Life sciences – Agricultural

Genetic analysis on Fertility Restoration ability of CMS lines using tetraploid Cotton (Gossypium sp.)

Subha.L

Assistant Professor
PB&G, SWMRI
Kattuthottam, Thanjavur.

***Abstract*- An investigation was made on fertility restoration, genetic architecture, breeding behaviour, recombination potential and interrelationship of yield, yield contributing traits involving 40 hybrids and 13 parents in cotton. A line x tester (5 x 8) model of mating design was followed by generation mean analysis of six generations built up in three chosen crosses. From the line x tester analysis, it was inferred that all the traits were governed by non- additive gene action. The study of gene effects for yield and yield contributing traits in generation mean analysis revealed that all the traits were controlled by additive, dominance and epistatic interaction besides duplicate gene action and complementary gene action. As non - additive gene action was present for most of the traits studied, biparental mating or recurrent selection would be advantageous for harnessing the dominant gene action. Due to the presence of dominant gene action selection has to be postponed to later generations. To harness epistasis along with additive effects one or two cycles of recurrent selection followed by pedigree breeding would be effective to obtain expected improvement in yield and its component characters. Pollen fertility analysis showed that all the forty combinations produced by utilizing five lines and eight testers had high fertility status. Male sterile lines had low production of different high molecular polypeptides in contrast to their maintainer lines. The importance of anther protein in deciding the fertility was brought to renown.**

***Keywords*: Gossypium Sp., Cytoplasmic Male Sterile lines, Male sterility, Fertility restoration, line x tester analysis, Pollen fertility**

INTRODUCTION

Cotton, the 'White gold' enjoys a pre-eminent status among all the cash crops in our country, being the principal raw material for a flourishing textile industry. India is the pioneer in the world to exploit cotton hybrids, ultimately leading to flourishing of cotton mill with no starvation for fibre and at present around 120 varieties of cotton belonging to all four botanical species (Gossypium arboreum, G. herbaceum, G. hirsutum and G. barbadense) are being cultivated. Of these, only 25 varieties account for 98 per cent of the total output. The other 65 varieties have poor fibre strength and are of short fibre length. Some of these varieties were once popular, but have now outlived their usefulness. There is a need to de-notify these varieties and develop their substitutes. The success in development of cotton hybrid largely depends on the availability of the effective restorer and precise basic knowledge on the genetics of fertility restoration of Cytoplasmic Male Sterile lines. (Tuteja et al., 2006)

Meyer (1975) developed the first acceptable Cytoplasmic Male Sterile line and its restorer. But there are many difficulties in its utilization for commercial production of F_1 seeds. One of the major problems in the production of F_1 hybrid seed production through CMS approach is the lack of good sterile lines and strong restorers to restore significantly desirable fertility in hybrids. The high level of interest in CMS is due to its economic importance in the production of hybrid seeds in reducing the cost of hybrid seed production. The CMS is highly stable and reliable than GMS, CGMS and is not affected by environmental factors. Considering the above mentioned facts, the present investigation was under taken using thirteen diverse parents and their forty hybrids obtained through line x tester mating design with the objectives to evaluate the gca of parents and sca of crosses and to identify desirable cross combinations possessing high fibre quality and yield for commercial exploitation and to study the inheritance of fertility restorer genes.

MATERIALS AND METHODS

The experiment was carried out at the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai during 2005 to 2007. Seventeen accessions in Gossypium hirsutum, one accession in Gossypium barbadense, three restorers, nine cytoplasmic genic male sterile (A) lines and corresponding maintainer (B) lines formed the material for the present study. All the nine cytoplasmic male sterile lines viz., 5A, 6A, 7A, 12A, JCMS BN, JCMS K2, LRA 5166, SUMAN and VIKRAM were used as female parents. Other entries representing seven accessions in G. hirsutum viz., Badnawar, CICR 4, LRK 516, SRT 1, KC 2, MCU 12, MCU 13 and one accession in G. barbadense (TCB 209) and six restorers R 5, R 6, R 7, R 10, Demeter and Mex were used as male parents. The details about the parents and their origin are furnished in Table.1 and 1a

Synthesis of hybrids

Hybridization work was taken up during Rabi 2005. Normal cultural and manurial (80:40:40 Kg N:P:K/ha) practices were followed. Three meter long ridges were formed at 70 cm apart and seeds were planted following hills method of planting. Three

seeds were put in a hill at one side of the ridge spaced at 30 cm. After germination, thinning was done leaving one healthy seedling per hill. Ten plants were maintained in a ridge after thinning.

