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Research Article

Confirmation of genome introgression in the inter-specific hybrid progenies of *Coffea* species through SRAP marker technique

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Abstract: Gene introgression in *Coffea arabica* has become essential to introduce resistant genes in arabica through diploid species such as *Coffea canephora* var. robusta and tree coffee species. The present study had also involved the work on similar aspects of the inter-specific hybrid progenies (10year-old) of a tetraploid dwarf cultivar 'Cauvery' (a Catimor line) and diploid cultivar CxR. SRAP marker technique developed by Cravero *et al.* was followed for characterization and confirmation for the presence of diploid genes transmitted from cultivar CxR. Findings revealed that out of thirty six SRAP primer combinations screened, 16 primer combinations were highly polymorphic for formation of an apparent amplification pattern and produced 147 distinct bands among the parents, F_1 and F_2 hybrid progenies. A total of thirty seven different types of markers generated based on the gel patterns of parents, F_1 robusta hybrids and four different types of F_2 hybrids were informative markers for parents and hybrid identification. Among a total number of 138 fragments obtained between F_1 robusta and F_2 arabica types, 118 (85.50 percent) fragments were shared between them. The amplification pattern between F_1 robusta and F_2 intermediate type exhibited the presence of 81.95 percent monomorphic fragments out of 133 fragments amplified besides, 13 and 11 fragments unique fragments found in F_1 robusta and F_2 intermediate type respectively. Similarly, F_1 robusta and F_2 off type plants indicated 10 fragments exclusively in F_1 robusta and 9 fragments in F_2 off type while, 112 fragments were present in both out of 131 fragments amplified. The similarity matrix indicated a close relatedness of all the four types of F_2 hybrids with the female parent than the male CxR. .
Keywords: *Coffea Arabica, Coffea canephora* var. robusta, F_1 and F_2 hybrid progenies

INTRODUCTION

The combination of plant with attractive Jasmine like fragrant flowers and seeds so called beans produced out of crimson red ripe cherry is generally known as "Coffee". Coffee being a vital non-alcoholic beverage, it is commercially cultivated in different parts of the world situated along the tropical regions [1]. It supports the growers financially as well as improves the economic condition of several coffee growing countries by earning foreign exchange. Coffee belongs to the family – Rubiaceae, genus- *Coffea* that possesses more than 70 species out of which commercially cultivated species are *Coffea arabica* var. arabica and *Coffea canephora* var. robusta. *Coffea liberica* is grown on a small scale [2]. Beside this, there are some *Coffea* species of Indian origin namely; *C. travancorensis, C. bengalensis, C. khasiana, C. wightiana occuring* in the forests of Kerala, Tamil Nadu, Meghalaya and Assam. *C. arabica* is a tetraploid and *C. canephora* diploid species. Arabica carries 2n=4x=44 and Robusta 2n=22 chromosomes [3].

The arabica varieties like Caturra, Catuai, Tupi, in Brazil, SL.28 in Kenya, Kents and S.795 in India, Sarchimor in Costa Rica, Java in Cameroon and variety Colombia in Colombia, have been developed through pure line and pedigree selection [4]. In recent years the potential of tissue culture and genetic manipulation of *Coffea* using recombinant DNA technology and tissue culture techniques has been investigated to develop the plant material of breeder's choice [5, 6]. Subsequently, molecular characterization using DNA markers became an easy and most reliable technique in coffee breeding and selection program to develop high yielding, excellent bean quality and disease resistant (especially rust) cultivars [7].

