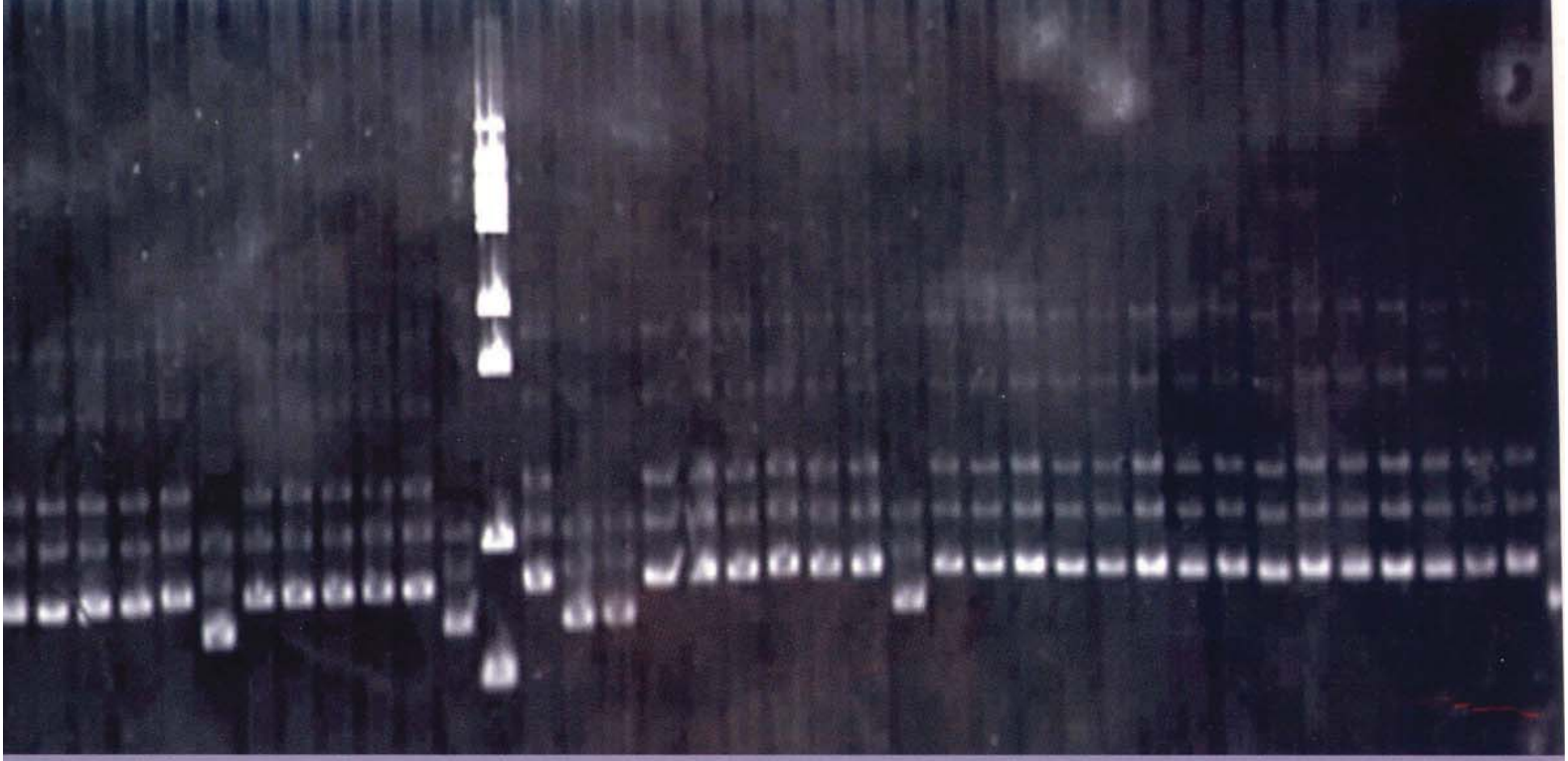

TMC MM 1.5: Molecular characterization of cotton germplasm using DNA markers

INTRODUCTION

The project is aimed to characterize cotton germ plasm using molecular tools for its proper utilization in breeding programs. Molecular markers increasingly play an important role in crop improvement programs Characterization of germplasm using molecular markers would ultimately help in broadening the genetic base of cotton.