At the time of anthesis, flowers were collected from male parents for dusting the pollen on the sides of stigma of male sterile plants to get 126 cross combinations. Simultaneously, the male sterile lines were dusted with pollen from B lines to get sufficient quantity of female parents. The crossed bolls were collected separately for each cross and ginned to get the F_1 seeds.

Ten plants in each of 126 F_1 hybrids were raised during Kharif 2006 at Agricultural College and Research Institute, Madurai in a non - replicated test cross nursery adopting a spacing of 70 cm between rows and 30 cm between plants. All the plants were scored for their pollen fertility status. Those combinations that had high pollen fertility were raised separately along with their parents and standard check SRT 1 in a randomized block design replicated thrice adopting a spacing of 70 x 30 cm. Each row of 6 m had twenty plants. In each combination, ten plants were selected at random for recording biometrical and quality traits. The main emphasis of this investigation was laid on fertility restoration. The other criteria such as high yield, heterosis and high mean of parents were not taken in to account for selection of hybrids for studying generation mean analysis.

GENERATION OF BREEDING MATERIALS

The main emphasis of this investigation was laid on fertility restoration The other criteria such as high yield, heterosis and high mean of parents were not taken in to account for selection of hybrids for studying generation means analysis. Therefore specific hybrids such as LRA 5166 × KC 2, LRA 5166 × R10 and SUMAN × Mex were selected because one of the parent of these combinations possessed the desirable trait of fertility restoration ability and jassid resistance.

Since build up of generation in all the forty fully fertile cross combinations required enormous space, and other resources, only three cross combinations viz., LRA 5166 × KC 2, LRA 5166 × R10, SUMAN × Mex were selected for studying the interaction effects through Generation means analysis. These three cross combinations were raised in a crossing block during Rabi 2006. The F_1's were used as females and the pollen from P_2 parents was dusted separately to obtain B_2 (F_1/P_2). B_1 was produced by using F_1's and dusting F_1's pollen to P_1 as female (P_1/F_1). Fresh crosses were also effected among the parents simultaneously to obtain F_1 seeds. Parents (for P_1 corresponding maintainer line) as well as F_1's were selfed to produce fresh seeds of P_1, P_2 and F_2 generations. Thus all the six generations viz., P_1, P_2, F_1, F_2, B_1, and B_2 for each of the three cross combinations were constituted for generation means analysis.

All the six generations of three crosses were sown during Rabi 2006 in a randomized block design with two replications. The crosses were first randomized within a block and the different generations of a cross were randomized within a cross. All the recommended practices were followed throughout the crop period.

The following biometrical characters viz., plant height, number of sympodia per plant, days to first flowering, number of bolls per plant, boll weight, number of seeds per boll, ginning percentage, lint index, seed index and single plant yield and quality traits viz., micronaire value, 2.5% span length, 50% span length, uniformity ratio, fibre strength and elongation percentage were recorded. Anther traits were also recorded for parents and F_1 hybrids. Observations were recorded on five randomly chosen plants in F_1 hybrids and parental genotypes in each replication and mean values were utilized for statistical analysis.

POLLEN FERTILITY

The pollen fertility was estimated for all the 126 F_1's in test cross nursery to assess the fertility restoration. Similarly pollen fertility was estimated for all the plants in all the generations to study the genetics of fertility restoration.

At the time of flowering fully bloomed flowers were taken from each plant and fixed in 70 per cent alcohol at morning time prior to anthesis. Anthers were crushed in one percent Iodine – Potassium Iodide (I-KI) solution and allowed for two minutes. The round well stained pollen grains were counted as fertile and irregular shaped and poorly stained pollen grains were counted as sterile ones.

Three flowers representing top, middle and lower portions were examined for each plant and the mean of the three flowers over the entire five microscopic fields was worked out and the fertile pollen expressed as percentage for that plant. In cotton, based on the number of stained and unstained pollen grains the fertility status was computed as follows (Baker, 1993)

ANALYSIS OF ANTHER PROTEIN BY SDS – PAGE

Anther of five male sterile lines viz., LRA 5166, JCMS BN, JCMS K2, SUMAN and VIKRAM and their respective maintainers were used for this study. Total protein content was estimated as per the method of Lowry et al. (1951).Analysis of the protein was carried out by SDS-PAGE according to the method of Laemmli (1970).

