields (Jyotiba, *et al.*, 2010). These aroused the interest for developing superior hybrids in Asiatic cotton. The competitive demand for fibre warrants to improving the productivity of cotton crop in such

situation which is difficult to achieve through conventional hybridization and selection. Heterosis breeding seems to be good approach in these directions (Pathak and Gill, 2011).

The studies of heterosis from early 1950s onwards indicated higher level of heterosis in *G. arboreum* (a) x (a) and *G. herbaceum* (h) x (a) crosses. The first ever success story of heterosis breeding in tetraploid cotton encouraged cotton breeders to explore the possibility of similar attempts in diploid cotton (Palve, *et al.*, 2011), that resulted in released hybrids *viz.*, G.Cot.DH7 and G.Cot.DH9, they have not covered sizable area (Patel, *et al.*, 2000); due to the problem of seed production in *desi* hybrids, the high cost of conventional hybrid seed is a limiting factor for poor/ marginal farmers to grow hybrids (Patel, *et al.*, 2000; Jyotiba, *et al.*, 2010). Hence, in case of cotton the system of male sterility is of great significance in practical, as it avoids laborious process of emasculation and it can add in production of hybrid seed. However, with the availability of GMS lines in *G. arboreum* seed production cost can be reduced with increased purity (Patel, *et al.*, 2000). Diploid hybrids are high yielding with short to medium fibre (22 to 25 mm) and GOT around 35-39 per cent. In spite of these qualities, diploid hybrids cover an insignificant area due to non availability of a suitable cost effective system for large scale production of quality F₁ seed (Gedam, *et al.*, 2011). Hybrid seed production of *desi* cotton hybrids is uneconomical due to low boll setting through conventional method of hybrid seed production mainly because of the low boll setting (Singh, *et al.*, 1994) attributed to small size of flowers and close adherence of brittle nature of staminal column in flowers, which easily breaks during emasculation, delicate flower biology (Chaporkar, 1998) particularly flower stalk (Meshram, 1992; Meshram and Wadodkar, 1992, 1997). In addition, flower buds suffer more damage from emasculation that result in to low retention of crossed flowers which reflect in to low yield and high cost of hybrid seed (Chaporkar, 1998).

Therefore, GMS(Plate II,Figs. 3-4) was thought to be a best, economical and alternative method for hybrid seed production technique in cotton and especially in diploid cotton to release higher yields(Jyotiba, *et al.*,2010) as it can reduce the cost of hybrid seed production at least by 40 to 50 per cent(Bhatt,1995), however, seed produces had to rogue out 50 per cent of the plant population after flowering(Meshram, 1992).The use of GMS for hybrid seed production has several advantage over hand emasculation system like, no carpel damage, higher boll setting per cent and reduced cost of hybrid seed production(Pathak and Gill,2011). This trait was combined with a genetically male sterile line through back crossing to develop a female parent for use in hybrid breeding programs. The F_1 developed on such female inbred produced fuzzy linted seed with normal fibers (Dagaonkar,*et al.*,2007).

The incidence of male sterility in *G. arboreum* cotton due to continuous selfing (Leak, and Prasad,1912), malformation and sterilities affecting all floral parts except calyx, affecting anther and with the development of ovules (Kottur and Patel,1920) and non dehiscent anthers (Trought ,1928) *etc.* were also found. Crossing steriles with fertile did not restored fertility, instead new forms different from the original parents were observed (Kottur 1912a,b). Single factor difference was found for sterility and fertility (Hutchinson and Gadkari, 1935), the former being dominant in *G. arboreum* var. 'Million Dollar' (Hutchinson and Gadkari, 1935). Similarly male sterility was also reported in *G. herbaceum* (Kumar, 1937) due to sterility factor affecting gametes which are normal in appearance resulting due to retrogressive mutations of the normal to sterile type. Sterile plants with rudimentary anthers but with normal female organs(Fisher,1961) in BC_1F_2 generation of *G. arboreum* were observed(Bahavandoss and Veluswamy,1969). Petaloidy transformation of anthers in to petal like leafy structure (Plate II, Figs. 5-6) was observed (Hutchinson and Ghose,1937; Chen and Meyer,1979;

Thombre,1986) , however, because of its instability it is not used in hybrid seed production(Hutchinson and Ghose,1937). Apart from these a case of male sterile plant in *G. arboreum* was also reported (Sandhu, *et al.*,1989).

Sources of GMS in *G. arboretum* :

Earlier reviews (Khadi,2010,2011) reported following sources GMS in *G. arboretum*

1. **Hisar source:** *G. arboreum* race *bengalense*-DS-5: a spontaneous mutation (GMS 1) bearing white small flowers with petal spot was isolated. Semi closed corolla observed in GMS 1 was over come in GMS 2 (Singh and Kumar, 1993; Tuteja, *et al.*, 2005, b).

2. **Akola source:** The GMS line GAK 423A is developed by transferring the genome of *G. arboreum* variety 'AKH 4' into *G. anomalum* cytoplasm (Meshram, 1992; Meshram and Wadodkar, 1992,1997; Meshram, *et al.*, 1994,1998). This source has yellow, larger flowers than DS 5 GMS and possesses dark petal spot. Most of *G. herbaceum* and *G. arboreum* lines restore fertility when used as males. In addition to above, one more source as below is also reported (Mehetre and Patil, 2001, 2004).

