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Marker-assisted selection in American cotton genotypes using biochemical and molecular profiling techniques

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ABSTRACT

Biochemical and molecular markers have proven to be powerful tools for discerning bio-systematic, bio-geographic, and phylogenetic relationships. Bio-systematic information can be important for guiding traditional breeding programs, gene transfer, inter-specific hybridization, and gene conservation. The polymorphism is a result of variation in genome of an individual plant or animal organism. The present investigation an experiment is conducted to explore the magnitude of polymorphism in cotton genotypes using biochemical and molecular marker techniques. In present investigation, the cotton lines exhibited the polymorphism is analyzed based on relative mobility of bands. The relative mobility (Rm) values ranged higher in protein banding pattern followed by carboxylase esterases, peroxidases and polyphenol oxidase activity studied by SDS-PAGE and PAGE electrophoresis techniques. In respect of the molecular investigation using RAPD, the primers used exhibited satisfactory amplification essential for discriminating the population. The most amplified primers exhibited an average polymorphism of about 73 per cent. The biochemical and molecular marker techniques are found to be quicker for screening of large gene pool and clustering the genotypes into different diverse clusters which are useful to select the parents for hybridization. These techniques can assist to plant botanist especially involved in plant breeding activities to speed up the conventional cotton improvement program. Further it can be concluded that the biochemical and molecular markers investigated in present experiment are adequate to judge the dissimilarity among the genotypes for speeding up the selection of parents with assumption that the banding profile is a measure of discrimination.

Keywords: *MAS, SDS-PAGE, Molecular Markers, Biochemical Markers and Cotton.*

Introduction

Cotton is one of the most important fiber and cash crop of India and plays a dominant role in the industrial and agricultural economy of the country. It provides the basic raw material (cotton fiber) to cotton textile industry. Cotton in India provides direct livelihood to about six million farmers and about 40 -50 million people are employed in cotton trade and its processing. In India, there are ten major cotton growing states which are divided into three zones, viz. north zone, central zone and south zone. North zone consists of Punjab, Haryana, and Rajasthan. Central zone includes Madhya Pradesh, Maharashtra and Gujarat. South zone comprises Andhra Pradesh, Telangana, Karnataka and Tamil Nadu. Besides these ten States, cotton cultivation has gained momentum in the Eastern State of Orissa. Cotton is also cultivated in small areas of non-traditional States such as Uttar Pradesh, West Bengal & Tripura.

At present various molecular markers used in cotton include Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Sequence Related Amplified Polymorphism (SRAP) and Single Nucleotide

Polymorphism (SNP). These markers play a crucial role in crop improvement program viz., Genetic diversity assessment, Construction of linkage map, QTL analysis for important traits in cotton and adopting the technique of Marker Assisted Selection (MAS) [15].

Keeping in view the above facts, the present investigation was undertaken with considering various technical and logistical factors that may limit the speed and scope of marker assisted selection (MAS) applicability by giving a practical approach to implement these techniques in cotton crop. Steady progress and advancement in DNA markers will make it more attractive for molecular breeding and plant genetics and ultimately help in cotton improvement conducted as an attempt to address the objectives to assess the biochemical and molecular diversity of thirty four elite lines of cotton.

Experimental Materials

The experimental material comprising of thirty-four genetically diverse and elite upland cotton lines from eight major cotton growing states viz., Maharashtra (twenty-two genotypes), Tamil Nadu (four genotypes), Gujarat (two genotypes), Punjab (two genotypes) and one each genotype from Andhra Pradesh, Madhya Pradesh, Rajasthan and Uttar Pradesh respectively collected from Senior Research Scientist, Cotton Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS).

