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Identification of Cotton F_1 Hybrids and their Parents through Molecular Marker under Salinity Stress

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Abstract

An experiment was carried out at Main Cotton Research Station, NAU, Surat, Gujarat, India during 2018–2020 to identify F_1 hybrids and their parents through SSR marker for salinity tolerance in cotton. The four cotton parents (two salt tolerant and two salt sensitive) were crossed in a diallel fashion to obtain twelve cotton hybrids and subjected to DNA isolation and PCR amplification with SSR markers. In the present study, six SSR markers (TMB0409, DPL0094, BNL686, JESPR153, CM45 and MGHES006) were identified to be polymorphic between parents and the hybrids. The SSR primer TMB0409, DPL0094, JESPR153 and CM45 identified two fragments each from different parents in two, two, four and eight cotton hybrids, respectively, which confirmed true hybrids. Hence, the SSR molecular marker, individually or in combination can be used to distinguish and confirm the hybrid and parents in cotton with special reference to salinity. The PCA analysis revealed that BNL686–1 (248 bp) allele contributed significantly to the quantum of variation as explained by PC1. Hence, this allele is able to serve as a benchmark for ascertaining the efficient pattern of grouping between genotypes. Further, the marker CM45 amplified a fragment specific to the saline tolerant parents which was absent in sensitive parents as well as a fragment produced in sensitive parent which was absent in the tolerant parents, hence the molecular marker CM45 may associate with the salinity tolerance in cotton and can be used for salinity tolerant breeding program after confirming in a large population.

Keywords: Cotton, dendrogram, hybrid, marker, PCA, saline, SSR

1. Introduction

Cotton is one of the most important commercial crops worldwide and is popularly known as “white gold”. The cotton is produced in major countries like the USA, China, India, Pakistan, Uzbekistan, Turkey, Brazil, Greece, Argentina, Australia and Egypt. The diversity of cotton cultivars and cotton agro climatic zones in India is considerably larger as compared to other major cotton growing countries in the world. Gujarat is one of the main cotton producing states of the country having a total area of 2.4 Mha and ranked second in the area. Gujarat state ranks first in cotton production with 9.0 M bales with average productivity of 673 kg ha^{-1} (Anonymous, 2020).

Plant breeder recombines characters present in different parental lines of cultivated and wild species for the improvement of agronomic and economic vital characters. Conventional breeding reaches this aim

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by screening the phenotypes of the plant for the desired character from segregating population, which is followed by a process of repeated back crossing, selfing and testing. For the screening of the phenotypic traits, breeders require an accurate screening method and availability of lines with distinct phenotypic characters, which is time consuming and difficult to achieve with classical methods. The genetically pure hybrid seeds supply to the farmers within the time limit is a key to the success of hybrid technology. Hence, a rapid and reliable technique to assess the purity of hybrid is required, which can be achieved by molecular marker analysis. DNA based markers include random amplified polymorphic DNA (RAPD) (Williams et al., 1990), microsatellite or simple sequence repeat (SSR) (Akkaya et al., 1992), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), restriction fragment length polymorphism (RFLP), (Liu and Furnier, 1993), and single nucleotide polymorphism (SNP) (Papp et al., 2003) are of greater significance for crop improvement. Asif et al. (2009) reported that RAPD and SSR being the most used molecular markers in the crops and can be used to confirm the parentage of their true F_1 hybrids. Similarly, Selvakumar et al. (2010), Dongre et al. (2011), Singh et al. (2015) and Rana et al. (2006) successfully used a set of polymorphic SSR primers to test the genetic purity of hybrid seeds and distinguished parents and hybrids successfully.

In plant breeding, these molecular markers are very helpful not only for confirmation of hybrids but also used for recognition, characterization, identification of genetic variations, marker assisted selection (MAS) (Zhang et al., 2003), linkage mapping (Jiang et al., 2000), genomic fingerprinting (Johar et al., 2018), genetic diversity (Rahman et al., 2002), removal of linkage drag in backcrossing (Kumar et al., 2020; Samuel et al., 2020) and to identify the traits which are not easy to measure by visual observation. These markers were used to distinguish the cotton varieties resistant to jassids, aphids and mites. RAPD marker for the male sterility gene has been identified in cotton (Wang et al., 2007). Abdelraheem et al. (2018) analyzed SSR, AFLP and SNP markers and 169 QTL were detected for drought and salt tolerance associated. Similarly, Cai et al. (2017), Mahmoud et al. (2018) and Sun et al. (2019) identified saline tolerant associated SSR markers in cotton. Patella et al. (2019) demonstrated the advantages of mutual integration of traditional and biotechnological methods and showed the added value of molecular markers for breeding programs by SSR markers not only in selecting the best parental plants for crossing based on their observed homozygosity and dissimilarity values but also in screening the resulting F_1 progeny to distinguish between the offspring resulting from cross-pollination and those resulting from self-pollination. The proposed research work was conducted with an aim to identify cotton hybrids and parents through SSR markers, which would be helpful for the cotton breeders.

2. Materials and Methods

2.1. Plant materials

The plant material for this study comprised of four parent genotypes viz. G.Cot.16, GSHV 185, L 1384 and TCH 1777. Variety G. Cot.16 and GSHV 185 were found salinity tolerant and L 1384 and TCH 1777 were found salinity sensitive. The crossing of these parents was carried out in full diallel fashion to obtain twelve hybrids. The crossing program and molecular marker studies for identification of cotton hybrids using the SSR marker system were carried out at Main Cotton Research Station, NAU, Surat, Gujarat, during 2018–2020.