3. **Rahuri Source:** 'MPKV-GMS' a GMS line of *G. arboreum* (Plate II, Figs.7-9)having a typical feature 'naked seed' as a special character(Mehetre and Patil, 2001, 2004) (NBPGR, New Delhi, registration No. IC 296576).

Inheritance of GMS in diploid cottons :

The Male sterile characteristic of *G. arboreum* race *bengalense* DS 5 is conditioned by ams_1 (Singh and Kumar, 1993) and ams_2 ams_2 (Tuteja, *et al.*, 2005,b) genes while it is under the control of two recessive genes in 'MPKV-GMS'(Mehetre and Patil, 2001, 2004). In addition $ar.ms$ (Meshram, *et al.*, 1997), ams_1 (Singh and Kumar, 1993) genes are also reported for GMS in *G. arboreum* .Two sources *viz.*, DS 5 and GAK 423 of

GMS were non allelic (Pathak and Gill, 2011). GMS in MSD 7, Hisar GMS and SRT 1GMS lines is under the control of the single recessive gene *ams₁* (Geddami, 2010).

Significant reduction was observed in sterile genotypes for flower morphological traits like flower pedicel length, staminal column length, style length, filament length, anther number and anther colour compared to their fertile genotypes (Geddami, 2013). Significant reduction in flower characters like petal size, and number (Mehetre and Patil, 2004) and size of anthers (Mehetre and Patil, 2004), non dehiscent anthers (Mehetre and Patil, 2004).

Transfer of GMS trait on to background of other varieties : GMS lines of diploid cottons *viz.*, GMS 4, GMS 2, GAK 20A, GAK 09, SGMS 2, SGMS 4, RGMS A 2, RGMS 3, SGMS 13, GMS 4-1, GAK 15A, GAK 26A, Sujay, GAK 423, GAK 8615 are reported upto 2002 (Sing, *et al.*, 2002). Genetic male sterility systems (Geddami, 2010) and genetics and morphological characters (Geddami, 2013) in diploid cotton were studied. GMS line RGMS-3 (NBPGR Registration No. IC 296646) was developed by transferring male sterility on background of a germ plasm line AC 6 that had good general combing ability (Kapoor, *et al.*, 2004). The study of large F₂ populations of two Hisar GMS and SRT 1 GMS Asiatic genotypes did not show significant differences from the male fertile counterparts for plant morphological traits such as plant height and leaf area. But both the GMS populations exhibited significant differences between male sterile and fertile plants with respect to flower morphological traits *viz.*, flower pedicel length, staminal column length, style length, filament length and anther number with each trait having lower mean values in male sterile plants and higher mean values in fertile plants of both SRT 1 GMS and HISAR GMS populations (Geddami, 2013). GMS from *G. arboreum* var. DS 5 was successfully transferred to *G. arboreum* var. Sujay and 4011 female parents of conventional hybrids G.Cot.DH 7 and G.Cot.DH 9, respectively (Patel,

et al., 2004).

Microsporogenesis in GMS lines :

Sterility in *G. herbaceum* due to chromosomal aberrations, formation of abortive pollen grains (Kumar, 1937) and anthers containing empty pollen grains in staminal column (Matuo and Mizuno, 1953) was reported. Comparative studies of microsporogenesis in *G. arboreum* DS 5 Male sterile and male fertile anthers indicated similar pattern of meiosis until release of microspores from tetrads. After release of microspores, certain changes were observed during further development of the microspores into pollen grains in GMS anthers. After release of microspores from tetrads, they enlarged considerably with dense cytoplasm taking dark stain, during further development microspore resulted in shriveling and shrinkage of cytoplasm (Kajjidoni, 1997).

A comparative histological study of GAKA 423 male sterile and fertile anthers of *desi* (*G. arboreum*) cotton revealed shriveling of microspores leading to deformed microspores without the complete development of pollen wall. The GMS anther sacs were completely appressed towards center enclosing the deformed microspores, which appeared as thick black undifferentiated band. The tapetum persisted during pre meiotic and meiotic stages and disintegrated after release of microspores (Kajjidoni, *et al.*, 2002).

Shriveling of microspores led to deformed microspores (Kajjidoni, 1997; Kajjidoni, *et al.*, 2002) without the complete development of pollen wall (Kajjidoni, 1997; Kajjidoni, *et al.*, 2002) led to pollen abortion (Kajjidoni, *et al.*, 2002). The GMS anther sacs were completely appressed towards the centre enclosing the deformed microspores (Kajjidoni, 1997) which appeared as thick black undifferentiated band (Kajjidoni, *et al.*, 2002). The tapetum persisted during premeiotic (Kajjidoni, 1997) and meiotic stages (Kajjidoni, 1997; Kajjidoni, *et al.*, 2002) and disintegrated after release of microspores (Kajjidoni, *et al.*, 2002).

