Cotton Improvement through Introgression and Heterosis Breeding

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Conventional breeding programme has resulted in development of numerous location specific cultivars, diverse genetic stock and development of cultivars with several useful traits against biotic and abiotic stresses in cotton. Breeding objectives for cotton improvement are formulated by taking into consideration the species in which improvement is sought. However, in India all four species have equal importance in judging their genetic improvement programmes. By and large breeding objectives in India vary according to the cotton growing zones and region specific requirements. The breeding objectives in cotton include higher yield, earliness, fiber quality, resistance to biotic stresses and abiotic stresses, quality of oil, wide adaptability, and synchronous maturity and to some extent on colored cotton.

However, the spectacular increase in yield coupled with significant reduction in insecticidal spray has made the Bt technology highly popular in India. Among the conventional breeding methods introgression breeding has played an important role in the improvement of cotton. As both the diploids have been considered as tolerant to biotic and abiotic stresses, tetraploidisation of diploid G.arboreum was done using colchicine. The presence of lower palisade layer of leaf lamina in diploid cotton conferred immunity to CLCuV, higher sucking distance and compact alignment of cortex cells was unearthed as basis of sucking pest resistance in diploid cotton. These traits have been transferred to G. hirsutum cotton (which usually lack lower palisade, sucking distance) by introgression with 4n G. arboreum. Introgression of AD genome into A2 and A1 genomes (4n G. arboreum x G. hirsutum and 2n G. herbaceum x G. barbadense) improved substantially fibre length and fibre fineness in diploid cottons. Further, genetic amelioration of G.arboreum was done for improving boll size, fibre length, fibre fitness and fibre strength by using G.hirsutum as a donor source. These introgressed G.arboreum genotypes are thought to be best candidates to substitute G.hirsutum genotypes and genetic transformation for insect pest resistance, as these are inherently resistant to sucking pests. Hence, this can be an efficient approach of hirsutisation of diploid cultivated species to elevate them to G.hirsutum status. These new genetic resources will help in preventing further genetic erosion of diploid cotton from Asiatic cotton eco-system and hence bring sustainability.

Apomixis

Apomixis is a phenomenon observed in many crop plants especially gramineae (Hanna and Bashaw, 1987) which can overcome all the demerits mentioned above for hybrid seed production. It enables production of seed without meiosis and fertilization. Apomixis helps to fix the hybrid vigour once a deplorable cross combination has been obtained resulting in hybrids which can be grown like varieties. Progenies derived from a tri-species cross of cotton involving G. arboreum, G. hirsutum and G. barbadense showed abnormal chromosome number and exhibited apomictic characters (Bhatade et al, 2004). Efforts have already been initiated on genetics and breeding strategies of apomixis in various crop species combined with molecular methods to analyse the apomictic and sexual mode of reproduction (Savidan, 2001). Already work has been initiated for utilization of apomictic lines in heterosis fixation of Bt cotton hybrids. The facultative apomictic condition in cotton has been observed in inter specific cross G. hirsutum x G.barbadense x G.arboreum (4n).

The number of locules in cotton boll has positive relationship with boll weight. The cultivated G.hirsutum genotypes are characterized by three to five locules. However, G.arboreum genotypes possess more of three loculed and less of four loculed bolls. There is improvement in introgressed derivaties with respect to number of locules in bolls. Introgression of G. arboreum race bengalense with race cernum and B genome G. anomaloum yielded a stable five loculed G. arboreum (ABC-5) genotype.

Among the fifty species of Gossypium, the Australian species belonging to Hibiscoidia and Sturtia possess unique feature of glandless seed and glanded plant, but this species belongs to C & G genomes and are phylogenetically remote from upland cotton and from diploid indigenous cottons. The distant hybridization of G. arboreum, G. hirsutum and G. herbaceum cotton with G. australe resulted glandless seed and glanded aerial parts conferring gossypol free cotton seed oil and resistance to pests and diseases.
Hybrid Development and Seed Production Techniques in Cotton

For the development of hybrids and seed production the male sterility systems like GMS, CGMS, TGMS are found handy.

Methods of production of hybrid cotton seed can be classified as per the following scheme.