2.2. Isolation of DNA

Seeds of sixteen cotton genotypes including parents in the present study were used for DNA isolation using the CTAB method as suggested by Sambrook et al. (2001) with minor modifications. Two seeds were cut and shells were removed. The inner material was ground and defatted with hexane. The defatted fine powder was crushing with liquid nitrogen in pestle and mortar. The ground sample was immediately transferred to a 2.0 ml eppendorf tube. One ml of pre–warmed CTAB extraction buffer [50 mMTris base (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 0.1 ml β–mercaptoethanol, 2% CTAB and 1% PVP] was poured and the content was thoroughly mixed by inversion. Tubes were kept in a water bath at 65°C for one hour and the contents were mixed after every 15 min. After cooling 800 µl of chloroform:isoamyl alcohol (24:1) was added and content was thoroughly mixed by inversion for one minute and centrifuged at 10000×g for 15 min at 10°C. The clear supernatant was transferred into another clean 1.5 ml eppendorf tube and washed twice with an equal amount of chloroform:isoamyl alcohol (24:1) as above. The top aqueous phase appeared after centrifugation and was transferred to a new 1.5 ml tube. To this solution, an equal volume of ice cold isopropanol was added, mixed well by gentle inversion and kept at –20°C for overnight. After chilling treatment, tubes were centrifuged at 10000×g for 15 min at 10°C. A pellet of DNA was formed after a short spin. The supernatant was decanted and the DNA pellet was washed with 500 µl of 70% ethanol and centrifuged again at 10000×g for 5 min at 10°C. A pellet of DNA was recovered, air dried and dissolved in 100 µl of 1X TE buffer and centrifuged for a short run of 10 s to collect DNA at the bottom. RNase treatment was given to DNA by adding 4 µl of RNase (100 µg ml^{-1}) and kept at 37°C for one hour. DNA was precipitated by adding 10 µl of 3 M sodium acetate and 200 µl of absolute ethanol. The content was mixed gently and centrifuged at 10000×g for 10 min at 10°C. The liquid was decanted after centrifugation and the pellet was allowed to air dry. DNA was then finally dissolved in 100 µl of 1X TE buffer and mixed thoroughly. The content was centrifuged at 10000×g for 2 min to collect the dissolved DNA at the bottom. An aliquot of stock was stored in the refrigerator at –20°C till further use.

2.3. PCR amplification

The PCR reaction was carried out with extracted genomic DNA. A set of 56 SSR primer pairs belonging to TMB, BNL, NAU, JESPR and MGHES series were synthesized from Eurofins

Genomics India Pvt. Ltd. and used to access the polymorphism in parents. Further screened primer sets were subjected to hybrid confirmation. SSR amplification was performed in 25 µl reaction volumes containing 10X PCR buffer (include 25 mM $MgCl_2$), 2.5 mM dNTPs (each of dATP, dGTP, dCTP and dTTP), 10 pmole μl^{-1} SSR primer and 5 U μl^{-1} *Taq* DNA Polymerase and 20 ng μl^{-1} genomic DNA in 200 µl PCR tube. PCR profile was 94°C for 5 min., then 35 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 1 min and finally 72°C for 10 min.

2.4. Resolution of PCR products

All the PCR products along with the DNA ladder (100 bp to 1 kb) were run on 2.0% agarose gel containing 4 µl of ethidium bromide (1 mg ml^{-1}). Running buffer containing Tris–buffer, boric acid and EDTA (pH 8.0) was used for electrophoresis. Twenty µl of PCR product was mixed with 4 µl of 6X loading dye and loaded onto the well. The gel was run at a constant current of 62 V to separate the amplified bands. The separated bands were documented under a UV transilluminator and photographed by Gel documentation system (UVITEC, Cambridge) and analyzed. The molecular weight of different amplified fragments was determined using UVITECH software with a reference DNA ladder.

2.5. SSR data analysis

The week and spurious bands were excluded from the analysis. Bands were scored as 1 (present) and 0 (absent). Similarity coefficient was calculated using the Jaccard index and a cluster analysis was performed by Unweighted Pair Group Method using Arithmetic Average (UPGMA) using the NTSYS–pc analytical software (Rohlf, 1998). Polymorphism Information Content (PIC) and SSR primer index (SPI) were calculated according to the formula described by Smith et al. (1997), Bootstein et al. (1980) and Garcia et al. (2004). Clustering and multivariate analysis was done based on SSR data using the online web tool ClustVis as described by Metsalu and Vilo (2015).

3. Results and Discussion

In the past few years, molecular markers were used significantly to evaluate plant material for different purposes. Researchers have developed various molecular marker systems according to the need of their analysis. The DNA based marker analysis is free from environmental factors and hence it has great importance in agricultural sciences.

3.1. Molecular marker analysis

In the present study, an SSR marker was used to study the molecular pattern of four cotton parents and its twelve F_1 hybrids. A total of 56 SSR primers were used for the amplification of four cotton parents. Among these, only six SSR primers (TMB0409, DPL0094, BNL686, JESPR153, CM45 and MGHES006) were found polymorphic for parents and further used for amplification of parents and hybrids.

The SSR marker TMB0409 showed two alleles and both alleles showed polymorphism with a 0.75 PIC value and 1.49 SPI value (Table 1). All parents and hybrids were amplified by TMB0409 and results are presented in Figure 1. TMB0409 could identify parent G. Cot 16 (T_1) with a specific fragment of near 229 bp and the same was amplified in the hybrids G. Cot 16×GSHV 185 (T_1T_2) and GSHV 185×G. Cot 16 (T_2T_1) where G. Cot 16 (T_1) was used as a parent. Further, this primer could amplify a fragment of 96 bp which was specific to L 1384 (S_1) and this fragment was also amplified in the hybrids where L 1384 (S_1) was used as a parent. The TMB0409 primer amplified 229 bp amplicon in hybrids TCH 1777×G. Cot 16 (S_2T_1) and G. Cot 16×TCH 1777 (T_1S_2), which was specific to parent TCH 1777 (S_2). Hybrids G. Cot 16×L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed two amplified fragments of 229 bp and 96 bp which were also specific to both parents G. Cot 16 (T_1) and L 1384 (S_1).