In 2007-08, initially 150 STMS primer-pairs were screened on a set of six germplasm lines. A total of thirty-seven primer-pairs giving optimum band amplification were identified. The annealing temperature for these was further standardized at 12 different temperatures in order to optimize the best temperature for amplification. Ninety cotton germplasm lines were used for characterization. The results indicated that all the lines could be discriminated from one another using 37 STMS primers. Twenty-four selected germplasm of working collection belonging to *G.hirsutum* were subjected to DNA based RAPD, ISSR and SSR (Microsatellite) analysis. The study revealed that MAR-868 was the most diverse among tetraploid genotypes with bootstrap support 100%. Also 24 elite cotton varieties were successfully discriminated on the basis of their RAPD pattern. Thirty-six used primers amplified a total of 332 bands with an average of 8.97 bands per primer. Out of 332 bands 306 were found to be polymorphic. They showed 90.52% polymorphism and the average number of polymorphic bands per primer were observed 8.9.

In 2008-2009, 236 STMS primer pairs were screened on a set of eight germplasm lines of diploid cotton *(Gossypium arboreum* and *Gossypium herbaceum)*. One hundred three germ plasm lines of G. *arboreum* were characterized using 26 STMS primer pairs and 104 germplasm lines of *G.herbaceum* were characterized using 10 STMS primer pairs. Molecular characterization of 100 cotton *(G.hirsutum)* core collections using PCR based 28 STMS loci was completed.

OBJECTIVES

- Standardization of PCR conditions for STMS primers and for known molecular tags for economic traits.
- Molecular characterization of cotton germ plasm using PCR based markers and known molecular tags for economic traits.
- Cataloguing of cotton germplasm accessions based on molecular marker data and using molecular tags for economic traits and diversity analysis.

SALIENT FINDINGS

At NRC on DNA Fingerprinting, 300 microsatellite primer-pairs were screened on a set of eight cotton lines to identify the ones give optimal and polymorphic amplification products. A total of 40 primer-pairs giving optimum band amplification were identified for further molecular characterization of the working collection of cotton. One hundred fifty-six germ plasm lines (Table 1.5.1) belonging to seven working groups (yield, variety, okra leaf, oil, boll weight, mean hallo length (MHL) and bacterial blight resistance) were characterized using 40 primers. DNA from each of the accession was extracted following the CTAB method standardized in the lab and subsequently purified and quantified fluorometrically. PCR amplification products were resolved using polyacrylamide gel electrophoresis. A total of 173 amplification products were obtained, of which 149 were found to be polymorphic resulting in 86% polymorphism. The number of polymorphic markers per primer ranged from 1 (BNL3594, MUCS082, CIR291, MUCS422, MUCS46) to 8 (MGHES73, JESPR215, JESPR66) with an average of 3.73 polymorphic bands per primer. Resolving power of primers and polymorphism

information content (PIC) were calculated to find their efficiency to distinguish genotypes unambiguously. Microsatellite profiling of 156 accessions for two representative loci is depicted in figure 1.5.1 and 1.5.2. Pair-wise genetic relationships were determined on the basis of Jaccard's coefficient and average pair-wise similarity was found to be 63% in the 156 working collection studied. Accessions IC-357200 and IC-356527 showed the maximum similarity (98%), while IC-356975 and IC-357682 CY showed the least pair-wise similarity (36%). All the selected accessions of working collection could be grouped into six major clusters namely, Cluster I, II, III, IV, V and VI comprising of 120,2,28,1,3 and 2 accessions, respectively (Figure1.5.3). Majority of accessions were in Cluster I which could be further divided into three sub c1usters- sub-cluster I (35 accessions), sub-cluster II (59 accessions) and sub-cluster III (26 accessions). Band statistics like per cent polymorphism, polymorphism information content and resolving power of each marker locus was determined. Resolving power of a primer was found to be the best statistics for unequivoca identification. Nineteen microsatellite marker loci were found to be useful in discriminating all 156 accessions of working collection based on resolving power. The probability of identity of fingerprints of two accessions by chance was found to be 2.95 x 10-17and 9.34 x 10-16 with a set of 40 and 19 primers, respectively which is quite low.

