Title: Molecular characterization of collon Germplasm using DNA Markers

Code No.	TMC/MMI/1.5
Year	2007-08
Name of the PI and Lead centre	Dr. M. K. Rana NRCDFp, New Delhi
Name of the CCPI and centre	Dr. A. B. Dongre CICR, Nagpur

INTRODUCTION

Modern technologies such as gene tagging and marker aided selection are additional tools for crop improvement specialists. Molecular markers have gained more significance with globalisation and introduction of Plant Variety protection. It is essential to document and catalogue our existing germ plasm using molecular markers, which are also environmentally stable and reliable. In addition, molecular cataloguing helps in avoiding conservation of duplicates if any, in the germplasm.

OBJECTIVE

Molecular characterization of Cotton germplasm using PCR based markers and known molecular tags for economic traits.

ACTIVITIES

Working germplasms characterized -

OIL-55, OIL-1142, OIL-1167, OIL-1190, OIL-1511, OIL-1756, OIL-1856, OIL-2040, OIL-2601, OK-2861, OK-2863, OK-2885, MAR-28, MAR-717, MAR-868, MAR-2480, MAR-2484, OK-2860, MAR-1 030, MAR-1295, MAR-1297, MAR-1429, MAR-1668, MAR-2234

1. Molecular markers applied: RAPD, ISSR and SSR,
2. No. of primers tried: RAPD -20, ISSR -19 and SSR -25.
3. No. of primers worked: RAPD -20, ISSR - 17, SSR 21.
4. Material used for analysis: Leaf

Varieties characterized

DDhC 11, JK4, RS 2013, Sahana RS 875, DLSa-17, F1861, Y-1, LH 1556, LD 694, LD 327, JAYADHAR, RHC 004, J, Tapti, RS 810, HS-6, H-117, HD 123, RARS, LAM, LRA-5166, K2(MB), G. Cot 10, SRT GMS-1, BN-1

1. Molecular markers applied: RAPD
2. No. of primers tried: RAPD- 38,
3. No. of primers worked: RAPD-36
4. Material used for analysis: Leaf

EXECUTIVE SUMMARY

Three molecular marker systems utilized in the study (RAPD, ISSR and SSR) provided a wider coverage of genome, delineating the genetic distance between the cultivars. Average number of bands produced per loci was 5-13 (RAPD), 6-15 (ISSR), and 2-6 (SSR) respectively. The range of similarity coefficient as found by RAPD was 0.73-0.96, by ISSR 0.76-0.95 and 0.72-0.96 by SSR, SSR Marker showed highest polymorphism i.e. 67.64%. In all marker systems, all accessions were found to form two major clusters I and II, Dendrogram study of RAPD, ISSR and SSR also revealed that the MAR-868 forms separate cluster, which suggested MAR-868 to be the most diverse among tetraploid genotypes with bootstrap support 100%. The rare allele and unique fingerprint obtained in the study have a number of potential applications including the determination of cultivar purity, efficient use and management of genetic resources collection and the establishment of property rights. They may also be utilized for tagging & mapping of either simple or complex traits. Based on the clustering pattern and genetic relationship obtained from the present study, breeders can identify the diverse cultivars from different clusters and employ them in their further breeding programs or the results obtained in this study can be used for the selection of parents to generate mapping population

SALIENT FINDINGS

Molecular characterization using RAPD

S. No.	RAPD analysis	Observations
1.	Total number of primers used	20
2.	Total number of working germplasm analyzed	24
3.	Number of reproducible primers	20
4.	Size range of amplified products	200 bp - 3000 bp

20 RAPD primers were used to find out the genetic diversity in Twenty-four selected germplasms belonging to G. *hirsutum (9* of Oil percentage, 11 Marker for internode length and 4 of Okra leaf structure).

The 24 germplasms were successfully discriminated on the basis of their RAPD pattern. 20 primers amplified gave total 203 bands with an average of 10.15 bands per primer. Out of 203 bands 126 were found to be polymorphic. They showed 62.44 percent polymorphism and the average number of polymorphic bands per primer were observed 6.30 Different primer produced different level of polymorphism among the different varieties. The number of DNA amplified fragment per primer ranged from 5 (OPA-04 and OPA-14) to 14 (OPA-5 and OPA-8).

The most informative RAPD primer was OPA-5 with 10 polymorphic bands (Fig: 1) and least informative were (OPA-4 and OPA-14) with three polymorphic bands. The size of the fragments ranged was between 200bp to 3000bp.