After amplification with SSR primer DPL0094 two alleles were observed (Figure 2) and both were polymorphic with 0.75 PIC value and 1.50 SPI value (Table 1). Fragments amplified with

Table 1: Amplification and analysis details of SSR primers

Sl. No.	Name of Primer	SSR motif	Forward (FP) and Reverse Primer (RP) sequence (5'–3')	Expected (bp)	Allele size (bp)	No. of alleles	PIC value	SPI value
1.	TMB0409	(CA)10+ (GA)5	F CAGAGGACGAAGGTAGCAG	221	96–229	2	0.75	1.49
			R TGGTGGGTTTCACTTTCACA					
2.	DPL0094	(AG)29	F CCCAAACCACATTCATTTCG	246	186–291	2	0.75	1.50
			R AGATGTCTGTGATGAGTTTGGAGA					
3.	BNL686	(GA)22	F ATTTTTCCCTTGGTGGTCCT	158	168–248	2	0.71	1.42
			R ACATGATAGAAATATAAACCAAACACG					
4.	JESPR153	(CTA)18	F GATTACCTTCATAGGCCACTG	129	115–200	2	0.73	1.46
			R GAAAACATGAGCATCCTGTG					
5.	CM45	(AG)17	F GATGCCAGTAAGTTCAGGAATG	147	97–146	2	0.75	1.49
			R GCCAACTTATATTCGGTTCCT					
6.	MGHES006	(CCA)7	F TCGCTTGACTTTCCATTTCC	189	90–180	2	0.75	1.49

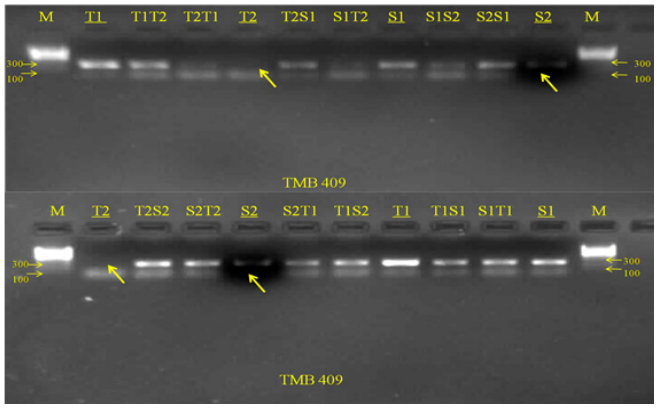

Figure 1: Amplification patterns of different cotton hybrids and their parents with TMB0409 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2 : L 1384×TCH 1777, S_2S_1: TCH 1777× L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777× G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

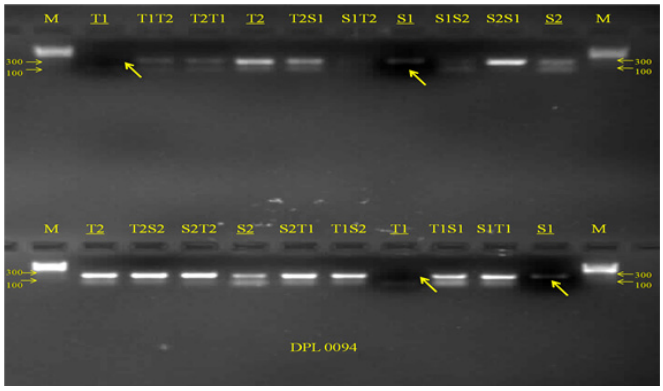

Figure 2: Amplification patterns of different cotton hybrids and their parents with DPL0094 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2 : L 1384×TCH 1777, S_2S_1: TCH 1777× L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777× G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

DPL0094 SSR primer could identify the parent G. Cot 16 (T_1) having a specific fragment of near 186 bp in hybrids G. Cot 16×GSHV 185 (T_1T_2) and GSHV 185 × G. Cot 16 (T_2T_1). In hybrids GSHV 185×L 1384 (T_2S_1) and L 1384×GSHV 185 (S_1T_2) nearly 291 bp fragment was amplified, which was specific to parent L 1384 (S_1). In hybrids, L 1384×TCH 1777 (S_1S_2) and TCH 1777×L 1384 (S_2S_1) about 291 bp amplicon were specific to parent L 1384 (S_1). Similarly, the DPL0094 produced the amplicon in hybrids GSHV 185×TCH 1777 (T_2S_2) and TCH 1777×GSHV 185 (S_2T_2) of about 136 bp and 291 bp which was present in both parents GSHV 185 (T_2) and TCH 1777 (S_2). DPL0094 amplified about 291 bp amplicon in hybrids TCH 1777×G. Cot 16 (S2T1) and G. Cot 16×TCH 1777 (T_1S_2), which was specific to the parent TCH 1777 (S_2). Hybrids G. Cot 16×L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed both fragments of about 186 bp and 291 bp which was specific to the parents G. Cot 16 (T_1) and L 1384 (S_1) respectively.