Identification of good combiners

Good general combining ability is mandatory for parents, while specific combining ability is the final measure in choice of parents. Apart from this, the female parent should have good boll weight, good seed index, more seeds per boll, ease of emasculation and availability of genetic marker for easy grow out test.

Hand emasculation and pollination: Doak’s method (Doak 1934) of hand emasculation with some modifications and pollination is still being used in conventional hybrid seed production in India. Patel (1955), Srinivasan and Gururajan (1983), Mehta and Patel (1983), Mehta et al. (1983), have reviewed the literature and suggested modifications to basic Doak’s method. Flower buds, that are likely to open the next day are emasculated during afternoon or evening hours by removing anther column. The emasculated flowers are tagged with red tissue paper bag or twine thread for identification of the bud for pollination the next day and to identify crossed boll at the time of harvest, respectively. The stigma is receptive between 8-12 am. Pollen from one male flower is used to pollinate 4-5 emasculated female flowers. To maintain high genetic purity, uncrossed flowers are removed every day and uncrossed bolls (if any) are removed at the time of harvest. Similarly, male plants are removed after crossing period is over. During peak flowering period 25-30 persons are required for effective crossing operation. Though commonly adopted, this method is not suitable for developing diploid hybrids since the flower buds of these are small and the style is short and brittle.

Use of male sterility

Hand emasculation and pollination is laborious and results in high cost of hybrid seed. Use of male sterility can make hybrid seed much cheaper and its utilization for commercial hybrid seed production is a better approach for teraploid and diploid hybrids.

Male sterility in Tetrploid

Genetic male sterility (GMS): A total of 11 loci have been identified controlling GMS and 10 of them are in G. hirsutum and one in G. barbadense. Four genes, namely Ms4, Ms5, Ms10, and Ms11, are dominant and produce complete sterility while the remaining seven are recessive. Among the recessive genes, ms1, ms2, ms3 cause male sterility when present singly while ms4, ms6, ms8 and ms9 behave as duplicate recessive genes. GMS system involving ms2ms5 ms7ms9 found in Greg MS, is the only stable source, utilized in India. All G. hirsutum genotypes which carry Ms4 or Ms6 (or both genes) are restorers. Any G. hirsutum line can be converted into GMS system by repeated back crossing with alternate selfing and selection. Maintenance of GMS lines involves sib mating between male sterile (ms2ms5 ms7ms9) and fertile (ms2ms5 Ms6 ms8 or Ms2ms5 ms7ms9) plants. The seed production plots of GMS female contain 50 per cent fertile plants which need to be rogued out during flowering but before pollination. For this reason GMS was considered non profitable mechanism for hybrid seed production.

Cytoplasmatic Genetic Male Sterility: Interaction between nucleus of G. hirsutum and cytoplasm of G. arboreum, G. anomalum, G. harknessii, G. longicalyx, G. aridum, G. trilobum produced male sterility (Meyer 1973, 1975; Meshram et al. 1992). However, G. harknessii (D2 CMS), G. trilobum (D6 CMS) and G. aridum (D4 CMS) are sources of cytoplasms which induce stable male sterility for practical hybrid seed production. Most of the G. hirsutum lines are maintainers (B lines) for these CMS lines (A lines). Any B line can be converted into MS line by repeated backcrossing and selection. Restorer line can also be converted through backcross method by alternate selfing and selection with the background of sterile cytoplasm. The restoration in D2 CMS involves monogenic dominant action with one enhancer gene (Sheetz and Weaver, 1980). Similarly in D6 CMS, restoration is monogenic dominant. It has been found that with the same set of parents, the performance of hybrids is superior, when F1 seed is produced by emasculation and pollination as compared to seed produced by employing cytoplasmic genetic male sterility system.
Male Sterility in Diploids

Asiatic hybrids between cultivated diploid species have produced very high level of exploitable heterosis (up to 200%) when crossed through conventional technique. The hybrids DDH 2, G. Cot Dh 7 and G. Cot DH9 give high yields but have seed production problems. There are at present two sources of GMS.

1. **Hissar source**: The recessive GMS in G. *arboreum* cotton variety, DS 5 (GMS-1) has white small flowers with petal spot. The GMS-1 was isolated as a spontaneous mutation. Semi-closed corolla is drawn back in GMS-1 and it has been over come in GMS-2.