RESULTS AND DISCUSSION

Results on pollen fertility (fertility restoration), quantitative and fibre traits were analysed statistically. Pollen fertility in per cent for 126 hybrids obtained by crossing nine Cytoplasmic male sterile lines with fourteen genotypes. (Table. 2) There was complete pollen sterility (zero fertility) in 86 out of the total 126 hybrids obtained by crossing nine CMS lines with fourteen genotypes. The remaining forty hybrids were completely pollen fertile. The pollen fertility ranged from 80.41 per cent (T_3 x L_3) to 99.73 per cent (T_7 x L_2). These forty fertile hybrids were actually the hybrids of the cross of each of the five CMS lines viz., L_1, L_2, L_3, L_4 and L_5 with each of the genotypes, T_1, T_2, T_3, T_4, T_5 and with the three restorer lines T_6, T_7 and T_8. The hybrids produced by using the two restorer lines. T_7 and T_8 and with those of CMS lines L_1, L_3, L_4 and L_5 had the maximum pollen fertility of 99.00 per cent and above. All the highly fertile forty cross combinations produced by utilizing five CMS lines and eight genotypes were assessed for ten economic characters, five fibre traits and screened for jassid resistance under field and screen house conditions.

The data obtained on the mean values of genotypes (Parents and hybrids) for each of the sixteen characters were analyzed statistically as per Randomized Block Design and the genotypic differences were found to be highly significant for all the characters. The analysis of combining ability variance results for 10 quantitative traits viz., days to first flowering, plant height, number of

sympodial branches per plant, number of bolls per plant, number of seeds per boll, ginning percentage, lint index, seed index, single plant yield and five fibre traits viz., micronaire value 2.5% span length, uniformity ratio, fibre strength and elongation percentage showed significant differences for all the characters. The differences due to sources lines were significant for all the characters except 2.5% span length. Differences due to testers were significant for all the characters. The line x tester interaction mean squares were significant for all characters except for the traits, micronaire value, elongation percentage and jassid resistance. The magnitude of the SCA variances were higher than GCA variances for all the characters. The highest ratio of GCA: SCA variances were obtained for 2.5% span length (0.064). Uniformity ratio had the lowest GCA: SCA variances (0.018).(Table 3)

The proportional contribution of lines were higher for boll weight (67.28) followed by number of sympodia per plant (63.82), days to first flowering (54.09) and single plant yield (47.56) whereas among the testers 2.5% span length contributed the highest (95.02). In the hybrids the trait uniformity ratio contributed higher than either parent (57.72).(Table 4). Observation recorded on pollen fertility in F_2, B_1 and B_2 generation of three cross combinations were analyzed. All the 120 plants studied in B_2 generations were fertile. A total of 200 F_2 plants and 120 B_1 plants studied for fertility were classified into fertile and sterile one based on fertility per cent. In F_2, the ratio for fertile: sterile was 3:1 in the entire cross and in the test cross in B_1, the ratio of fertile: sterile was 1:1 in all the three cross combinations. The chisquare table value is 3.84 for 5% significance. The calculated chisquare value for the F_2 and B_1 generations in all the three crosses were found to be less than the table value and hence non – significant and the null hypothesis is accepted. It can be concluded from above that the observed phenotypic ratio confirms to the theoretical segregation ratio of 3:1 in F_2 and 1:1 in the B_1 generations respectively. (Table 5). Anther protein in the male sterile lines and their maintainers were analyzed electrophoretically and their protein banding is presented in plates 12 and 13. Differences in protein banding are discussed below. All the male sterile lines had increased synthesis of 14KDa polypeptide. The maintainer lines of the CMS lines were characterized by the absence of synthesis of 14KDa polypeptide. Synthesis of 48KDa was only marginal in the CMS lines. The lower molecular polypeptides 14KDa and 24KDa were found synthesized in all the lines. The male sterile lines had very low quantity of various proteins. Maintainer lines had higher level of protein contents. Synthesis of 24, 38 and 48KDa polypeptides was found in the maintainer lines JCMS BN and JCCMS K2. Synthesis of low molecular weight polypeptide viz., 14KDa and 24KDa were found in the male sterile lines.