Selvaraj and Aruna Devi [8] investigated the interrelationships among twelve *Coffea* species through

Biosystematical studies and demonstrated that morphologically all the species of *C. arabica, C. canephora, C. liberica, C. excelsa, C. abeokutae, C. stenophylla , C. eugenioides, P. bengalensis, C. congensis, C. salvatrix, P. kapakata,* and *P. wightiana we*re dissimilar. Prakash *et al.,* [9] evaluated S.288, a selfed progeny of the oldest arabica genotype S.26, believed to be a spontaneous inter-specific hybrid of *C. arabica* and *C. liberica,* along with 17 other accession developed from F_2 and F_4 generations of S.288 x Kents crosses, for transmission of liberica genes to these progenies. He opined that *C. liberica* could be the probable progenitor for evolution of natural hybrid (S.26). Poncet *et al.,* [10] developed the anchor markers using linkage map of each species of *Coffea* and a common set of DNA markers to align the map to provide information on genome evolution and mapping of qualitative and quantitative genes. Moncada [11] had characterized thirty accessions of genus *Coffea* from CENICAFE gene bank in Colombia applying 34 microsatellite markers and observed high level of diversity in diploid species. Prakash *et al.,* [12] identified AFLP markers closely associated with rust resistant genes S_H3 that is spontaneously transmitted to *C. arabica* accession S.288 from *C. liberica*. About 101 lines generated from the arabica genotypes 'Matari' and 'S.288' were analyzed using AFLP markers. Herrera *et al.* [2] revealed that most of the BC_1 hybrids analyzed were tetraploid probably due to production of gametes with 22 chromosomes and the progeny of tetraploid x diploid crosses had higher quantum of gene introgression, whereas, BC_1 hybrids developed by mating of Triploid x F_1 inter-specific hybrid exhibited an unusual trend.

Mishra *et al.* [7] indicated inheritance of maximum number of bands from female parents rather than the male in hybrids using Sequence Related Amplified Polymorphism (SRAP) a new molecular marker technology. He also detected higher degree of polymorphism in diploid species than tetraploid arabica. Batista *et al.,* [13] discovered the genetic structure, adaptive variation and evolution of *Hemileia vastatrix* plant pathogen causing coffee leaf rust with the application of population genomics and also detected the divergent alleles through population analysis of virulent genes.

The review of literatures on crop improvement in coffee clearly indicated that the research undertaken earlier in the field of genetics and plant breeding are scanty particularly, in relation to F_1 progenies of coffee cultivars. Though some studies have been carried out previously on F_1 progenies developed through hybridization programmes in the other coffee growing counties such as, Brazil, Columbia, Kenya, Ethiopia etc., but the F_1 hybrids used in India in the present research work were neither developed nor studied elsewhere. Hence, the present study was aimed at molecular genetic analysis of an interspecific hybrid progenies (F_1 and F_2).

MATERIALS AND METHODS

Among various markers available for genetic analysis in plants, molecular markers are more efficient, precise and reliable for discriminating closely related species and cultivars and therefore, widely used in marker assisted breeding. Among the many types of molecular markers, sequence-related amplified polymorphism (SRAP) has been demonstrated to be a useful tool in genetic analysis of different plant species [14-17].

SRAP is a PCR based marker system that preferentially targets coding sequences randomly distributed throughout genome [14]. Forward and reverse primers used in SRAP preferentially amplify exonic and intronic regions of the genome respectively and uncover polymorphic sequences resulting from variations in the length of introns, promoters and spacers among different populations and genotypes. SRAP is highly reproducible and comparatively less expensive than other types of markers [18]. The potential of SRAP marker has not yet been tested in coffee hence, in the present study, SRAP marker approach was employed in genetic analysis of an inter-specific hybrid progenies of tetraploid and diploid coffee species.

Plant materials used for molecular genetic analysis

An inter-specific hybridization was undertaken involving tetraploid *C. arabica* c.v. Cauvery (4n=44) and triploid C. canephora c.v. CxR (3n=33). The resultant F_1 hybrids have distinct morphotypes where, one had resemblance with the maternal parent 'Cauvery' and the other largely similar to the paternal parent CxR with intermingling features of Cauvery. F_2 progeny was derived from the F_1 CxR type of plants (exhibiting morphological similarity with CxR parent plants). Based on their phenotypic features, F_2 plants were grouped into four different types as follows:

- Cauvery type- with phenotypic appearance of arabica variety 'Cauvery'
- CxR robusta type- showing similarity with robusta plants of larger leaves and bush type character
- Intermediate type- exhibiting admixture of arabica and robusta features
- Off-types with abnormal leaf and fruits

The plant materials chosen for the study are presented below (table-1).