Molecular basis in GMS lines : Molecular analysis between of GMS sterile and fertile plants produced polymorphism using 34 Primers like OPAB 19, OPH 20, OPI 2, OPI 3 and OPI 7. Of these polymorphic primers OPI 3 produced a male sterile specific fragment of 486 bp size and it is sequenced and converted into a trait specific Sequence Characterized Amplified Region (SCAR) (Geddham, 2010; Geddham, *et al.*, 2012). Characterization of the Hisar GMS and SRT 1GMS lines by RAPD markers indicated that out of 60 random decamer primers, 34 were found to be polymorphic generating 60.73 per cent polymorphism between male sterile and male fertile plant. Polymorphic primers OPAB3, OPAB4, OPAB5, OPAB19, OPH20, OPI2, OPI3 and OPI7 showed notable differences in the amplicon profile of MS and their fertile counterparts. Two distinct clusters, one each of MS and fertile plants were formed that indicated genetic differences between them. The primer OPI3 was found to be male sterile specific in repeated PCR as it consistently produced a specific fragment of 486 bp only in the sterile plants which has been later converted into a locus specific Sequence Characterized Amplified Regions (SCAR) marker (Geddham, 2010; Geddham, *et al.*, 2012, b).

Twenty near isogenic GMS lines of diploid cotton were screened using 119 random decamer primers. Out of 314 amplicons amplified, 187 were found to be polymorphic with an average of 3.83 fragments/primer of which 2.28 were polymorphic. Primers PPBO4 and OPZ 14 showed genetic diversity between male fertile and sterile plants of all diploid plants (Bharati, *et al.*, 2010).

Heterosis for yield and growth characters in GMS based hybrids : Diploid cottons are inherently low yielders. So, there is lot of scope to improve their genetic potential for yields (Jyotiba, *et al.*, 2010). These aroused the interest for developing superior hybrids in Asiatic cotton. Heterosis breeding seems to be good approach in these directions (Kapoor, *et al.*, 2002; Patil, *et al.*, 2009). Attempts (Kapoor, *et al.*, 2002;

Patil, *et al.*, 2009; Kajjidoni, *et al.*, 1999, a, b; 2002, 2004, Rajput, *et al.*, 1997, 1998, Maisuria, *et al.*, 2007, Geddham, *et al.*, 2012; Mokate, *et al.*, 2005, Govindraju, 2002, Nanjundan, *et al.*, 2003, Tuteja, *et al.*, 2000, Mokate, *et al.*, 2012, Patil, *et al.*, 2012, Kajjidoni and Patil, 2003, Khadi, *et al.*, 2003; Narayanan, *et al.*, 1999) made by different cotton breeders, realized heterosis for different growth, yield and yield contributing traits as well as for fibre quality traits/parameter as summarized below.

The F₁ hybrids were developed using GMS lines *e.g.*, DS5, (Kajjidoni, *et al.*, 1999, 2002, 2004), GAKA 423 (Kajjidoni, *et al.*, 1999, 2002, 2004), GAK A423 A (Rajput, *et al.*, 1997), GAKA 423 (Jyotiba, *et al.*, 2011), Million GMS (Jyotiba, *et al.*, 2011), NCAGA 4, NCAGA 26; Kajjidoni, *et al.*, 1999; Maisuria, *et al.*, 2007), MSD7n or (Geddham, *et al.*, 2012), MSD7nkd (Geddham, *et al.*, 2012), MSD10 (Geddham, *et al.*, 2012) and MSD11 (Geddham, *et al.*, 2012). Based on their performance, appreciable amount of heterosis is observed for different characters *e.g.*, days to first flowering (Rajput, *et al.*, 1997), anther number (Kajjidoni, *et al.*, 1999), Plant height (Kajjidoni, *et al.*, 1999, Rajput, *et al.*, 1997; Maisuria, *et al.*, 2007), sympodia (Geddham, *et al.*, 2011; Kajjidoni, *et al.*, 1999; Maisuria, *et al.*, 2007), seed cotton yield (Patel, *et al.*, 2000; Jyotiba, *et al.*, 2010; Geddham, *et al.*, 2011; Mehetre and Patil, 2004; Kajjidoni, 1999, 2002; Rajput, *et al.*, 1997; Geddham, *et al.*, 2012; Mokate, *et al.*, 2005), bolls/plant (Patel, *et al.*, 2000; Jyotiba, *et al.*, 2010; Geddham, *et al.*, 2011; Kajjidoni, 1999; Rajput, *et al.*, 1997; Govindraju, 2002; Maisuria, *et al.*, 2007), boll weight (Patel, *et al.*, 2000; Mehetre and Patil, 2004; Kajjidoni, 1999; Nanjundan, *et al.*, 2003), lint/boll (Patel, *et al.*, 2000), and seeds/boll (Kajjidoni, 1999), GOT¹⁷⁸, ginning outturn (Jyotiba, *et al.*, 2010; Kajjidoni, 1999), negative (Geddham, *et al.*, 2011; Kajjidoni, *et al.*, 2002; Geddham, *et al.*, 2012; Singh, *et al.*, 1994), ginning percentage (Maisuria, *et al.*, 2007; Geddham, *et al.*, 2012; Mokate, *et al.*, 2005), seed index (Patel, *et al.*, 2000; Jyotiba, *et al.*, 2010) (Negative seed index) Kajjidoni, 1999, lint index (Patel, *et al.*, 2000;