2. **Akola Source**: The GMS line GAK-423A is developed by transferring the genome of G. *arboreum* (AKH 4) into G. anomalum cytoplasm (Meshram and Wadodkar 1992). 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. GMS has enabled the exploitation of heterosis in diploids and easy hybrid seed production.

Up to 185% of useful heterosis has been reported by Rajput et al., (1998) in GMS based diploid hybrids. Superior performance of diploid hybrids over tetraploid by 10 to 102 per cent was observed in the preliminary study conducted by Narayanan et al. (1989). 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.

Thermo sensitive Genetic male sterility

The discovery of environment sensitive genetic male sterility system in rice laid the foundation for replacing the three line hybrid production system using A, B and R line by two line system (Yang et al., 1990, Lopez & Virmani, 2000). The work on TGMS in rice has been extensively done in India (Reddy et al. 2000). In cotton a spontaneous mutant of G. *arboreum* GMS line was observed showing sensitivity to temperature regimes (Khadi et al., 2001). Gradual alteration from sterility to fertility occurred when temperature reduced to less than 18°C. These lines have been stabilized and are helpful to overcome the problem of crossing for maintenance of male sterile line and hence no fertile segregants need to be rogued out. Prevention of linkage drag along with male sterile cytoplasm during transfer as well as overcoming the problem of sterility restoration is some of the other advantages of this male sterility system. Histological studies in Petunia showed that post meiotic male sterility is caused as a result of non-release of microspores after the tetrad stage. This in turn is due to non-degradation of callose wall surrounding the tetrad (Shahmay et al., 1971). In cotton TGMS, it is assumed that Callase enzyme may become active only at lower temperatures enabling the degradation of Callose wall making lines to be fertile by releasing the pollen grains. Work is under progress towards exploitation of the system for commercial hybrid seed production.

Diploid GMS lines have been found to be thermosensitive as partial male sterile plants were observed when minimum temperature went down to 18 °C. Individual plant selection for minimum temperature regimes have been made and further studies will be taken up to fix the TGMS lines.

MSH 345 showed cleistogamy type of flowers, ensuring complete self fertilization. This culture is also characterized by big, round bolls with 4-5 locules and boll weight of 3.2 –3.5 g. It would open up avenues in cotton breeding researches ensuring purity of genetic lines.

In the present era of eco-friendliness, wherein the scientists are involved in developing eco-friendly procedures in various fields, development of naturally coloured cottons is expected to help reduce the hazards of dyeing. The dyeing and finishing process require plenty of water. The disposal of solutions after dyeing and finishing causes environmental pollution and some of the dyeing agents containing carcinogens cause adverse effect on health of the workers. Naturally coloured cotton brings medical remedy for over fifty different somatic and psychosomatic disorders of man, hence the importance of naturally coloured cotton.

Heterosis breeding

The success of heterosis breeding in maize provided an impetus to work on different species combinations of cotton for evaluating F1 superiority over better parent (Heterobeltiosis) and commercial check variety (standard heterosis).

The hybrid vigor or heterosis in cotton has been critically reviewed by Davis (1978); Singh et al. (1980); Bhale (1987); Narayanan et al. (1989); Joshi (1997), and ICAC (1997). India is pioneer in development and commercial cultivation of hybrids and has the distinction of spreading hybrid technology (mostly intra specific) hybrids to 40 per cent of its cotton area which was mainly concentrated in South and Central India. Inter-specific hybridization between *hirsutum* and *barbadense* has also been successful by developing Varalaxmi the world’s first inter-specific hybrid.
Today, the hybrids cover the entire country. 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.

Though, Mell (1884) and Balls (1908) reported heterosis in tetraploid cotton and Kulkarni and Khadilkar (1929) in diploid cotton, it was Patel (1971) who demonstrated heterosis at commercial level. The first hybrid cotton H4 (Shankar 4) developed by him was an intra hirsutum (G-67 × American Nectariless). In the same year Katarki (1971) showed heterosis in interspecific tetraploid hybrid, through the release of Varalaxmi (Laxmi × SB 289E). The two events transformed the entire cotton scenario of India. C.T. Patel is called Father of Hybrid Cotton Technology.

The introgression breeding, hybrid development along with adoption of Bt technology played a big role in doubling cotton production and the improvement of fibre quality in the country.

REFERENCES