Table1.5.1 : List of 156 germ plasm **lines of G. *hirsutum* belonging to** seven **trait groups used for characterization**

Sr. No.	Group Name	Accessions Name
1	Yield Group (YG)	1. EC-132030, 2.IC-358371 LYY, 3. EC-128340CY, 4. IC-358215LYC, 5. Ec138570 CY, C,(28 accessions) 6.IC-357011 CC, 7. EC-137592CY, 8. IC-358036, 9. EC 141294CY, CC, 10. IC-357298, 11. IC-359042CC, 12. IC-359963, 13. EC-559033, 14. EC-450616, 15. EC-126424, 16.IC-358280CY, MH, PURE, 17.IC-359828, 18. IC-358345, 19. EC-138569CY, C, 20.IC-356900, 21. IC359858, 22. IC-357196LYY, 23. EC-128334, 24.IC-356935LYY, 25.IC-359863, 26. EC-110788, 27. EC-559034,28.IC-358306.
2	Boll Weight Group (BW) (21 accessions)	29.IC-359062, 30. EC-128334, 31. EC-138340CY, 32. IC-359883, 33.IC-357857, 34. IC-357599, 35. EC-128333, 36. IC-357540, 37. IC-356587, 38. IC-356531, 39.IC-356543, 40.IC-358004, 41.IC-357011, 42. EC-285601, 43. IC-357749, 44. IC-359042, 45.IC-359108, 46. EC-138317, 47.IC-357000, 48. EC-268434, 49. LAM GUNTUR.
3	MHLGroup (9 accessions)	50.IC-358358, 51.IC-356665, 52.IC-357200, 53.IC-357243, 54. IC-356527, 55.IC-357258, 56.IC-357218, 57.IC-357205, 58.IC-357196
4	Okra Group (OK) (14 accessions)	59. EC-170338, 60. EC-170340, 61. EC-142762, 62. EC-152280, 63. EC-158938, 64. EC-128578, 65. EC-142762, 66. EC-170340, 67. EC-128578, 68. EC-152285, 69. EC-142762, 70. EC-170340, 71. EC-142762, 72. EC-142762
5	Bacterial Blight Resistance Group (BBR) (18 accessions)	73.IC-357555, 74.IC-358789, 75. IC-357571 , 76.IC-359059, 77.IC-357554, 78. IC-357461 , 79.IC-358215, 80.IC-358449, 81.IC-356785, 82.IC-358653, 83.IC-356725, 84.IC-358218, 85.IC-358382, 86.IC-357008, 87.IC-356825, 88. IC-357296, 89. IC-356975. 90. IC-356825.
6	Variety Group (VAR) 53 accessions)	91. IC-359032 CC, H, 92.IC-358002, 93. IC-359051, 94. IC-359061, 95. IC-359048, 96.IC-359029, 97.IC-359055, 98.IC-359060, 99.IC-359034, 100.IC-359038, 101. IC-358781 , 102.IC-359046, 103. IC-359041 , 104.IC-359036 CY 105.IC-359026CC, 106.IC-359023, 107.IC-358790, 108.IC-359040, 109.IC-359065, 110.IC-359054, 111.IC-359047, 112.IC-359031, 113.IC-359036, 114.IC-359027, 115.IC-359051 H, LYC, 116.IC-358789, 117.IC-359022 CC, H 118.IC-358905 CC, 119.IC-359056, 120.IC-359039CC, MH, 121.IC-359044 CY, H 122.IC-359056, 123.IC-357981 CC, MH, 124.IC-358773 CC, MH, 125.IC-359035 H, LYC, 126.IC-359048, 127. IC-359028, 128, IC-359055, 129. IC-359066 CY, 130. IC-359043 LYY, H, 131. IC-358856, LYC, 132. IC-359062 CY, H, 133. IC-359056, 134. IC-356901 CC, H, 135.IC-359021 CC, MH, 136.IC-359059CC, H, 137.IC-359042CC, H, 138. IC-359023CC, H, 139. IC-357038 LYC, H, 140. BN RED, 141. BN FREGa, 142. SUMAN, 143.IC-359045CY. MH.
7	Oil Group (13 accessions)	144. IC-356750 CY, 145.IC-358271 CC, CY, 146.IC-358002, 147.IC-357705CC, 148.IC-358248CY, 149.IC-356570CC, 150.IC-358026CC, 151.IC-357657CY, 152.IC-358305CY, 153.IC358371, 154.IC-357682CY, 155. EC-140905, 156. EC-140904.