Jyotiba, *et al.*, 2010) as well as fibre quality traits (Geddami, *et al.*, 2011) Jyotiba, *et al.*, 2011/ fibre properties (Mehetre and Patil, 2004) viz., 2.5 per cent span length (Jyotiba, *et al.*, 2010), fibre length (Kajjidoni, *et al.*, 2002, Geddam, *et al.*, 2012, Maisuria, *et al.*, 2007; Tuteja, *et al.*, 2000), fibre strength (Jyotiba, *et al.*, 2011; Maisuria, *et al.*, 2007, Geddam, *et al.*, 2012; Tuteja, *et al.*, 2000), fineness (Geddami, *et al.*, 2012), Micronaire (10⁶g/inch) (Maisuria, *et al.*, 2007; Geddam, *et al.*, 2012), fibre elongation (Geddami, *et al.*, 2012), Uniformity Ratio (UR) (Jyotiba, *et al.*, 2010, Geddam, *et al.*, 2012; Tuteja, *et al.*, 2000).

Heterosis over mid parent (Pathak, and Gill, 2011; Palve, *et al.*, 2011; Kajjidoni, *et al.*, 1999; Rajput, *et al.*, 1997), better parent (Pathak, and Gill, 2011; Palve, *et al.*, 2011; Patil, *et al.*, 2009; Kajjidoni, *et al.*, 1999; Rajput, *et al.*, 1997) and heterobeltosis in GMS based and conventional crosses (Kajjidoni and Patil, 2003) *etc.*, was documented. Highest direct effect in positive direction - bolls/plant and lint index (Kapoor, *et al.*, 2002) in negative direction GOT (Kapoor, *et al.*, 2002; Mehetre and Patil, 2004).

Comparison GMS based hybrids with standard varietal and conventional hybrid checks : Compared with conventional check hybrid G. Cot. DH 7 (Patil, *et al.*, 2000), Chamatkar 222 (Jyotiba, *et al.*, 2010), 'Swadeshi 1 and 5 (Mokate and Shinde, 2004) G. Cot. MDH 11 (Mokate and Shinde, 2004) and varietal check Y 1 (Mokate and Shinde, 2004), AKA 8401, (Rajput, *et al.*, 1997), RaHS 14 (Jyotiba, *et al.*, 2011), DLSa 17 (Jyotiba, *et al.*, 2010), AAH (Gedam, *et al.*, 2012), Gsav (Gedam, *et al.*, 2012) JLH-794 (Mokate and Shinde, 2004; Mokate, *et al.*, 2005), Suvarna (Mokate, *et al.*, 2005). The performance of reconstituted hybrids was statistically *on par* with the conventional hybrids in respect of almost all the economical as well as technological characters. The performance of reconstituted hybrids was statistically *on par* with the conventional hybrids (Patil, *et al.*, 2004; Mokate, and Shinde, 2004). From the above review

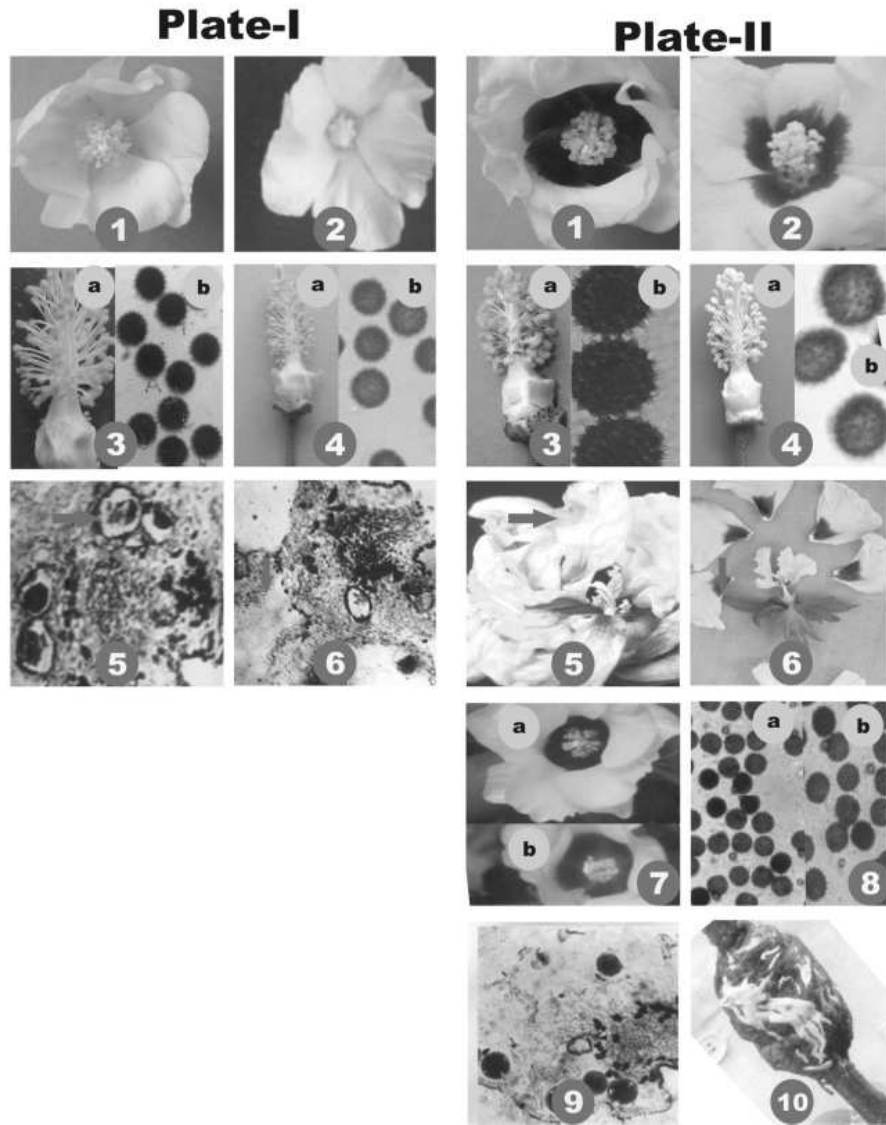
potential hybrids identified for exploitation of hybrid vigour as under:

