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Madhuri B Gawande
Ph.D. Scholar, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

SJ Gahukar
Professor & Officer Incharge, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

DR Rathod
Assistant Professor, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Aamrpali Aakhare
Assistant Professor, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Yashoda Etther
Ph.D. Scholar, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

RL Bhakre
Ph.D. Scholar, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Correspondence
Madhuri Gawande
Ph.D. Scholar, Biotechnology Center, Department of Agril Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Study of parental polymorphism for fibre properties in cotton (*Gossipium hirsutum*) using SSR markers

Madhuri B Gawande, SJ Gahukar, DR Rathod, Aamrpali Aakhare and Yashoda Etther and RL Bhakre

Abstract
The present investigation has been undertaken to study the polymorphism between the three diverse cotton genotypes i.e. MCU5 a long staple popular variety, PKV Rajat and AKH-081 popular university varieties of *Gossipium hirsutum*. The variation among these parents was characterized using 116SSR markers. Out of 116 SSR markers tested, 64 SSR markers were shown polymorphism between the parents PKV Rajat, AKH-081 and MCU-5.

Keywords: Cotton, parental polymorphism, fibre properties SSR markers

Introduction
Cotton (*Gossypium spp.*) is the most preferred natural fibre in the world and plays a major role in the economy of agriculture and industry. Cotton accounts for 45 per cent of the world fibre and supplies 10 per cent of world's edible oil (Rathore, 2005) [4]. India has a pride place in the global cotton scenario due to several distinct features such as largest cotton growing area, cultivation of all the four cultivated species, large area under tetraploid cotton, one of the largest producers of long and extra-long staple cotton, possibly the only country to grow hybrid cotton, native home of old cultivated cotton and wide diversity in agro-climatic conditions under which cotton is grown.

It is grown mainly for its fibre used in manufacturing of clothes for mankind. Seed is the most important part of the plant. In case of yield enhancement of lint, fibre qualities i.e. staple length, staple strength and micronair are very important for crop improvement. Fibre is a basic raw material in the textile industry. It is an industry raw material in which cotton marketing is based on measurable quality properties and one where technological changes are being rapidly implemented.

Molecular markers allow breeders to dissect complex traits without having to measure the phenotype every time, thus reducing the need for extensive field testing over time and space which avoid unreliable phenotypic assays and accelerate accurate identification of genotypes with agronomically important traits. DNA-based molecular markers hold great promise in breeding for qualitative traits like fibre quality (Gupta 2006) [3], (Zeng *et al.* 2009) [6], (Boopathi *et al.* 2011) [1].

Materials and Methods
Three different genotypes out of which MCU-5 is the first extra-long staple and high strength variety of *G. hirsutum* group released in India during 1968 for Tamil Nadu state, PKV Rajat and AKH-081 were more suited under high density planting system (HDPS) of cotton cultivation. AKH-081 is one of the good cotton yielding genotype which was released in 1987 by cotton research unit, Dr. PDKV, Akola were chosen as parents to make crosses *viz.,* MCU5 × AKH-081and PKV Rajat x MCU-5 to develop mapping population. Genomic DNA was extracted from young tender leaves from a random sample of five plants from each parent following the standard cTAB method (Doyle and Doyle, 1990) [2]. The DNA quantification was done by after extraction was confirmed by running it on 0.8% agarose gel (containing ethidium bromide @ 0.5 mg/ml) in a horizontal gel electrophoresis system, Isolated genomic DNA was amplified using different available markers and parental polymorphism survey was done on PAGE (Polyacrylamide gel electrophoresis) (Sambrook and Russell, 2006) [5]. PCR optimization for SSR markers was done by varying concentration of template DNA, Taq polymerase, dNTPs, primers and MgCl. The amplification reaction with SSR primers was carried out in a final volume of 25 µl.

PCR reaction mix containing the following.