The cytoplasmic – genic male sterility in cotton to be practically useful in hybrid cotton seed production will have to ensure The male sterile line intended for use in hybrid seed production to be totally male sterile with no breakdown. Restorer lines to be so effective as to restore cent per cent pollen fertility. Female parent i.e., CMS lines used in hybrid seed production to give an increased seed set. Some pollinator lines carry genes which have the ability to restore the viable pollen producing ability of sterile plants, when crossed to CMS lines. Such genes are called “restorer genes”. For producing commercially useful hybrid by using male sterile lines it is most essential to have a pollinator line which possesses not only the nicking effects, hybrid vigour etc. but also fertility restorer genes (Patel and Mehta, 1984). Fertility restoration capacity primarily depends on the effect of restorer genes and their interaction with cytoplasm of CMS lines. A knowledge on the nature of gene action for fertility restoration in a CMS system is very much essential as it helps in the identification of an efficient and effective restorer to be used in hybrid seed production programme. In the present study of inheritance of fertility restoration in the F_2 and B_1 generations of three cross combinations viz., L_1 x T_1, L_1 x T_6 and L_5 x T_7, it has been found that a segregation pattern of three fertile and one sterile in F_2 indicated that fertility restoration was controlled by a single dominant gene. The test cross progenies, segregating into one fertile and one sterile, confirmed the monogenic control of fertile restoration in cotton based on the chisquare value. Earlier genetic studies brought out the single gene control of fertility restoration in cotton (Weaver and Weaver, 1977, Sheetz and Weaver, 1980, Meyer, 1980 and Silva et al., 1981). However, Silva (1978) reported that fertility restoration was controlled by three dominant genes. Thombre and Mehetre (1979) and Thombre (1986) reported that duplicate dominant genes controlled fertility restoration in cotton.

The polypeptide profile of total anther in male sterile and maintainer lines of different cytoplasmic origin were analyzed and the difference in protein banding is discussed here under.Clear differences between male sterile and maintainer lines were exhibited for anther protein. All the five male sterile line produced very low quantity of different proteins. There was a heavy synthesis of the high molecular 48 and 38KDa polypeptides in the maintainer lines. Tripathi et al. (1981) reported a decrease in number and intensity of protein bands from sterile anthers.

FUTURE THRUST

Looking into the current demand for cost effective quality fibre in the country, the break even that cotton growers can achieve with less scope for good price for their harvest under the global market scenario, shall be only through cotton varieties that yield good quality cotton fibre without additional burden on cost of cultivation. In order to achieve the emphasis for reduction in cost of cultivation, major thrust for development of cytoplasmic male sterile lines with efficient restorers and the development of host plant resistant is significant. The International Cotton Genome initiative is active for the last five years in unraveling the cotton genome. The various candidate genes could be identified and sequenced for extensive exploitation. The sensitivity of the work implicates the great reduction of cost of cultivation to make Indian cotton globally competitive.

REFERENCES:

1. Baker, R. 1993. **Cytological technique**. McMillan Publishers, Mathuen, London, 35p.
2. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature 227:** 680 – 685Meyer, V. G. 1975. Male sterility from Gossypium harknessii. **J. Hered., 66:** 23-27.
3. Meyer, V. G. 1980. Registration of DES. 146c. Cotton Germplasm. **Crop Sci., 20:** 417.
4. Patel, J. C. and N. P. Mehta. 1984. Identification and transfer of restorer genes to cotton strains for use as pollinator. **Cotton Dev.,** 49-50.

5. Silva, F. P. 1978. Genetic study of fertility restorations for cytoplasmic male sterile cotton originating from G. harknessii G. hirsutum, Dissertation Abstr. Int.
6. Silva, F. P. D., J. E. Endrizzi and L. S. Stith. 1981. Genetic study of restroration of pollen fertility of cytoplasmic male sterile cotton. **Revista Brasilera de genetica, 4 :** 10-15.
7. Sheetz, R. H. and J. B. Jr. Weaver. 1980. Inheritance of a fertility enhancer factor from Pima Cotton when transferred into upland cotton with Gossypium harknessii cytoplasm**. Crop Sci., 20:** 272-275.
8. Thombre, M. V. 1986. Fertility restoration in the cytoplasmic genetic male sterile cotton (G. hirsutum). **Curr. Sci., 48:** 172.
9. Tripathi, D.P., S. Mehta and N.G.P.Rao. 1981. Soluble protein and isoenzymes from anthers of diverse male steriles in sorghum. **Indian J. Genet., 41:** 170-177
10. Thombre, M. V. and S. S. Mehetre. 1979. Cytoplasmic male sterility in American Cotton, (G. hirsutum). **Curr. Sci., 55:** 161.
11. Tuteja, O. P., Sunil Kumar, Mahendar Singh and B. M. Khadi. 2006. Identification and characterization of new fertility restorers in cytoplasmic genetic male sterility (CGMS) of cotton (Gossypium hirsutum L.) derived from Gossypium harknessii. **Indian. J. Genet., 66 (1):** 53-54
12. Weaver, D. B. and J. B. Jr. Weaver. 1977. Inheritance of pollen fertility restoration in cytoplasmic male sterile upland cotton. **Crop Sci., 17:** 494-499.