SGMDH 1 (Patil, *et al.*, 2000), SGMDH 2 (Patil, *et al.*, 2000), DS5 x 30802 (Kajjidoni, *et al.*, 1999a), DS5 x 2631 (Kajjidoni, *et al.*, 1999a, b), DS5 x B-Desh (Kajjidoni, *et al.*, 1999a), GAK423A x GHBHV1037-88 (Rajput, *et al.*, 1997), GAK423A x HD107 (Rajput, *et al.*, 1997), GAK423 x AKA9302 (Rajput, *et al.*, 1997), GAK423A x GH1457-87 (Rajput, *et al.*, 1997), MSD 7 nor x RAHS 14 (Geddami, *et al.*, 2011). RAH 4 (Jyotiba, *et al.*, 2010), RAH 7 (Jyotiba, *et al.*, 2010), RAH-8 (Jyotiba, *et al.*, 2010), RHAH 1040 (Govindraju, 2002), RHAH 1022 (Mokate, *et al.*, 2012), RHAH 1010 (Mokate, *et al.*, 2012), AKDH 92 (Patil, *et al.*, 2012), AKDH 91 (Patil, *et al.*, 2012), AKDH 93 (Patil, *et al.*, 2012) AKDH 92, AKDH 91, AKDH 93 were stable for seed cotton yield while AHDH 92 for bolls/plant and G.P (Patil, *et al.*, 2012), MSD 7 nor x Jayadhar (Jyotiba, *et al.*, 2010). Significant G+ (G x E) and (G x E) indicated differential response of genotypes under different environment (Patil, *et al.*, 2012).

GMS based interspecific (a x h) G. arboreum (a) x G. herbaceum (h) hybrids. : DS 5 and GAK 423A x *G. herbaceum* as males SM 88, R.51, Kumpta, Dig 6.3.1.3., Suj3.1.3.3, Jaydhar, DB3-12, Surti Broach and Suj4.3.4.4 (Kajjidoni, *et al.*, 1999b). Significantly higher anther number and lower GOT as compared to conventional hybrids (Kajjidoni, *et al.*, 1999, b).

G. arboreum GMS MSD 7 nor, MSD 7 nkd, MSD 10, MSD 11 as females and *G. herbaceum* as males DDhc 11 Jayadhar and RAHS 14 checks hybrids a x a AAH 108, G. cot MDH 11 and *G. arboreum* varieties GSaV 1056 DLSa 17 (Geddami, 2010). The hybrids 14C 543, 11C 534 and 16C 534 were stable for cotton yield (Nanjundan, *et al.*, 2003). Hybrid Million-GMS x Jayadhar found promising (Jyotiba, *et al.*, 2010).

Use of GMS lines in interspecific hybridization : An interspecific hybrid between *G. arboreum* (GMS) and *G. thurberi* (Mehetre and Patil, 2003) and *G. arboreum* x *G. stocksii* (Mehetre



Description of figures

- Plate I. *Flower morphology, anthers, pollen and anther histology G. hirsutum* GMS 'Gregg'
 Figs.1-2 Fully opened flowers, 1.fertile and2. sterile
 Figs. 3-4: 3= fertile, 4= sterile, a= androecium showing anther development, b= pollen,
 Figs.5-6. Anther histology : 5= sterile anthers with conspicuously shrivelled and vacuolated pollen grains at maturity and 6= fertile anthers showing normal dehiscence.