After amplification with BNL686 SSR primer, two alleles could be observed (Figure 3) and both alleles showed polymorphism with a PIC value of 0.71 and SPI value of 1.423 (Table 1). Amplified amplicons with BNL686 primer could identify parent G. Cot 16 (T_1) with a specific fragment of nearly 246 bp in hybrids G. Cot 16×GSHV 185 (T_1T_2) and GSHV 185×G. Cot 16 (T_2T_1). In hybrids GSHV 185×L 1384 (T_2S_1) and L 1384×GSHV 185 (S_1T_2) 171 bp fragment was amplified by BNL686 SSR primer, which was present in both parents GSHV 185 (T_2) and L 1384 (S_1). In the hybrids, L 1384×TCH 1777 (S_1S_2) and TCH 1777×L 1384 (S_2S_1) 168 bp amplicon was produced by BNL686 SSR primer, which was present in both parents L 1384 (S_1) and TCH 1777 (S_2). Similarly, the BNL686 produced the amplicon of about 168 bp in hybrid GSHV 185×TCH 1777 (T_2S_2) and TCH 1777 × GSHV 185 (S_2T_2), which was present in both parents GSHV 185 (T_2) and TCH 1777 (S_2). The BNL686 primer amplified the amplicon of about 248 bp in hybrids TCH 1777×G. Cot 16 (S_2T_1) and G. Cot 16×TCH 1777 (T_1S_2), which was specific to the parent G. Cot 16 (T_1). Hybrids G. Cot 16×L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed a fragment of about 248 bp, which was specific to the parent G. Cot 16 (T_1).

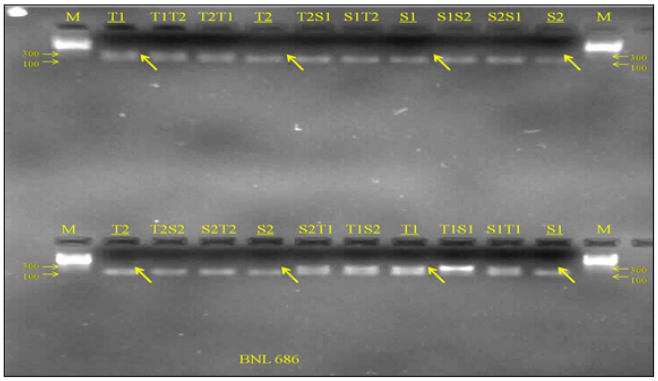

Figure 3: Amplification patterns of different cotton hybrids and their parents with BNL686 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2: L 1384×TCH 1777, S_2S_1: TCH 1777×L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777×G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

SSR primer JESPR153 could amplify two alleles and both alleles showed polymorphism (Figure 4) with a PIC value of 0.73 and SPI value of 1.46 (Table 1). Fragments amplified with JESPR153 SSR primer could identify parents G. Cot 16 (T_1) and GSHV 185 (T_2) with a specific fragment of near 200 bp and 115 bp respectively and similarly these fragments could

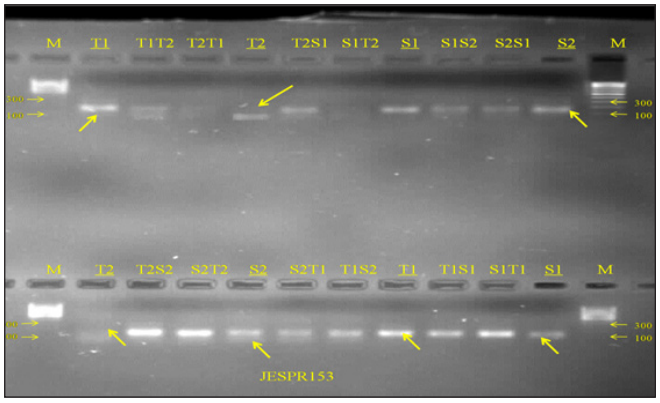

Figure 4: Amplification patterns of different cotton hybrids and their parents with JESPR153 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2: L 1384×TCH 1777, S_2S_1: TCH 1777× L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777× G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

also amplify in hybrids G. Cot 16×GSHV 185 (T_1T_2) and GSHV 185×G. Cot 16 (T_2T_1). In the hybrids, GSHV 185×L 1384 (T_2S_1) and L 1384×GSHV 185 (S_1T_2) the amplified fragments of about 115 bp and 194 bp with JESPR153 SSR primer were specific to the parent GSHV 185 (T_2) and L 1384 (S_1). In hybrids, L 1384×TCH 1777 (S_1S_2) and TCH 1777×L 1384 (S_2S_1) about 193 bp amplicon were produced by JESPR153 primer, which was also present in both the parents L 1384 (S_1) and TCH 1777 (S_2). Similarly, the JESPR153 primer produced an amplicon of about 193 bp in hybrid GSHV 185×TCH 1777 (T_2S_2) and TCH 1777×GSHV 185 (S_2T_2), which was also specific to the parent TCH 1777 (S_2). The JESPR153 primer amplified of about 115 bp amplicon in hybrids TCH 1777×G. Cot 16 (S_2T_1) and G. Cot 16×TCH 1777 (T_1S_2), which was specific to parent TCH 1777 (S_2). Whereas, hybrids G. Cot 16 × L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed a fragment of about 200 bp amplified by JESPR153 primer, which was present in both parents G. Cot 16 (T_1) and L 1384 (S_1).

The SSR primer CM45 could amplify two alleles (Figure 5) and both alleles showed polymorphism with a PIC value of 0.75 and SPI value of 1.49 (Table 1). CM45 amplified the fragment of near 97 bp in hybrids G. Cot 16 × GSHV 185 (T_1T_2) and GSHV 185×G. Cot 16 (T_2T_1), which was present in both the parents G. Cot 16 (T_1) and GSHV 185 (T_2). In hybrids, GSHV 185×L 1384 (T_2S_1) and L 1384×GSHV 185 (S_1T_2) about 97 bp and 146 bp fragments, were amplified by CM45 primer which were specific to the parents GSHV 185 (T_2) and L 1384 (S_1) respectively. In the hybrids, L 1384×TCH 1777 (S_1S_2) and TCH 1777×L 1384 (S_2S_1) about 146 bp amplicon were produced by CM45, which was present in both the parents L 1384 (S_1) and TCH 1777 (S_2). The CM45 produced two amplicons of about 97 bp and 142 bp in hybrids GSHV 185×TCH 1777 (T_2S_2) and TCH 1777×GSHV 185 (S_2T_2), which were specific to the parents GSHV 185 (T_2) and TCH 1777 (S_2) respectively.