Table 1: Parents and hybrid combination analyzed by using SRAP marker

Parents	Hybrids of Cauvery x (CxR)	
Cauvery/Catimor C x R Triploid form (3n=33)	F_1 hybrids	Cauvery type
		CxR type
	F_2 generation	Cauvery type
		Robusta type
		Intermediate type
		Off- type

Fresh young leaves from ten individual plants were collected from both the parents and their F_1 and F_2 progenies for isolation of DNA. Among F_1 population two different types i.e. few plants of arabica (Cauvery) and the remaining of robusta (CxR) phenotype were used in addition to 10 individual plants belonging to four different types of F_2 progeny as described earlier were used.

Methods of DNA extraction

Genomic DNA was isolated from fresh young leaves using a modified CTAB method as described earlier by [7]. About 200 mg of fresh leaf tissue was ground to fine powder in liquid nitrogen, transferred to a 30 ml tube containing 5 ml preheated extraction buffer (2 percent CTAB (w/v), 100 mM Tris-HCL (pH 8.0), 25 mM EDTA, 2M NaCl and 0.1 percent beta-mercaptoethanol). The tubes were incubated at 60 ºC for one hour with occasional shaking. After incubation, the tubes were cooled to room temperature and centrifuged at 6000 rpm for 20 min. The supernatant was transferred into a new tube and extracted twice with chloroform-isoamyl alcohol (24:1). The supernatant was transferred to 2 ml tubes, precipitated with 0.7 volume of isopropanol at room temperature for 30 min., and then centrifuged at 8000 rpm for 20 min at 4°C. The pellet formed after centrifugation was washed with 75 percent (v/v) ethanol for 10 min and dissolved in 60 µl of Tris-EDTA (1-10 mM). The concentration of DNA was measured using 0.8 percent agarose gel stained with ethidium bromide as well as via a UV spectrophotometer at 260 nm. The ratio of the absorbance at 260 and 280 nm ($A_{260/280}$) was used to assess the purity of DNA. The re-suspended DNA was then diluted in sterile distilled water to obtain 10 ng/µl concentrations for use in amplification reactions.

Methods of Amplification of SRAP markers

SRAP primers used in this study consist of 13 forward & 16 reverse primers of and their sequences are presented [14] (table-2). Primers were selected for further analysis based on their ability to detect clear and distinct polymorphic amplification products in various samples. Sixteen SRAP primer combinations that produced clearly readable and distinct polymorphic fragments in parents and hybrids were further selected for PCR amplification. Polymerase chain reaction was carried out in an Eppendorf master cycler (Eppendorf, Germany).

The SRAP analysis was conducted by adapting the procedure described [9] with minor modifications as described earlier [7, 11]. The reaction mixture of 20 µl containing 1x reaction buffer (75mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01 percent Tween 20), 30 ng template DNA, 200 µM dNTP mixture, 2.5 mM $MgCl_2$, 3 µM each of forward and reverse primers, 1.0 U *Taq* DNA polymerase and sterile doubled-distilled water. The amplification conditions selected for SRAP included 4 min initial denaturation at 96 ºC; 5 cycles consisting of 1 min denaturation at 94 ºC, 1.15 min primer annealing at 35 ºC; and 2 min extension at 72 ºC, followed by 30 cycles consisting of 1 min denaturation at 94º C, 1.15 min primer annealing at 50º C and 2 min elongation at 72 º C and a final extension of 15 min at 72 ºC.