Plate II

Flower morphology, anthers, pollen and anther histology of G.arboreum GMS Akola and MPKV sources

- Figs.1-2: Fully opened flowers, 1.fertile and2. sterile
 Figs. 3-4: 3= fertile, 4= sterile, a= androecium showing anther development, b= pollen
 Figs.5. Petaloidy 'anthers transformed into tiny petal like structure' recorded in *G. arboreum* during segregation of *G. arboreum* x *G. anomalum*:
 Fig.6. Dissected petaloid flower. Arrow showing 'petaloidy'
 Figs.7 9. *G. arboreum* MPKV-GMS (Regn. No: IC 296576) ,7 flowers (a) fertile and (b) sterile, 8= pollen(a) fertile and (b) sterile, 9= sterile anthers with conspicuously shrivelled and vacuolated pollen grains at maturity
 Fig.10. Profuse rooting induced in vegetatively propagated (Air Layerage) branch of *G. arboreum* MPKV-GMS

and Patil, 2004) was obtained

Seed production using GMS lines : Most of the *G. arboreum* and *G. herbaceum* lines restore fertility in GMS lines when used as pollinators. Asiatic hybrids between cultivated diploid species have produced very high (Kajjidoni, *et al.*, 2003 and Khadi, *et al.*, 2003), level of exploitable heterosis (up to 200 per cent, 185 per cent (Rajput, *et al.*, 1998) when crossed through conventional technique. India is the first country in the world to release first hybrid AAH 1 of diploid cotton (Khadi, 2011). Superior performance of diploid hybrids over tetraploid by 10 to 102 per cent was observed (Narayanan, *et al.*, 1999). Sufficient seed set by using GMS has been obtained and seed production can be made economical. India is the first country to release a GMS based hybrid (AAH-1) in diploids (Khadi, *et al.*, 2003).

Seed production traditionally, is carried out by expensive hand emasculation and pollination techniques, with resultant higher seed costs (Kopulwar, *et al.*, 2007). The hybrids DDH 2, G. Cot Dh 7 and G. Cot DH9 give high yields but have seed production problems (Patel, *et al.*, 2000), and hence have not covered sizable area. The spread of hybrid is limited because of problem of hybrid seed production due to poor hybrid seed setting (Mokate, *et al.*, 2004). Sufficient seed set by using GMS lines has been obtained and production can be made economical (Khadi, 2011). Hence use of GMS for economic hybrid seed production in *desi* cotton is recommended (Singh, *et al.*, 1992) as use of GMS for hybrid seed production has several advantages over hand emasculation system like no carpel damage, higher boll setting percentage and reduced cost of hybrid seed production among others. Hence presently, nuclear male sterility for hybrid development in *desi* cotton is largely being used (Pathak and Gill, 2011).

Technology for production of F_1 hybrid cotton seed of both conventional and GMS based hybrids is in practice for producing hybrid seed on commercial scale. Profit and profitability of

hybrid seed production of GMS based *desi* cotton AAH 1 hybrid (Nirania, *et al.*, 2009) indicated economically beneficial. Improved cross boll setting observed in converted GMS *G. arboreum* var. Sujay and 4011 female parents of conventional hybrids G. Cot. DH 7 and G. Cot. DH 9, respectively from *G. arboreum* var. DS 5. The performance of reconstituted hybrids was statistically at par with conventional hybrids in respect of all the economical and technological characters that revealed an advantage of GMS over conventional (Patel, *et al.*, 2004).

Among all 3 GMS lines, GAK 423 exhibited earliness for flowering and fruiting behavior in September. Boll setting, boll retention and seeds per crossed boll were significantly influenced by period and time of pollination. The stigma receptivity judged on the basis of boll setting percent was maximum at time of pollination from 11 a.m. to 1 p.m. in September and from 12 noon to 2 p.m. in October and November and it might be due to varying climatic conditions during these three months. This information may be useful for properly synchronizing males and females and suggested that pollination can be extended upto 2 p.m. for getting high seed yield and quality particularly in *desi* cotton hybrid seed production programme (Burghate, *et al.*, 2009).

The data on, the average cost of one kilogram hybrid (F_1) and net return/ha of F_1 hybrid production of GMS based *desi* cotton AAH 1 hybrid undertaken during 2005-2006, 2006-2007 revealed that hybrid seed production is profitable in addition to advantages like increased income and improved the socio-economic condition of the farmers, increased employment opportunities, optimum utilization of farm labour during off-season and attraction of entrepreneurship (Nirania, *et al.*, 2009). Use of GMS can considerably reduce the cost of hybrid seed production by elimination of manual labour cost by elimination of manual labour cost required for emasculation (Kajjidoni, *et al.*, 1999b and a). Cost of hybrid seed produced by using GMS was lower (Rs300/kg) as compared (Rs700/kg) seed

produced by conventional method (Mehetre, *et al.*, 2002).

Since the stigma receptivity of GMS lines *viz.*, GAK 423, GAK 8615 and DGMS 1 (judged on the basis of boll setting per cent) was maximum from 11 am to 1 pm and 12 noon to 2 p. m during September and October to November, respectively, it is suggested that pollination could be extended upto 2 pm in hybrid seed production plot of *desi* cotton hybrid (Burghate, *et al.*, 2010a). Further, for getting high seed yield and quality particularly in *desi* cotton hybrid seed production programme proper synchronization of males and GMS females lines can be done using information of climatic condition for planting to achieve earlier flowering in September rather than October followed by November (Burghate, *et al.*, 2010b).