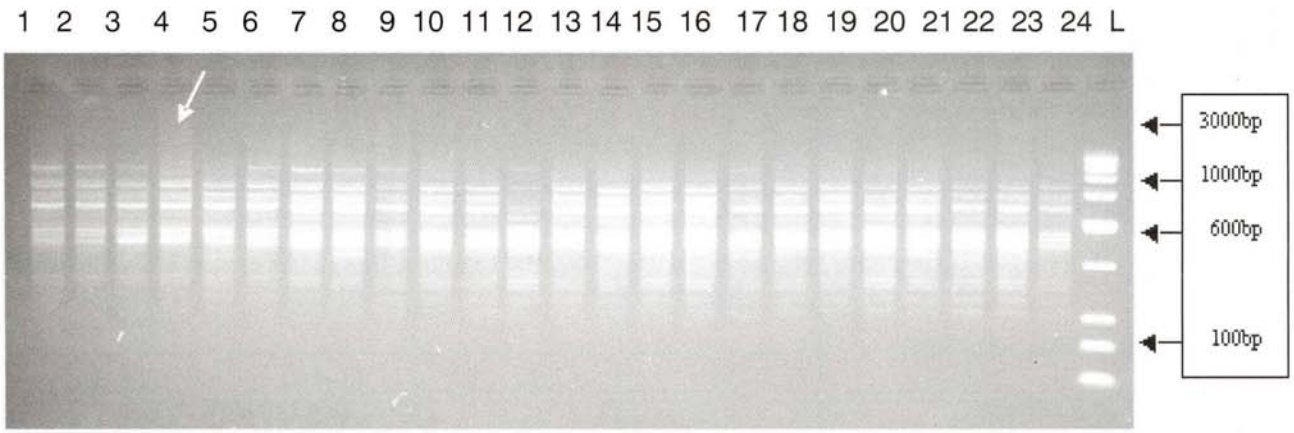

Fig 1: RAPD profile of 24 working germplasms obtained with prime OPA-05, M = Low range DNA Ladder used as Molecular weight Marker.

Molecular characterization using ISSR

S. No.	ISSR analysis	Observations
1.	Total number of primers used	19
2.	Total number of working germplasm analyzed	24
3.	Number of reproducible primers	17
4.	Size range of amplified products	250 bp - 3000 bp

The 24 germplasms were successfully discriminated on the basis of ISSR pattern. Seventeen primers amplified total provided 166 bands with average of 9.7 bands per primer. Out of 166 bands, 91 bands were found to be polymorphic. They showed 54.05 percent polymorphism and the average numbers of polymorphic bands per primer were observed was 5,3. Different primers produced different level of polymorphism among the different varieties. The number of DNA amplified fragment per primer ranged from 6(IS-2) to 15 (IS-8).

The most informative primer was IS-14 with 10 polymorphic bands (Fig: 2a & b) and least informative was (IS-12) with two polymorphic bands. The size of fragments ranged between 250bp to 3000bp.

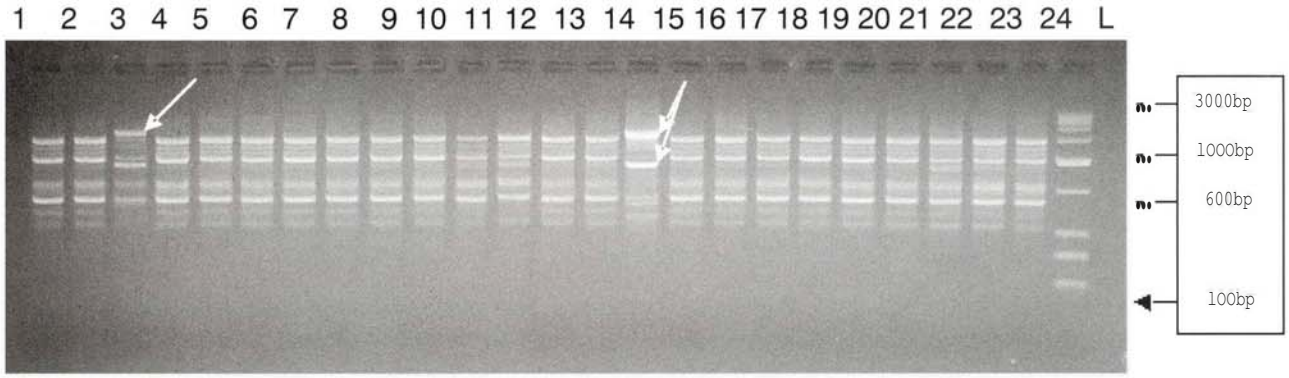

Fig 2a: ISSR profile of 24 working germplasms obtained with primer IS-04, M= Low range DNA Ladder used as Molecular weight Marker.

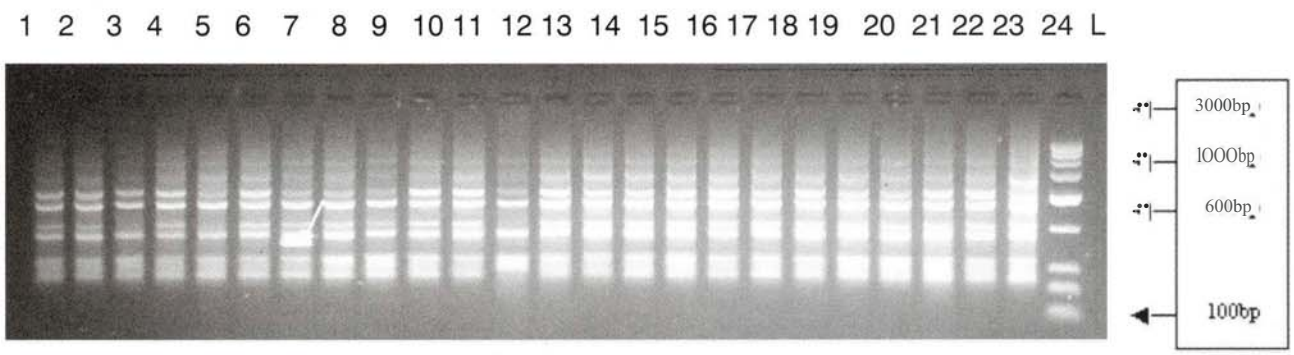

Fig 2b: ISSR profile of 24 working germplasms obtained with primer IS-07, M= Low range DNA Ladder used as Molecular weight Marker