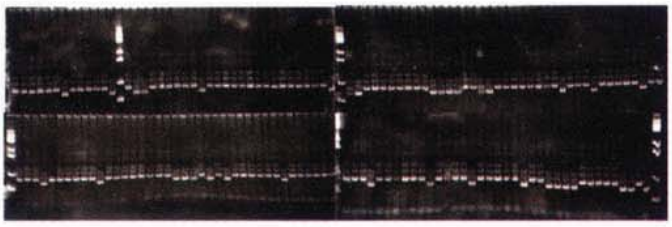

Fig.1.5.1 : Amplification profile of 156 germ plasm lines using microsatellite markers for the locus JESPR 151

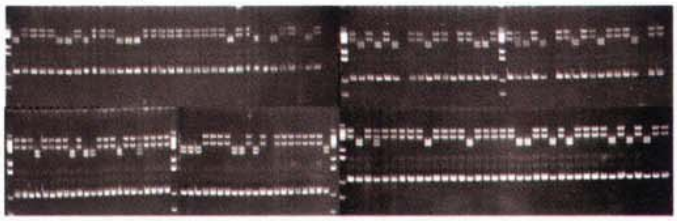

Fig. 1.5.2 : Amplification profile of 156 germplasm lines using microsatellite markers for the locus MGHES 40

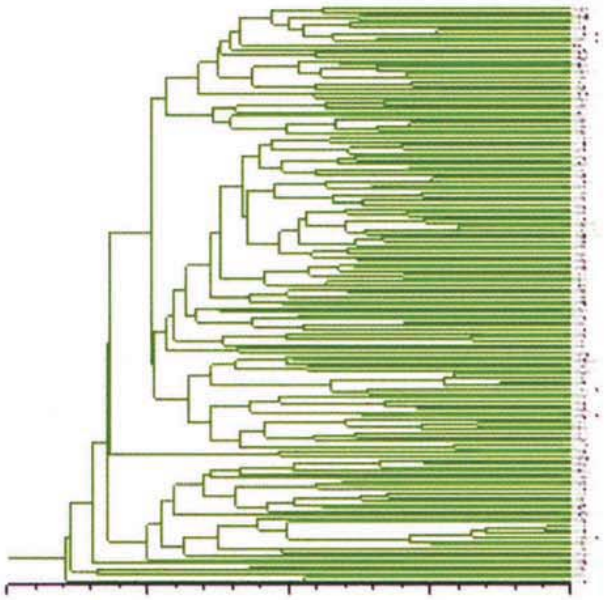

Fig 1.5.3 : Jaccard's similarity based dendrogram of 156 cotton germplasm lines belonging to seven working collection using STMS markers

At CICR Nagpur, hundred accessions of working collection of *G.hirsutum* were successfully discriminated on the basis of their banding pattern. Twenty eight (28) STMS primers (Table 1.5.2) were used which produced a total of 139 bands with an average of 4.96 bands per primer. Out of 139 bands, 121 were found to be polymorphic which resulted in 86.12 per cent polymorphism and the average number of polymorphic bands per primer was observed to be 4.32. The number of DNA amplified fragment per primer ranges from 3 (JESPR 208) to 7 (MUCS 164 and BNL 2986), whereas the average size of the fragments ranged between 100bp to 900bp. A representative profile of 100 accessions fingerprinted with primer M-04 is depicted in figure 1.5.4.