Methodology

Profiling Techniques: SDS-PAGE Gel Electrophoresis method was followed for the separation of total seed proteins for the identification of cultivars by using cotton seedlings [1,9]. Whereas, the RAPD analysis is carried out with the help of standard protocol [7] at Biotechnology center under University Department of Agricultural Botany, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Data Analysis: The data obtained from all the profiling techniques were used to estimate the similarity on the basis of the number of shared amplified bands. Similarity was calculated with SIMQUAL function of NTSYS that computes a variety of similarity and dissimilarity coefficients for qualitative data.

Clustering and Selection of parents: Based on genetic distance estimates, the population is categorized into various clusters using the clustering technique of un-weighted pair group method of arithmetic means (UPGMA) [10]. The parents are selected based on statistical distances between the clusters as the index of genetic diversity is required. Hence the crosses between the genotypes belonging to the clusters having higher magnitude of average inter cluster distances may yield better segregants. The mean statistical distance may be considered arbitrarily as a guide line [2].

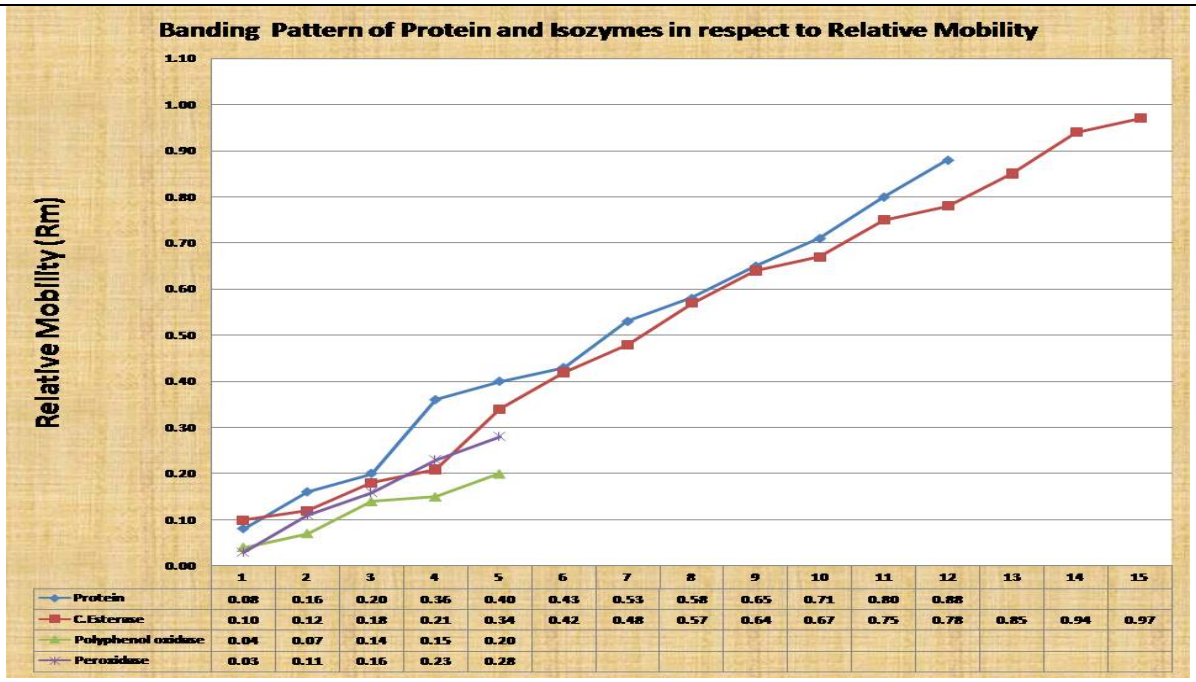
Results and Discussion

Protein Profiling (SDS-PAGE): The success of electrophoretic procedures depends on the wide ranging polymorphism of seed protein and isozymes and the fact that these proteins represent primary gene products. Analysis of protein composition has proved to be a good indicator unless altered by growth condition only to a relative minor extent. SDS-PAGE of proteins is the most commonly used method to discriminate the varieties [5,6]. The protein banding pattern is unique for the particular genotype and is independent of seed vigor and physiological seed activity.