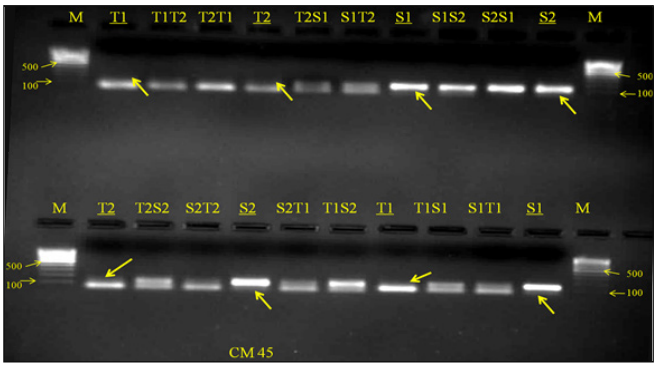

Figure 5: Amplification patterns of different cotton hybrids and their parents with CM45 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2: L 1384×TCH 1777, S_2S_1: TCH 1777× L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777× G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

The CM45 primer amplified of about 142 bp and 97 bp amplicons in hybrids TCH 1777×G. Cot 16 (S_2T_1) and G. Cot 16×TCH 1777 (T_1S_2), which were specific to the parents TCH 1777 (S_2) and G. Cot 16 (T_1) respectively. Similarly, hybrids G. Cot 16×L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed two amplified fragments with CM45 primer of about 97 bp and 142 bp, which were specific to the parents G. Cot 16 (T_1) and L 1384 (S_1) respectively.

The SSR primer MGHES06 amplified two alleles (Figure 6) and both alleles showed polymorphism with a PIC value of 0.75 and SPI value of 1.49 (Table 1). MGHES06 SSR primer identified fragments nearly 180 bp and 90 bp in the hybrids

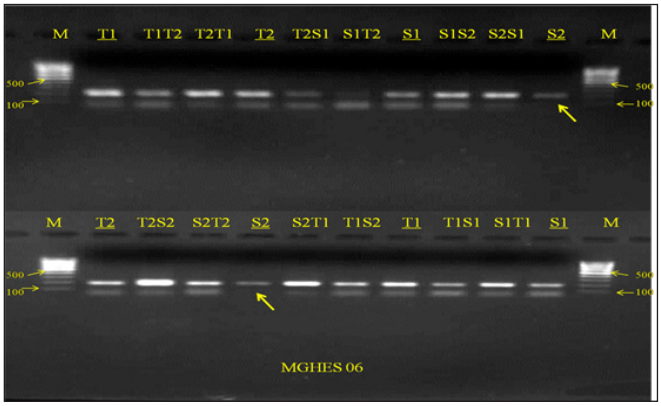

Figure 6: Amplification patterns of different cotton hybrids and their parents with MGHES06 marker

M: ladder (100–1000 bp), T_1: G. Cot 16, T_2: GSHV 185, S_1: L 1384, S_2: TCH 1777, T_1T_2: G. Cot 16×GSHV 185, T_2T_1: GSHV 185×G. Cot 16, T_2S_1: GSHV 185×L 1384, S_1T_2: L 1384×GSHV 185, S_1S_2: L 1384×TCH 1777, S_2S_1: TCH 1777× L 1384, T_2S_2: GSHV 185×TCH 1777, S_2T_2: TCH 1777×GSHV 185, S_2T_1: TCH 1777× G. Cot 16, T_1S_2: G. Cot 16×TCH 1777, T_1S_1: G. Cot 16×L 1384, S_1T_1: L 1384×G. Cot 16

G. Cot 16×GSHV 185 (T_1T_2) and GSHV 185×G. Cot 16 (T_2T_1), which was present in both parents G. Cot 16 (T_1) and GSHV 185 (T_2). In hybrids, GSHV 185×L 1384 (T_2S_1) and L 1384×GSHV 185 (S_1T_2) about 180 bp and 90 bp fragments were amplified by the SSR primer MGHES06, which was present in both parents GSHV 185 (T_2) and L 1384 (S_1). In the hybrids, L 1384×TCH 1777 (S_1S_2) and TCH 1777×L 1384 (S_2S_1) about 180 bp amplicon were produced by MGHES06, which was specific to the parent TCH 1777 (S_2). Similarly, the SSR primer MGHES06 produced the amplicon of nearly 180 bp in hybrids GSHV 185×TCH 1777 (T_2S_2) and TCH 1777×GSHV 185 (S_2T_2), which was specific to the parent TCH 1777 (S_2). The MGHES06 amplified about 180 bp amplicon in hybrids TCH 1777 × G. Cot 16 (S_2T_1) and G. Cot 16×TCH 1777 (T_1S_2), which was specific to the parent TCH 1777 (S_2). Whereas, hybrids G. Cot 16×L 1384 (T_1S_1) and L 1384×G. Cot 16 (S_1T_1) showed fragments of about 180 bp and 90 bp which were present in both parents G. Cot 16 (T_1) and L 1384 (S_1).