Table 2: Sequences of SRAP forward and reverse primer and primer combinations used in parents and hybrid analysis

Forward primer (5' – 3')	Reverse primer (5' – 3')	Polymorphic primers combination	
Me1TGAGTCCAAACCGGATA	Em2 GACTGCGTACGAATTTGC	Forward	Reverse
Me2TGAGTCCAAACCGGAGC	Em3 GACTGCGTACGAATTGAC	Me1	Em4 /Em12
Me3TGAGTCCAAACCGGAAT	Em4 GACTGCGTACGAATTTGA	Me2	Em4/Em6/Em12/Em14
Me4TGAGTCCAAACCGGACC	Em5 GACTGCGTACGAATTAAC	Me3	Em3/Em9/Em11
Me6TGAGTCCAAACCGGACA	Em6 GACTGCGTACGAATTGCA	Me4	Em11 / Em16
Me9TGAGTCCAAACCGGAGG	Em9 GACTGCGTACGAATTCAG	Me6	Em5
Me10TGAGTCCAAACCGGAAA	Em10 GACTGCGTACGAATTCAT	Me9	Em10
Me11TGAGTCCAAACCGGAAC	Em11 GACTGCGTACGAATTCTA	Me10	Em13
ME12TGAGTCCAAACCGGAGA	Em12 GACTGCGTACGAATTCTC	Me11	Em16
	Em13 GACTGCGTACGAATTCTG	Me12	Em16
	Em14 GACTGCGTACGAATTCTT		
	Em16 GACTGCGTACGAATTGTC		

The PCR products obtained from SRAP analysis were analyzed via electrophoresis on 2.0% (w/w) agarose gels containing 0.5 µg ethidium bromide/ml in 1x TAE buffer as previously described [7]. The amplified bands were visualized and photographed using the UV-transilluminator (SYNGENE) and documented using the Gene Snap software program. All the three PCRs were repeated at least twice to confirm the reliability and repeatability of each PCR amplified band. The SRAP-amplified bands obtained with different primers were scored for presence (1) or absence (0) in data matrix form. Ambiguous bands that could not be easily distinguished were not scored the total number of bands, distribution of bands among the parents and hybrids, polymorphic bands, parental and hybrid specific bands and average number of bands per primer were manually calculated. The similarity of samples was calculated as follows: Similarity = $2N_{AB}/N_A+N_B$, N_{AB} is the number of bands shared by individuals A & B &, N_A & N_B are the number of bands in individuals A & B respectively.

RESULTS AND DISCUSSION

SRAP polymorphism among parents and hybrids

A total of thirty six SRAP primer combinations were screened, of which 16 primer combinations were found to be highly polymorphic (table-3) and produced a clear amplification pattern. These 16 primer pairs produced 147 distinct bands among the parents, F_1 and F_2 hybrid progenies. The number of amplified fragments ranged from four (Me3-Em9) to 13 (Me1-Em4), with a mean of 9.18 bands per primer combination (table-4). The size of the amplified products ranged from 75 to 4200 bp. Of the total 147 amplified bands, 94 (63.94 percent) were polymorphic, with a mean of 5.87 polymorphic fragments primer^{-1} combination. Percent of polymorphism ranged from 33.33 percent (Me6-Em5) to a maximum of 87.5 percent (Me4-Em16) with a mean of 64.98 percent. Out of 16 polymorphic SRAP primer combinations used, five primer combinations showed more than 80 percent polymorphism (table-4). The mean number of fragments amplified in parents and hybrid samples ranged from 6.31 in C x R parent to 8.37 in arabica type F_2 progeny (table-4).

Fragment distribution and Marker types

Table 3: Percentage of polymorphism in parents and their hybrid progenies

Sl. No.	Primers	Percent polymorphism
1	B 12	63.63
2	F 5	33.33
3	D 16	87.50
4	C 3	71.42
5	B 14	83.33
6	C 11	45.45
7	A 12	58.33
8	D 11	40.00
9	C 9	75.00
10	B 4	85.71
11	B 6	54.54
12	A 4	69.23
13	I 10	83.33
14	K 16	50.00
5	J 13	57.14
16	L 16	81.81
Mean	-	64.98