The maintenance of the male sterile lines. : Maintenance of GMS line (A line) is by backcrossing with the heterozygote B lines (Maintainer lines) is followed, however, the progeny produced are 50 per cent fertile and 50 per cent male sterile. Identification marker genes that are closely linked to *ms* genes and affect some vegetative characters that will be helpful for identification of sterile plant either at seedling stage, or at least few days before flowering like curved stigma mutant (Badigannavar, *et al.*, 2003). However, such desired combinations are not available till date. Hence, in seed production field in case of male sterile parent, 3-4 seeds should be sown/hill because 50 per cent of the population (male fertile) is removed when flowering starts, thus number of GMS plants available for crossing is very low, it adversely affects the quantity of hybrid seed produced. Moreover, vegetative propagation (Mehetre, *et al.*, 2003a) and air layering (Mehetre, *et al.*, 2002) on branches of GMS (Plate II, Fig. 10) lines is also found as an alternative to above methods for rapid seed multiplication of *G. arboreum* GMS based hybrids. Potentiality of 'petaloidy' controlled by single dominant gene needs to be investigated further for rectification of 'instability'

(Hutchinson and Ghose, 1937) as it is possible by selection through segregating generations of interspecific crosses (Mehetre, *et al.*, 2003b) as it is crossable with wild and cultivated cottons (Thombre and Mehetre, 1981).

Further screening of genotypes and refinement of micro propagation techniques are required to increase the rate of *in vitro* multiplication up to the level where it can be used for commercial purposes (Girhotra, *et al.*, 2001). In addition the RAPD markers associated with MS and putative SCAR marker specific to male sterility may facilitate for the utilization of the GMS system in hybrid breeding in the Asiatic cotton (Geddani, 2010; Geddani and Khadi, 2012). SCAR marker specific to male sterility can be considered as putative markers for linkage studies and for identification of male sterile and fertile plants in the GMS based hybrid seed production plots at the early stages of the crop growth.

GMS based hybrid released for cultivation : Earlier reports (Khadi, 2011; Patel, *et al.*, 2000; Pathak and Gill, 2011; Patel, *et al.*, 2004; Palve, *et al.*, 2011; Geddani, *et al.*, 2011) listed the so far released *intra arboreum* (a x a) hybrids using GMS as female parents are: MDCH 212 (Chaporkar, 1998), CISAA 2 (CICR HY 2) and AKDH 7 Raj DH 9 (Kapoor, *et al.*, 2004), G.Cot.MDH 11 (Patel, *et al.*, 2004), Moti (Bhatia, *et al.*, 2005), KR 64 (Raj and Arya, 2012), PKVDH 1 and PKV Suvarna, AAH 1 and CICR 2 (Pathak and Gill, 2011).

Photoperiod sensitive genic male sterility (PGMS), thermo sensitive genic male sterility (TGMS) and Environment sensitive genic male sterility (EGMS) : Thermo sensitive genic male sterility (TGMS) reported in diploid cotton (Khadi, *et al.*, 2001, 2003c,d; Govindraju, *et al.*, 2004; Ma, *et al.*, 2012; Laxman, 2009; Sekhar, *et al.*, 2012). TGMS lines DTGMSa 5h and DTGMSa 23ak, those were sterile under reduced minimum temperature below 18°C get in to

fertile (Govindraju, *et al.*, 2004). Histological studies indicated that the intact callose wall around tetrad are responsible for male sterility, which get disintegrated when temperature reduced to below 18p C and produced fertile pollen. A *G. arboreum* TGMS 1-1 line showing sterility till temperature reaches 24p C and reverting back to complete fertility when temperature failed down to 18p C (Palve, *et al.*, 2011) .TGMS trait is controlled by a single gene(Geddami and Khadi,2012; Sekhar and Khadi,2010).

Histological basis of TGMS : The process of microsporogenesis in male sterile anthers was parallel to fertile anthers until shortly after meiosis in the same TGMS line Later the microspores were released from the tetrad by dissolution of the callose walls in sterile and fertile anthers. The first indication of abnormal pollen development in sterile anther was early disintegration of tapetum in sterile as compared to fertile anthers .The abnormal pollen development in the sterile anther was the vacuolation of the microspore(Soddi,1995; Kajjidoni,1997; Sekhar and Khadi,2010) , associated with crushing of chromatin material coupled with shrinkage of microspore cytoplasm. The abnormality associated with the further development released microspores was most likely due to nutrient deficiencies (Bowman, *et al.*,1978; Khadi, *et al.*,1994; Soddi,1995; Kajjidoni,*et al.*,2002; Sekhar and Khadi,2012). The supply /inability of developing microspore to absorb the nutrients might be the reason for the pollen abortion in the MS line because of early disintegration of tapetum (Khadi, *et al.*,1994).

Biochemical basis of TGMS : Timing of callose activity play role in the formation and degradation of cell walls during microsporogenesis. High callose activity is required for the normal release of microspores from tetrad at late tetrad stage. In fertile anthers, the enzyme activity was strong as compared to the TGMS line. Hence the release of microspores

was not affected in fertile anthers as compared to TGMS line(Sekhar and Khadi,2012).

Molecular basis of TGMS : As evidenced from differential expression observed in sterile and fertile anthers, two NAU2176 and NAU 2096 markers linked to TGMS trait is confirmed(Sekhar and Khadi,2012).The primerOP13 was found to be male sterile specific in repeated PCR by constantly producing a specific fragment of 486 bp only in male sterile plants which further converted into SCAR markers. markers associated with male sterility and putative SCAR marker specific to male sterility may facilitate the utilization of the GMS system in in hybrid breeding in Asiatic cotton(Bharati, *et al.*,2010).