Cluster analysis grouped all accessions into two clusters (Figure 1.5.5 & 1.5.6). Cluster I consisted of 93 accessions whereas cluster II consisted of six accessions. Cluster I was divided into two sub clusters Ia and Ib at a similarity of 0.659. Subcluster Ia consisted of 86 accessions while Subcluster Ib consisted of seven accessions. Cluster II was divided into Sub-cluster IIa & Sub-cluster Iib at a similarity coefficient of 0.662. Subcluster Iia consisted of four germplasms i.e. KEKCHI, SENZE-PENA-TOBA, MACHA and S-4727 of which KEKCHI, SENZE-PENA-TOBA were from bacterial blight resistant group and were 100% identical for characteristics i.e. seed cotton yield (g), ginning outturn (%), mean halo length (mm) and boll weight (g) etc. Accession COKER-413 and M-1 were in the Sub-cluster Iib.

At NRC on DNA Fingerprinting and CICR Nagpur, 256 germplasm accessions were characterized out of which sixty were common in both the centres.

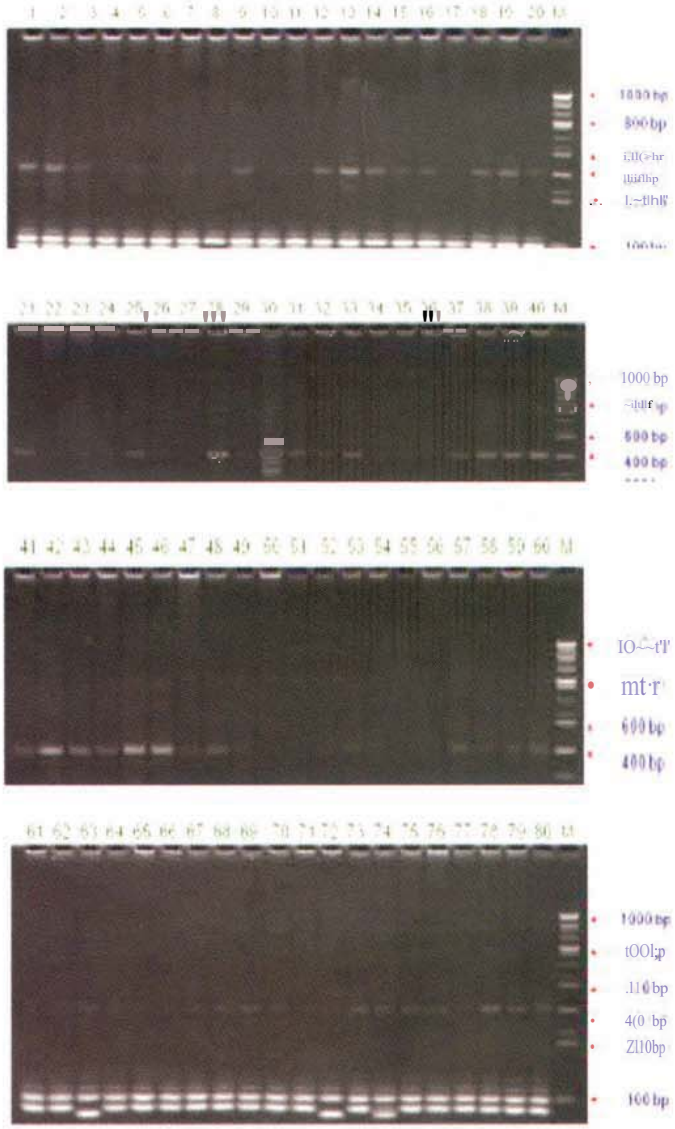

Fig.1.5.4 : STMS profile of 100 cotton working germplasm obtained with primer M-04 M is 100bp DNA size standard