In present investigation total twelve bands were observed in the SDS–PAGE Gel Cassette having the range of Relative mobility (Rm) ranging in between 0.08 to 0.88. The genotypes five genotypes exhibited all twelve bands, while, three genotypes had shown the minimum number of bands. The wide range of relative mobility values indicates the broad spectrum of protein fraction have been successfully utilized and detected by gel electrophoresis. This kind of variation, revealed that in the electrophoretic banding pattern could lead to the detection of genotype specific bands which can applied as a reliable biochemical marker for distinguishing the population. The migration velocity ostensibly provides the rational estimate of the degree of homology based on protein bands within and between related one amendable to statistical distance. The intensity of the band also varied among all the genotypes indicated the use of SDS page profile through electrophoresis for discrimination of genotypes of mustard [4] whereas reported in upland cotton [6,11,13].

Isozyme Profiling (PAGE): Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution [16]. In present investigation, the cotton genotypes were tested for polymorphism on the basis of Carboxylase Esterase, Peroxidase and Polyphenol oxidase profiles. For Carboxylase Esterases, the total fifteen bands (Rm range from 0.10 to 0.97), Peroxidases indicated five bands (Rm range from 0.04 to 0.21) whereas Polyphenol oxidase exhibited five bands (Rm range from 0.03 to 0.28) as given in Fig 1.

During the period of investigation it has been found that all three isozymes studied, possess very sensitive nature of enzymatic reactions, hence the results were not reproducible. Whereas many workers has used these SDS-PAGE and PAGE Electrophoresis profiling techniques useful as a rapid discriminating tool in various crops [6]. The isozyme markers can be genetically mapped onto chromosomes and then used as genetic markers to map other genes. They are also used in seed purity test and occasionally in plant breeding. There are only a small number of isozymes in most crop species and some of them can be identified only with a specific strain, that's why, the use of enzyme markers is limited [16]



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Protein	0.08	0.16	0.20	0.36	0.40	0.43	0.53	0.58	0.65	0.71	0.80	0.88			
C.Esterase	0.10	0.12	0.18	0.21	0.34	0.42	0.48	0.57	0.64	0.67	0.75	0.78	0.85	0.94	0.97
Polyphenol oxidase	0.04	0.07	0.14	0.15	0.20										
Peroxidase	0.03	0.11	0.16	0.23	0.28										

Fig.1 - Banding pattern analysis obtained by different profiling techniques

DNA Profiling (RAPD): In the present investigation, the random primers were tested for amplification out of them five was found useful for study giving sufficient amplification product. The polymorphism among the total forty bands (eleven monomorphic and twenty-nine polymorphic) estimated was about 73 per cent with the range of 100 to 58.34 per cent by well amplified primers. The RAPD study revealed that there is sufficient diversity among the genotypes. Whereas, it is observed that though the polymorphism was observed, it is a very preliminary and therefore there is need to increase more number of primers in the study to screen the maximum portion of genome. For more accuracy of variation of desirable trait, it is necessary to use advance techniques viz Restricted Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) etc., and these are more reliable. These results were sufficient to categorize the population into diverse clusters as compared to year long field trials which are exposed to various biotic and abiotic limiting factors for expression of potent germplasm lines [14]. Although these modern tools efficiently assists by providing the criteria for selection of the parents having broad genetic base to improve the gene pool of cotton by hybridization and sub sequent isolation of transgressive segregants.

Based on the assessment of diversity among the cotton germplasm lines, the profiling techniques employed during this investigation formed different clustering pattern as depicted in Fig-2. The maximum i.e. five clusters were formed based on dissimilarity assessed by profiling using Protein, Carboxylase esterase, Polyphenol oxidase and RAPD techniques whereas the population was classified into four clusters by peroxidase profiling. The mean cluster dissimilarity values were used protein (0.74), carboxylase esterase (0.64), peroxidase (0.20), polyphenol oxidase (0.20) and RAPD (0.61) profiling for selecting the parents having intra-cluster dissimilarity values above the means.