TGMS In *G. hirsutum*, EGMS male sterile line exhibiting male sterility during summer when temperatures were above 33-35p C while it reverted back when minimum temperatures 26-28p C reverted back to full fertile one. A single recessive gene was found for controlling this sterility. PGMS line in *G. hirsutum* that show pollen sterility when temperature rises continuously above 40p C(Palve, *et al.*,2011).A mutant with virescent that was fertile with an 11-12.5 h photoperiod when the temperature was higher than 21.5 °C, and was sterile with a 13-14.5 h photoperiod. Genetic analysis indicated that both traits were controlled by a single recessive gene or two closely linked genes. And the cytological observations and transcriptome profiling analysis showed that the degradation of pollen grain cytoplasm should be the primary reason why the mutant line performed male sterile under long day condition(Khadi, *et al.*, 2003c).

These negative effects of sterile cytoplasm can be avoided if environment sensitive genetic male sterility system (EGMS) is used for commercial hybrid seed production, because there will not be any strong sterile cytoplasm of wild species in this system. In upland cotton (*G. hirsutum*) also an EGMS line has been identified

at Agricultural Research Station, Mudhol which expresses full male sterility during summer conditions (when maximum temperatures are above 33°C and minimum temperatures range from 26-29°C) and same line reverts back to full fertility during *kharif* and *rabi* seasons (when maximum temperatures are below 33 °C and minimum temperatures range from 16 to 25 °C). The month wise acetocarmine test for sterility behavior showed complete absence of stained pollen grains during summer period. As many as 250 EGMS lines have been isolated and their stability for expression of male sterility confirmed for three years *i.e.*, 2006, 2007 and 2008 summers. This type of EGMS system would be of considerable value particularly in cotton where cost of hybrid seed production by conventional method of hand emasculation and pollination is very high (Laxman, 2009).

Male sterile (MS) mutants have been reported in many species of higher plants as the result of both spontaneous and induced mutations (Kaul,1998). Male sterility is conditioned by either cytoplasm-specific (CMS) or genetic male sterility (GMS) genes. In rice, male sterility is classified into four major groups: cytoplasmic male sterility (CMS), photoperiod-sensitive genic male sterility (PGMS), thermo-sensitive genic male sterility (TGMS) and other genic male sterilities (Kurata, *et al.*, 2005). CMS and PGMS/ TGMS have been used for hybrid seed production. However, genic MS lines have hardly been used due to the difficulty in purifying MS plants in segregating mixtures of MS and heterozygous plants. Several efforts have been made to develop a genic male-sterility system involving a closely linked phenotypic marker so that the male-sterile plants could be readily distinguished from normal plants in segregating populations. Initially, it was envisioned to produce F₁ seeds using the linked marker to indicate which plants were male sterile. However, this approach has not been generally successful (Galinat, 1975; Suh,

et al.,1991; Horner and Palmer,1995; Kaul, 1998)

The phenomenon of heterosis and its importance in crop improvement have been known since decades. Recognizing that commercial exploitation of hybrid vigor would, however, depends on economic viability of hybrid seed production. Varied genetic and nongenetic approaches for selective emasculation of one of the parent have been evolved and used. CGMS is worldwide used system among them .yet ,its cumbersome nature, labour expensive seed production method and restricted parental choice warrants an alternative has led to the discovery and study of TGMS AND PGMS in a model crop like rice. Development of stable and agronomically superior PGMS and TGMS lines using promising gene sources and identification of ideal location/ season for hybrid seed production and multiplication of PGMS and TGMS lines has facilitated two line theory (Siddique and Ali, 1999). The advantages of dominant male steriles for crop improvement due to its special features like a selfing generation is not required to identify homozygous plants and progenies of MS plants always gives 50 per cent dominant MS plants(Sorrells and Fritz,1982). Three programmes *viz.*, selected responses for S1, combined S1 and half sib and combined S1 and full sib families for recurrent selection schemes where intermating was done between selected S1 families and a dominant gene for male sterility (Knapp and Cox,1988).Use of recessive or dominant male sterile with hybrid eliminating haploid inducing lines is proposed in hybrid seed production(Stelly and Lee,1988). Use of GMS for hybrid seed production is a challenging area (Horner and Palmer,1995). Apart from use of GMS in hybrid development ,they can be advantageously used for cotton breeding different ways as suggested(Rao,*et al.*,1990; Horner and Palmer,1995; Siddique and Ali, 1999; Sorrells and Fritz,1982; Knapp and Cox,1988; Stelly and Lee,1988).

ACKNOWLEDGEMENTS

I thank Late Dr A. B. Joshi, Ex Director, IARI, New Delhi and Ex Vice Chancellor, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri for his encouragements and guidance during my life time. Help and cooperation of post graduate students from MPKV who perused their Ph. D /M.Sc.(Agri) Theses research work under author's supervision, co authors of my earlier research papers, and research technical staff of AICCIP, Rahuri is gratefully acknowledged.

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Recieved for publication : August 14, 2013

Accepted for publication : January 29, 2014