The distribution of amplified fragments in parents, F_1 and F_2 were computed (table-5). These 16 pairs of SRAP primers amplified 146 fragments which are distributed in twelve different types at variable frequency in both parents and two F_1 hybrid types (table-5). Among the different marker types, Type I marker which constitute the monomorphic fragments are more frequent (45.55 percent) followed by the type V (17.93percent) and Type II (13.79 percent) marker types. All together, these three marker types accounted for about 77.27 percent of total amplified fragments. With four different types of F_2 plants derived from F_1 robusta type of plants a combined analysis was made and a total number of 147 fragments were amplified (table.6). These 147 amplified fragments formed 37 different types of marker profiles based on their distribution among the parents, F_1 and F_2 hybrids. Among these 147 fragments, 64 (43.53 percent) fragments are monomorphic and belonged to marker type VI. The other two marker profiles such as type IV and type IX account for 12.92 percent and 10.88 percent of fragments respectively.

Table 4: Average numbers of amplified bands by the primers in parents and hybrids

Parents of F_1 hybrid	Mean number of amplified bands	Range
Female- Cauvery	6.56	3 – 9
Male- C x R	6.31	2 – 10
F_1 arabica	8.18	4 – 11
F_1 robusta	7.56	4 – 12
F_2 arabica	8.37	4 – 12
F_2 robusta	8.12	4 – 11
F_2 offtype	7.50	4 – 10
F_2 intermediate	7.56	4 – 11
Average	7.52	-

Identification of Parents and F_1 Hybrids

Twelve different types of SRAP markers obtained were critically analyzed for discriminating the parents and hybrids. Out of these twelve types of markers, Types II, III, XI and XII were good markers for hybrid identification (Table-5). Among the four types of markers mentioned earlier, the type II marker can unambiguously identify the hybrid status of both F_1 hybrids (arabica and robusta types) where as the marker type III and XII can independently determine the hybrid status of F_1 robusta type and F_1 arabica type respectively.

Table 5: Types of SRAP markers identified from inter-specific F_1 hybrid population

Marker types	Female parent Cauvery	Male parent CxR	F_1 Arabica	F_1 Robusta	Total number of bands	Percent different markers
I	+	+	+	+	66	45.2
II	–	+	+	+	20	13.69
III	–	+	–	+	7	4.79
IV	+	–	+	+	26	17.8
V	+	–	+	–	5	3.42
VI	–	–	+	–	5	3.42
VII	–	+	–	–	6	4.1
VIII	–	–	+	+	3	2.05
IX	–	–	–	+	1	0.68
X	+	+	–	–	1	0.68
XI	+	+	+	–	3	2.05
XII	–	+	+	–	3	2.05
Total	101	-	131	-	146	99.93

Further, marker types VI and IX are also very effective for differentiating the arabica and robusta type of F_1 hybrids. Similarly, the type VII is effective markers for identifying true male parent i.e CxR in the F_1 hybrid population. However, specific marker for identifying female parent (Cauvery) could not be identified in the present study using the limited SRAP primers.

Identification of Parents and F_2 Hybrids

Thirty seven different types of markers which were generated based on the gel patterns of parents, F_1 robusta hybrids and four different types of F_2 hybrids were analyzed (Table 6). Out of the 37 types of markers, five marker types such as Types XII, XIII, XXVI, XXVIII, and XXXII are informative markers for parents and hybrid identification. Among the five marker types, XII and XXXII can unequivocally identify male and female parent respectively due to the presence of male and female specific bands (Table 6). Similarly, the marker types XIII, XXVI and XXVIII can independently identify F_2 (robusta type) F_2 (arabica type) and F_1 (robusta type) respectively. However, among the 37 types of marker types, no specific marker type is identified for differentiating F_2 off type and F_2 intermediate type of plants in the population.