Master Mix	1x
10x *Taq* polymerase assay buffer with $MgCl_2$	2.5 µl
$MgCl_2$ (25 mM)	1.5 µl
dNTPs (10 mM)	0.25 µl
Taq polymerase (5 U/ µl)	0.1 µl
Sterile distilled water	15.65 µl
PCR Reaction	
Master Mix vol.	20.00µl
Primer (Forward)	1.5 µl
Primer (Reverse)	1.5 µl
Template DNA (30 ng)/ µl	2µl
Total Reaction volume	25 µl

PCR condition for SSR analysis included an initial pre denaturation step of 5 min at 94^0C and following 40 cycles of amplification: Final extension was carried out at 72^0C for 10 min. The amplified fragments were stored at 4^0C for short periods and at -20^0C for long duration. Amplified PCR product from each reaction was separated on 1.8% PAGE gel. 1 x TAE buffer at 130V, finally visualized and photographed using gel documentation. In the presentinvestigation, 116 SSR markers were used.

Results and Discussion

Parental polymorphism survey among three diverse parental lines of cotton was studied using the 116 simple sequence repeat (SSR) primer sequences reported in various research papers were screened to identify polymorphic markers to differentiate parents and hybrid under study. The size of amplicons produced by SSR markers was highly variable. Out of total 116 primers tested; sixty four primers showed polymorphic amplification Fig. 1, Table 1. Each polymorphic primer was tested at least twice to determine if both the polymorphism and banding pattern were reproducible. Though the parental genotypes were distinct for various important traits. The material consisting of 40 selected F_2 populations from promising two crosses derived from two F_1's crosses between PKV Rajat × MCU-5 of cross-I and AKH-081 × MCU-5 followed by sefling developed two resultant F_2 populations. The F_2 population were segregated for the trait of interest namely major morphogical, fiber qualities like staple length and its strength were employed for identifying SSR marker linked. Thus screening of contrasting parents involved in both the crosses under the study was the basic requirement for identifying the polymorphic microsatellite markers linked to traits specifically fibre quality.

Table 1: Details of Chromosome wise SSR marker used, tested, polymorphic markers & % polymorphism

Ch. No.	marker tested	Poly marker	Poly %	Name of Polymorphic marker
1	5	3	60.0	BNL2827, BNL3888, BNL2921
2	4	3	75.0	NAU895, BNL580, BNL2440
3	3	3	100.0	BNL2656, NAU769, BNL226
4	6	2	33.3	BNL2572, BNL4047
5	3	3	100.0	NAU1200,BNL3569, BNL3881
6	5	2	40.0	BNL3985, BNL1064
7	5	3	60.0	NAU474,NAU1043, BNL3652
8	5	2	40.0	BNL3627, BNL663
9	6	4	66.7	BNL219, BNL2847, BNL3582, BNL4028
10	4	2	50.0	NAU992, BNL1161
11	5	3	60.0	BNL1066,BNL1681,BNL1404
12	5	3	60.0	BNL3537, BNL1679, BNL1707
13	4	1	25.0	BNL4061,
14	6	3	50.0	NAU4024, BNL3502, BNL2263
15	5	3	60.0	BNL3902, BNL2920, BNL3920
16	4	2	50.0	NAU733, BNL2634
17	4	2	50.0	BNL2471, BNL3590
18	6	2	33.3	BNL1040, BNL3479,
19	6	2	33.3	BNL3535, BNL3347
20	4	1	25.0	BNL135
21	3	2	66.7	BNL3649, NAU555
22	3	3	100.0	BNL4030, NAU569, BNL448
23	2	2	100.0	BNL1317, NAU923
24	3	3	100.0	NAU1037, NAU1302, BNL269
25	4	1	25.0	BNL3806
26	6	3	50.0	BNL3031, BNL840, BNL2495
Total	116	64		

Ch. No: Chromosome number, marker tested: Number of marker tested, Poly marker: polymorphic markers, Poly%: Polymorphism percent, Name of marker: Marker used on respective chromosomes