Cai et al. (2017) reported that a total of 74 SSR markers showed 246 allelic variations ranging from 2 to 7 with an average of 3.32 per SSR marker. In addition to that, the polymorphic information content ranged from 0.0290 to 0.3729, with an average of 0.2381 which is concurrent with our results. The results revealed that TMB0409 could identify specific band in hybrids G. Cot 16×GSHV 185, GSHV 185×G. Cot 16, GSHV 185×L 1384, L 1384×GSHV 185, L 1384×TCH 1777, TCH 1777×L 1384, 1777×G. Cot 16, 1777×G. Cot 16 that corresponds to one parent. Further TMB0409 identified two fragments in hybrids GSHV 185×TCH 1777 and TCH 1777 × GSHV 185 each from different parents which indicated true hybrids. The DPL0094 amplified specific amplicon in hybrids G. Cot 16×GSHV 185, GSHV 185×G. Cot 16, GSHV 185×L 1384, L 1384×GSHV 185, L 1384×TCH 1777, TCH 1777×L 1384, TCH 1777×G. Cot 16 and G. Cot 16 × TCH 1777 to one parent as well as hybrids G. Cot 16×L 1384 and L 1384×G. Cot 16 produced specific fragment in both parents and hence it was true hybrid. The SSR primer BNL686 amplified specific fragments in hybrids G. Cot 16×GSHV 185 and GSHV 185 × G. Cot 16, TCH 1777×G. Cot 16, G. Cot 16×TCH 1777, G. Cot 16×L 1384 and L 1384×G. Cot 16 with respect to one parent. Liu et al. (2000) also reported 2 loci for the marker BNL686 with amplicon size range of near 189 and 144 bp in cotton which was similar to our results. The SSR primer JESPR153 produced specific amplicon in hybrids GSHV 185×TCH 1777, TCH 1777×GSHV 185, TCH 1777×G. Cot 16 and G. Cot 16×TCH 1777 with respect to one parent. In addition to that, hybrids G. Cot 16×GSHV 185, GSHV 185×G. Cot 16, GSHV 185×L 1384 and L 1384×GSHV 185 produced fragments from both the parents and confirmed true hybrids. Hybrids GSHV 185×L 1384, L 1384×GSHV 185, GSHV 185×TCH 1777, TCH 1777×GSHV 185, TCH 1777×G. Cot 16, G. Cot 16×TCH 1777, G. Cot 16×L 1384 and L 1384×G. Cot 16 produced two bands specific to each parent with SSR primer CM45 and proved the true hybrid of their respective parents. Singh et al. (2015) has also used JESPR153 to discriminate parents and hybrids in cotton for abiotic stress. The SSR primer MGHES06 produced fragments in hybrids L 1384×TCH 1777, TCH 1777×L 1384, GSHV 185×TCH 1777, TCH 1777×GSHV 185, TCH 1777×G. Cot 16 and G. Cot 16×TCH 1777 specific to one parent and confirmed hybrid. Hence, the SSR molecular markers could be used to distinguish and confirm the hybrid and parents in cotton. Similar reports were documented for cotton by Selvakumar et al. (2010), Dongre et al. (2011), Singh et al. (2015) and Rana et al. (2006).Further, the marker CM45 amplified a fragment specific to the salinity tolerant parents which was absent in sensitive parents as well as a fragment produced in sensitive parent which was absent in the tolerant parents hence, the molecular marker CM45 may be associated with the salinity tolerance in cotton and can be used for salinity tolerant breeding program after confirming in a large population. Mahmoud et al. (2018) and Zhang et al. (2003) also drawn similar conclusions when observed unique bands for salinity tolerance and fiber strength respectively.

3.2. Similarity coefficient and dendrogram

The NTSYSpc program was used to calculate Jaccard's similarity coefficient. The similarity coefficient of different cotton parents and their hybrids is presented in Table 2. The genetic similarity matrix revealed that the parents and hybrids showed similarities ranging from 0.42 to 0.99. The least similarity was observed between parents GSHV 185 and TCH 1777 (0.42) and the maximum similarity (0.99) was observed between genotypes G. Cot 16×GSHV 185 with GSHV 185×G. Cot 16, L 1384×TCH 1777 with TCH 1777×L 1384, TCH 1777×G. Cot 16 with G. Cot 16×TCH 1777, G. Cot 16×L 1384 with L 1384 × G. Cot 16.

The clustering pattern of the dendrogram constructed by Jaccard's similarity coefficient indicated differences among the different cotton parents and their hybrids. Based on the dendrogram two major and 5 sub clusters were formed. Major cluster I included all tolerant or moderately tolerant hybrids and parents, whereas major cluster II included two sensitive parents and two sensitive hybrids. Further, the dendrogram showed five sub clusters such as A, B, C, D and E were formed on the basis of their similarity coefficient (Figure 7). Sub cluster A included only parent G. Cot 16. Sub cluster B included the hybrids G. Cot 16×GSHV 185, GSHV 185×G. Cot 16, TCH 1777 × GSHV 185, TCH 1777×G. Cot 16, G. Cot 16×TCH 1777, G. Cot 16 × L 1384 and L 1384×G. Cot 16. The sub cluster C consist GSHV 185×L 1384, GSHV 185×TCH 1777 and L 1384×GSHV 185. The sub cluster D included only parent GSHV 185. The sub cluster E consists of parents L 1384 and TCH 1777 and their hybrids L 1384×TCH 1777 and TCH 1777×L 1384. Dendrogram depicted that the sub cluster E included L 1384 and TCH 1777 and their hybrids showed clear genetic divergence from other clusters. Similarly, sub cluster D included GSHV 185 showed genetic distinctness from sub cluster A, B and C. Sub cluster C near sub cluster D consists of hybrids of GSHV 185 as a parent. Further sub cluster A consisted of G. cot 16 was near to sub cluster B, which had hybrids with G. Cot 16 as one of the parents.