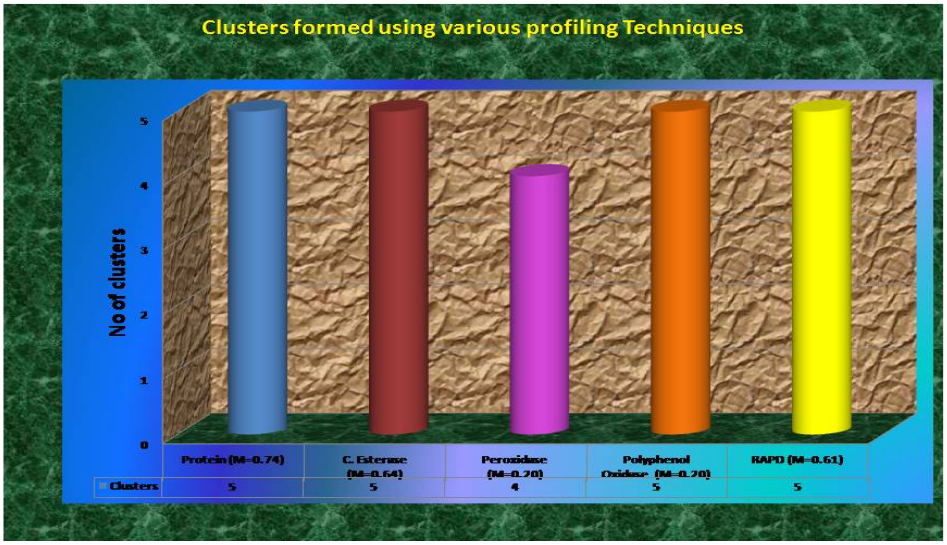


Fig.2 –Clustering pattern obtained using different profiling techniques

To overcome the limiting factor of environmental influence in important process of selection, the biochemical and molecular characteristics marking differences among the individuals are being commonly utilized by geneticists and plant breeder now–a–days and similar kind of activities to employ techniques biochemical and molecular markers in cotton has been reported by many workers. RAPD techniques have been used for many purposes including assessment of genetic variations in population, DNA fingerprinting, and determining the relationship between the genotypes of different and same species. In cotton RAPDs were used to distinguish the cotton varieties resistant to jassids, aphids, and mites. RAPD marker (R-6592) for the male sterility gene has been identified in cotton [8]. RAPD techniques are also used to evaluate the genetic relationship among cotton genotypes [12], to identify the QTLs for stomatal conductance and to construct linkage mapping in cotton [3] .

Marker assisted selection (MAS) is a procedure by which a phenotype is selected on the basis of genotype of a marker. Selecting the plants in the segregating population that have the suitable genes combinations is the important component of plant breeding. Once the markers tightly linked to the genes have been detected, breeders may use particular DNA marker to identify the plants carry the genes. The effectiveness and cost of MAS are influenced by the marker technique; therefore, it must be selected carefully. With the rapid development of genomics, many functional genes have been targeted. Molecular marker assisted selection can accelerate the breeding process by linking selection to functional genes. The SNP marker could be used for molecular assisted selection of cotton architecture. [17]

Conclusion

Presently, the enormous development of more efficient DNA markers will go on in the future, because they can serve as an important tool for the plant breeders and geneticists to develop the cultivars of cotton that are demanded by the society. The innovative tools studied in present investigation to screen the diverse cotton population using biochemical markers viz., seed protein, carboxylase esterase, peroxidase and polyphenol oxidase along with precise DNA technique viz., RAPD analysis can be deployed for speed-up the process of selection of parents in breeding programme to build the platform of marker assisted breeding. Whereas, there is need of standardizing and updating these techniques to adopt as a integral component of cotton improvement programs in future.

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