Sharing of bands between parents and F_1 Hybrids

The presence and absence of fragments in parents and F_1 arabica type hybrid was calculated and it was observed that F_1 arabica type hybrid shared 69 (47.26 percent) common fragments those are present in both male (CxR) and female (Cauvery) parents (table-6) Similarly, F_1 arabica type hybrid also shared 74 (50.68

percent) and 92 (63.01 percent) fragments with female and male parent respectively. The F_1 robusta type hybrid shared 66 (45.2 percent) common fragments with both the male and female parents where as 92 (63.01 percent) and 93 (63.69 percent) fragments were shared between F_1 robusta type and female (Cauvery) and male (CxR) respectively. Both F_1 arabica and F_1 robusta type hybrids share 115 (78.76 percent) amplified bands out of total 146 bands among themselves.

Sharing of bands between F_1 Robusta type and F_2 Hybrids

The sharing of fragments between F_1 robusta and the four morphologically different types of F_2 hybrids derived from it were computed separately and it was observed that a total number of 138 fragments were obtained between F_1 robusta and F_2 arabica types. Of the 138 fragments, 118 (85.50 percent) fragments are shared between them (table 6). Four fragments those are present in F_1 robusta hybrid could not be amplified in F_2 arabica hybrid. Similarly, 16 (11.59 percent) unique fragments were amplified in F_2 arabica hybrids which were not present in F_1 robusta hybrid. When the amplification pattern was compared between F_1 robusta and F_2 robusta type hybrid, it was observed that out of 136 total fragments amplified in both, 116 (85.29 percent) fragments were monomorphic and shared by them whereas 6 and 14 fragments are exclusively present in F_1 robusta and F_2 robusta respectively. The amplification patter was also compared between F_1 robusta and F_2 intermediate type which revealed that out of 133 amplified fragments 109 (81.95 percent) fragments are monomorphic and present in both F_1 robusta and F_2 intermediate type whereas 13 and 11 fragments are unique to F_1 robusta and F_2 intermediate type respectively. Similarly, comparison of amplification pattern between F_1 robusta and F_2 off type revealed that out of 131 fragments amplified, 10 fragments are exclusively obtained in F_1 robusta and 9 fragments in F_2 off type whereas 112 (85.49 percent) are present in both.

Genetic relatedness between parents, F_1 hybrids and their progenies

An important observation made in the present study is the relatedness among the parents F_1 hybrids and their progenies based on the marker profiles. From the similarity matrix, it was observed that both the parents i.e. Cauvery and CxR shared 0.67 similarities among them (table 7). Both F_1 arabica and F_1 robusta hybrids are closer to the maternal parent sharing 0.86 and 0.82 similarity respectively compared to the male parent with which they shared 0.73 and 0.78 similarity respectively. A high similarity value of 0.91 was obtained between F_1 arabica and F_1 robusta hybrids. All the four types of F_2 hybrids have displayed close similarity with the female parent Cauvery than the male parent CxR. Similarly, the similarity index between all the four F_2 hybrids with two F_1 hybrids (Arabica and robusta types) was more or less similar except that F_2 arabica exhibited higher similarity with F_1 arabica compared to F_1 robusta (table.7).

Table 6: Types of SRAP markers detected in the parents and their F_1 and F_2 progenies

Marker types	Female parent Cauvery	Male parent (C x R)	F_1 Robusta type	F_2 Arabica type	F_2 Robusta type	F_2 Off-type	F_2 Intermediate	Total number of bands	Percent different markers
1	–	–	–	–	+	+	–	1	0.68
2	–	–	+	+	+	+	–	1	0.68
3	–	+	+	+	–	–	–	1	0.68
4	+	–	+	+	+	+	+	19	12.92
5	+	–	+	+	+	–	–	1	0.68
6	+	+	+	+	+	+	+	64	43.53
7	+	+	+	+	+	–	–	1	0.68
8	+	+	–	–	–	–	–	1	0.68
9	–	+	+	+	+	+	+	16	10.88
10	–	–	–	+	+	+	+	1	0.68
11	–	–	–	–	–	+	+	1	0.68
12	–	+	–	–	–	–	–	5	3.4
13	–	–	–	–	+	–	–	1	0.68
14	–	+	+	–	+	+	+	1	0.68
15	–	+	+	+	+	–	+	3	2.04
16	+	–	+	+	–	–	–	1	0.68
17	–	+	+	–	+	–	–	1	0.68
18	–	–	+	+	+	+	+	2	1.36
19	+	+	–	+	+	+	+	2	1.36
20	+	+	–	+	+	–	+	1	0.68