Table 2: Jaccard's similarity coefficient among different cotton parents and their hybrids based on molecular analysis

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
G1	1.00															
G2	0.58	1.00														
G3	0.58	0.50	1.00													
G4	0.50	0.41	0.75	1.00												
G5	0.83	0.75	0.58	0.50	1.00											
G6	0.83	0.75	0.58	0.50	1.00	1.00										
G7	0.66	0.75	0.75	0.66	0.83	0.83	1.00									
G8	0.58	0.83	0.66	0.58	0.75	0.75	0.91	1.00								
G9	0.66	0.58	0.91	0.83	0.66	0.66	0.83	0.75	1.00							
G10	0.66	0.58	0.91	0.83	0.66	0.66	0.83	0.75	1.00	1.00						
G11	0.66	0.75	0.75	0.66	0.83	0.83	1.00	0.91	0.83	0.83	1.00					
G12	0.75	0.83	0.66	0.58	0.91	0.91	0.91	0.83	0.75	0.75	0.91	1.00				
G13	0.75	0.66	0.66	0.58	0.91	0.91	0.91	0.83	0.75	0.75	0.91	0.83	1.00			
G14	0.75	0.66	0.66	0.58	0.91	0.91	0.91	0.83	0.75	0.75	0.91	0.83	1.00	1.00		
G15	0.83	0.58	0.75	0.66	0.83	0.83	0.83	0.75	0.83	0.83	0.83	0.75	0.91	0.91	1.00	
G16	0.83	0.58	0.75	0.66	0.83	0.83	0.83	0.75	0.83	0.83	0.83	0.75	0.91	0.91	1.00	1.00

G1 : G. Cot 16, G2: GSHV 185, G3: L 1384, G4: TCH 1777, G5: G. Cot 16 × GSHV 185, G6: GSHV 185× G. Cot 16, G7: GSHV 185×L 1384, G8: L 1384×GSHV 185, G9: L 1384×TCH 1777, G10: TCH 1777× L 1384, G11: GSHV 185×TCH 1777, G12: TCH 1777×GSHV 185, G13: TCH 1777×G. Cot 16, G14: G. Cot 16×TCH 1777, G15: G. Cot 16×L 1384, G16: L 1384× G. Cot 16

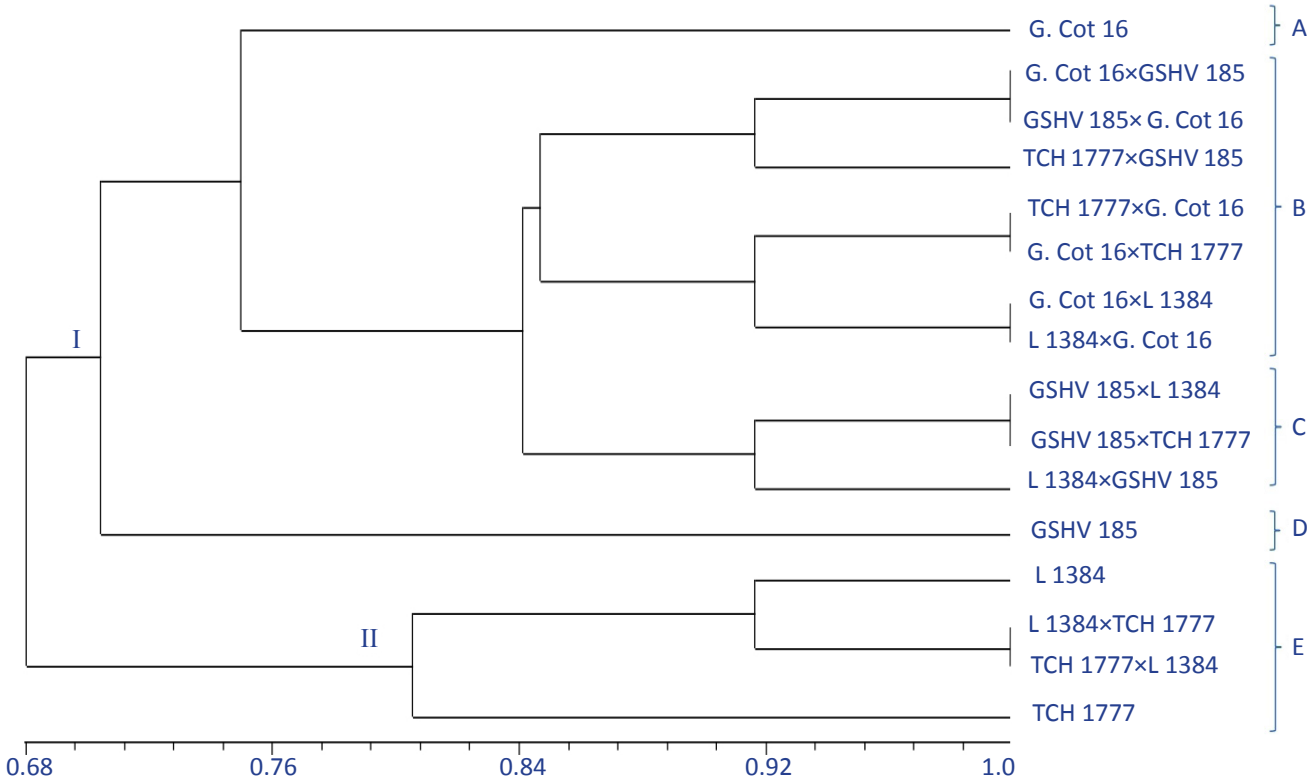

Figure 7: Dendrogram depicting the genetic relationship among the different cotton hybrids and their parents based on molecular analysis

It was clearly observed that the closely related parents gave rise to the related hybrids and grouped in the same cluster while distantly related parents gave hybrids grouped into different clusters. Joher et al. (2018) showed that genotypes with narrow genetic bases had higher similarity coefficient and vise a versa which concurrent with our analysis.