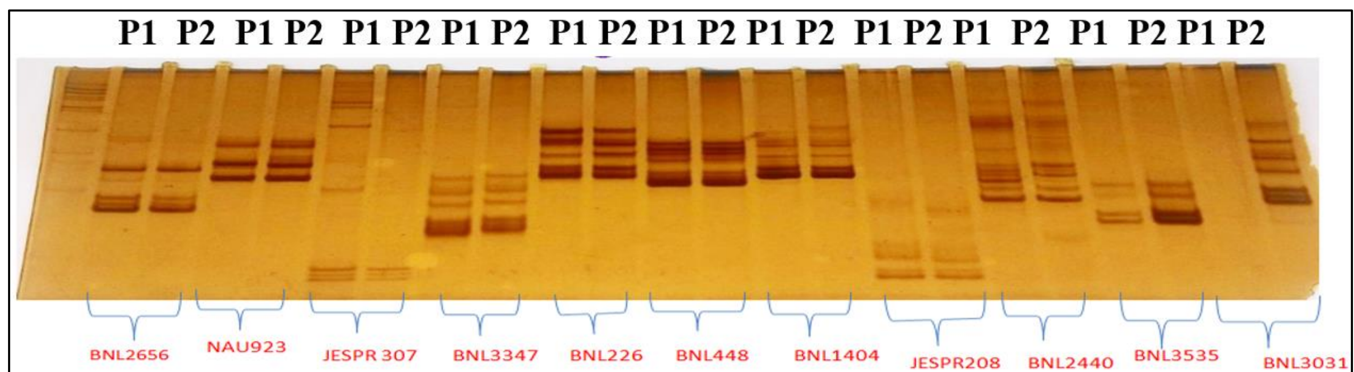

Fig 1: Representative gel image showing parental polymorphism between parents involved in cross-I (P1- PKV Rajat × P-2 MCU 5) using SSR marker M – Known DNA maker of 100 bpP1: AKH-081, P2 : MCU-5

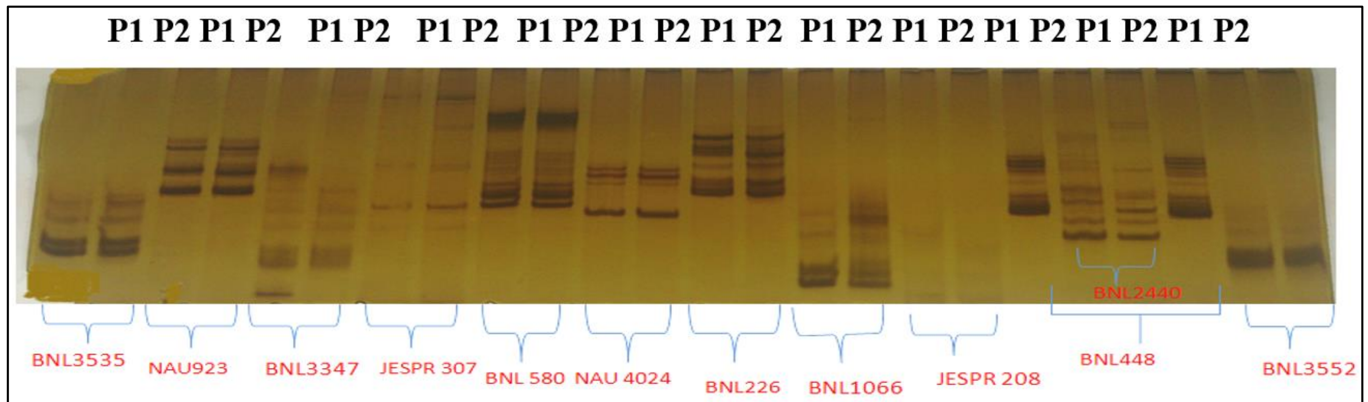

Fig 2: Representative gel image showing parental polymorphism between parents involved in cross-I (P1- PKV Rajat × P-2 MCU 5) using SSR marker M – Known DNA maker of 100 bpP1: AKH-081, P2 : MCU-5

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