Molecular characterization using SSR

S. No.	SSR analysis	Observations
1.	Total number of primers used	25
2.	Total number of working germplasm analyzed	24
3.	Number of reproducible primers	21
4.	8ize range of amplified products	50 bp -1000 bp

The 24 germplasms were successfully discriminated on the basis of 88R pattern. Twenty-one primers amplified total 113 bands with an average of 5.38 bands per primer. Out of 113 bands 81 were found to be polymorphic. They showed 67.64 percent polymorphism and the average number of polymorphic bands per primer were observed 3.85. Different primer produced different level of polymorphism among the different varieties. The number of DNA amplified fragment per primer ranged from 2 (8-44) to 10 (8-40 and 8-46).

The most informative primer was 8-46 with 9 polymorphic bands (Fig:3) and least informative were (8-33, 8-34, 8-35, 8-43, 8-44 and OPB-15) with one polymorphic band. The average size of the fragments was between 40bp to 1000bp.

Principle component analysis was also carried out to compliment the cluster analysis and to provide visualization of genetic relation ships among the genotypes.

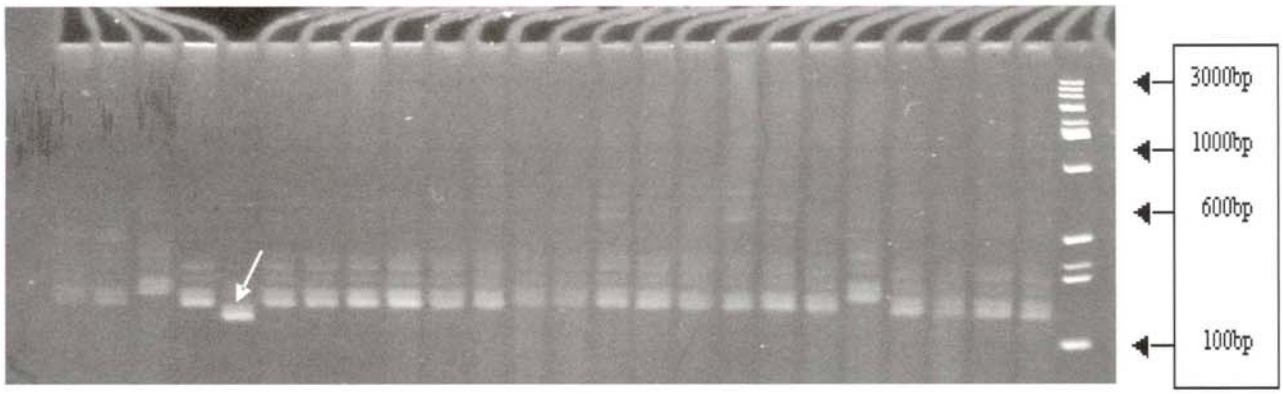

Fig 3: SSR profile of 24 working germplasms obtained with primer S-46, M= Low range DNA Ladder used as Molecular weight Marker.

List of RAPD, ISSR, SSR primers generated polymorphic amplicon and rare alleles

Sample No.	Primer No.	Total No. of Bands	No. of Polymorphic Bands	% Polymorphism	Rare alleles
OIL-2601 MAR-1429 OK-2863	OPA-1	09	07	77.8	03(1100bp) (1400bp) (1100bP)
OIL-1190	OPA-5	14	10	71.4	01 (2100bp)
MAR-868 MAR-1030	OPA-8	14	08	57.1	02 (2200bp) (2200bp)
OIL-1167 MAR-868 MAR-1297	OPA-10	12	06	50.0	03 (600bp) (1100bp) (600bp)
MAR-868	OPA-12	09	07	77.8	01 (1700bp)
MAR-1295	OPA-13	13	09	69.2	01 (800bp)
OIL-1190	OPA-20	11	07	63.6	01 (2200bp)
MAR-1297	ISSR-1	08	06	75.0	01 (3000bp)
OIL-1167 MAR-1297 MAR-1297	ISSR-4	09	05	55.5	03 (2200bp) (600bp) (2200bp)
MAR-868	ISSR-5	12	07	58.3	01 (2100bp)
OIL-1856	ISSR-7	10	06	60.0	01 (750bp)
MAR-1429 MAR_1429	ISSR-11	09	05	55.5	02 (1200bp) (2000bp)
OIL-1765 MAR-868	ISSR-14	12	10	83.3	02 (3000bp) (3000bp)
OIL-1511	SSR-46	10	9	90.0	01 (120bp)