3.3. *Principal component analysis (PCA) and heat map analysis*

Principal component analysis (PCA) was performed for molecular traits of twelve cotton hybrids and four parents and results are presented in Figure 8. Singular value decomposition (SVD) with imputation was used to calculate principal components. X and Y axis showed principal component 1 (PC1) and principal component 2 (PC2) that explained 34.9% and 26.3% of the total variance, respectively. From the PCA

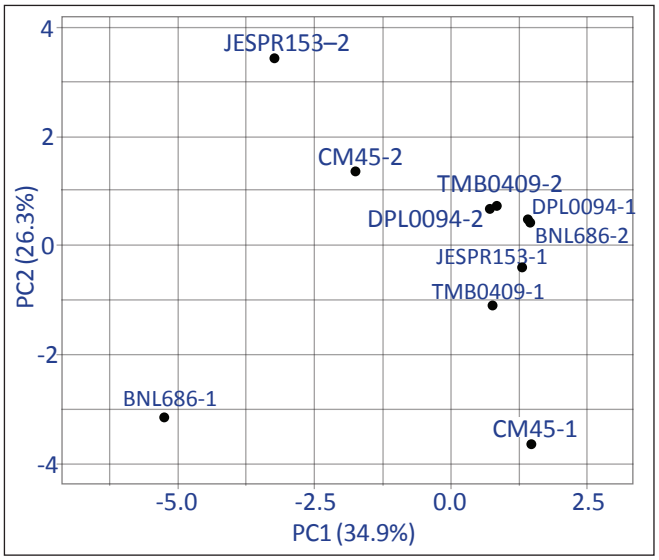

Figure 8: Principle component analysis based on the molecular marker (Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 and principal component 2 that explains 34.9% and 26.3% of the total variance, respectively. N = 12 data points.)

analysis, it was revealed that BNL686–1 (248 bp) contributed significantly to the quantum of variation as explained by PC1. In this way, this component is able to serve as a benchmark for ascertaining the efficient pattern of grouping between genotypes based on the allele BNL686–1 (248 bp) so as to distinguish hybrids and parents. It was further observed that JESPR153–2 (115 bp) was the main component of the PC2. Further two way cluster analysis was conducted to assess the quantum of genetic variability within and between the discrete groups based on the index of similarity and dissimilarity as indicated by the genetic distance between them. Distance coefficient between individuals was calculated using the Euclidean square distance method and different cluster analysis methods like Ward, Nearest and Farthest neighbors. Based on the dendrogram and heat map (Figure 9),

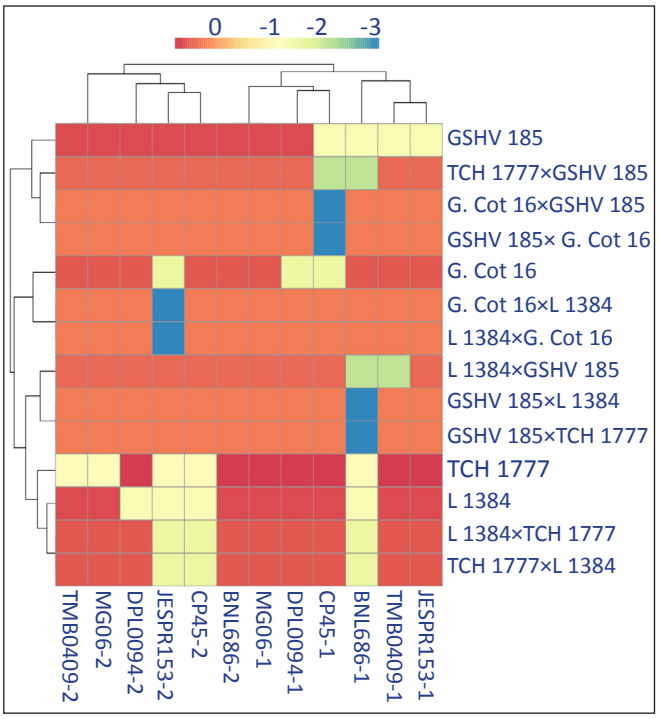

Figure 9: Heatmap analysis of molecular marker and genotypes (Heatmap shows unit variance scaling is applied to rows. Both rows and columns are clustered using correlation distance and average linkage)

it could be clearly expressed that all cotton genotypes were classified based on an index of similarity and dissimilarity of attributing traits (molecular marker). All genotypes could be grouped into three major clusters based on heat map and dendrogram. Cluster I contained seven genotypes (TCH 1777, L 1384, L 1384×TCH 1777, TCH1777×L1384, GSHV 185×TCH 1777, GSHV 185×L 1384, L 1384×GSHV 185), cluster II contained (L 1384×G. Cot 16, G. Cot 16×L 1384, G. Cot 16) and cluster III contained (GSHV 185×G. Cot 16, G. Cot 16×GSHV 185, TCH 1777×GSHV 185 and GSHV 185) based on heat map analysis. By comparing both clustering methods all parents and hybrids showed a similar pattern of grouping.

4. Conclusion

Molecular characterization of cotton hybrids and parents revealed that SSR molecular marker could be successfully used for confirmation of hybrids and their parents. Six SSR markers (TMB0409, DPL0094, BNL686, JESPR153, CM45 and MGHES006) showed polymorphism between parents and hybrids studied and were able to identify the relation between parents and hybrids. SSR marker CM 45 could be exclusively used for screening of salinity tolerant and susceptible cotton genotypes that may enhance the potency of cotton breeding programs.

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