Table 1.5.2 : List of STMS primers and polymorphic amplicons generated

Sr. No.	Primer	Total number of fragments	Polymorphic fragments	Percent polymorphism	Size range (bp)
1	MUGS 164	7	5	71.42	100bp-1 OOObp
2	MUGS 400	5	4	80	200bp-500bp
3	BNL 3992	6	6	100	50bp-800bp
4	JESPR 197	5	5	100	50bp-400bp
5	BNL 3594	4	3	75	150bp-400bp
6	BNL 2960	5	3	60	50bp-300bp
7	MUSS 082	4	3	75	200bp-500bp
8	GIR 166	4	2	50	150bp-500bp
9	GIR 285	6	5	83.33	150bp-400bp
10	GIR 291	4	4	100	400bp-600bp
11	BNL 2709	4	4	100	200bp-400bp
12	MUSS 018	5	5	100	400bp-800bp
13	GIR 288	6	6	100	100bp-600bp
14	MUGS 152	6	6	100	150bp-400bp
15	MUGS 422	5	4	80	150bp-500bp
16	BNL 1694	5	4	80	100bp-500bp
17	BNL 2986	7	6	85.71	100bp-900bp
18	BNL 3008	6	6	100	150bp-400bp
19	JESPR 135	4	3	75	100bp-200bp
20	JESPR 151	4	4	100	150bp-400bp
21	JESPR 152	5	5	100	100bp-600bp
22	JESPR 160	5	5	100	100bp-900bp
23	JESPR 197	4	3	75	50bp-200bp
24	JESPR 204	6	6	100	150bp-400bp
25	JESPR 206	5	4	80	200bp-600bp
26	JESPR 208	3	2	66.66	100bp-200bp
27	JESPR 215	5	5	100	150bp-600bp
28	JESPR 224	4	3	75	150bp-500bp
	Total	**139**	**121**	**2412.12**	
	Average	**4.96**	**4.32**	**86.12**	

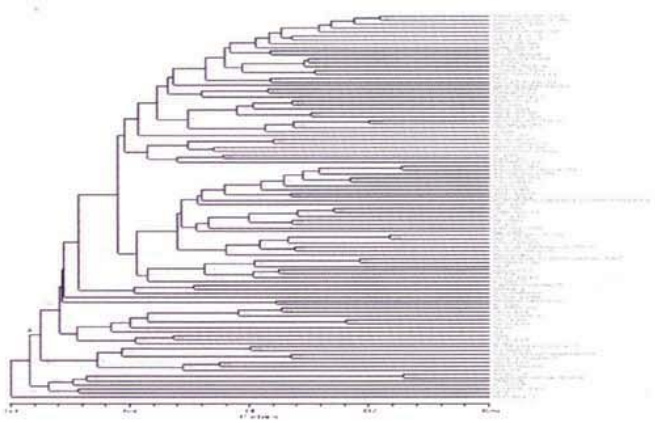

Fig. 1.5.5 : UPGMA cluster analysisbased dendrogram constructed from the STMS profiles depicting genetic relationships among 100 working germplasms of *G.hirsutum* (percent bootstrap values depicted inside the figure)

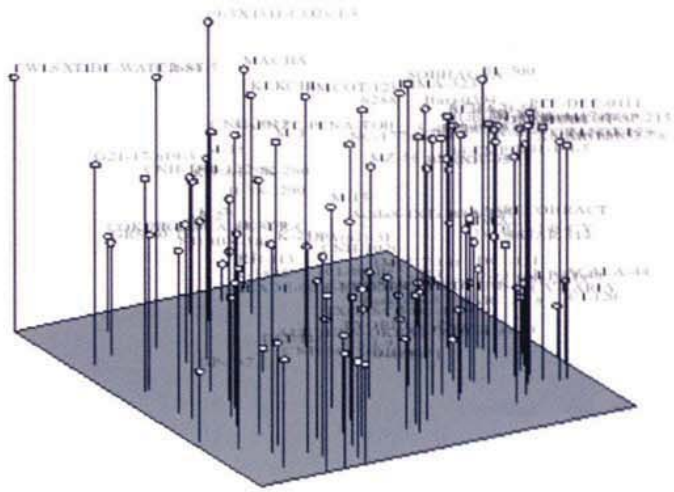

Fig. 1.5.6 : Principal Coordinate analysis of 100 working collection accessions based on STMS Markers