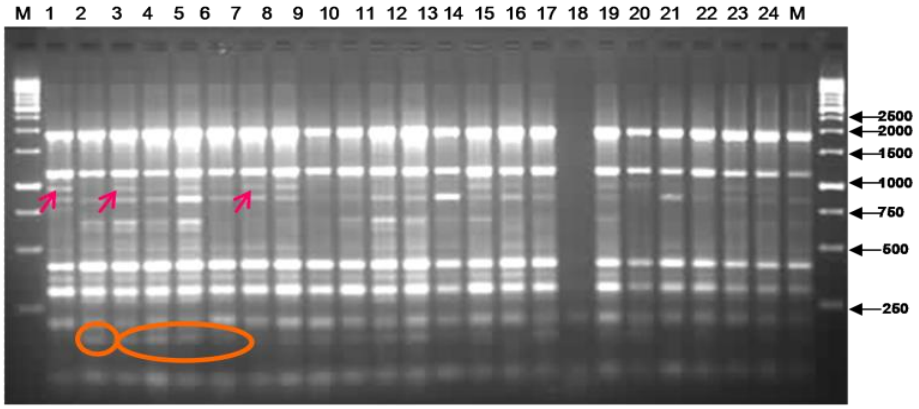
21	–	+	–	+	–	–	–	1	0.68
22	+	–	–	+	+	+	–	3	2.04
23	+	–	–	+	–	–	–	1	0.68
24	+	–	+	+	+	+	–	1	0.68
25	–	–	–	+	–	–	+	1	0.68
26	–	–	–	+	–	–	–	1	0.68
27	–	+	–	+	+	+	+	3	2.04
28	–	–	+	–	–	–	–	1	0.68
29	–	+	+	+	+	–	+	2	1.36
30	+	–	–	+	+	–	–	1	0.68
31	–	–	–	+	+	–	–	1	0.68
32	+	–	–	–	–	–	–	2	1.36
33	–	+	+	–	+	+	–	1	0.68
34	+	–	+	+	+	–	+	1	0.68
35	+	–	+	+	–	–	+	1	0.68
36	–	+	+	+	–	+	–	1	0.68
37	+	+	+	+	–	+	+	1	0.68
Total	-	-	-	-	-	-	-	147	99.97

Legend

1. C x R (pollen parent)	9. Cauvery x (CxR) F_2 (Arabica type)	17. Cauvery x (CxR) F_2 (off type)
2. Cauvery (mother parent)	10. Cauvery x (CxR) F_2 (Arabica type)	18. Cauvery x (CxR) F_2 (off type)
3. Cauvery x (CxR) F_1(Cauvery type)	11. Cauvery x (CxR) F_2 (Arabica type)	19. Cauvery x (CxR) F_2 (off type)
4. Cauvery x (CxR) F_1(Cauvery type)	12. Cauvery x (CxR) F_2 (Arabica type)	20. Cauvery x (CxR) F_2 (off type)
5. Cauvery x (CxR) F_1 (Cauvery type)	13. Cauvery x (CxR) F_2 (Robusta type)	21. Cauvery x (CxR) F_2 (Intermediate type)
6. Cauvery x (CxR) F_1 (CxR type)	14. Cauvery x (CxR) F_2 (Robusta type)	22. Cauvery x (CxR) F_2 (Intermediate type)
7. Cauvery x (CxR) F_1 (CxR type)	15. Cauvery x (CxR) F_2 (Robusta type)	23. Cauvery x (CxR) F_2 (Intermediate type)
8. Cauvery x (CxR) F_1 (CxR type)	16. Cauvery x (CxR) F_2 (Robusta type)	24. Cauvery x (CxR) F_2 (Intermediate type)

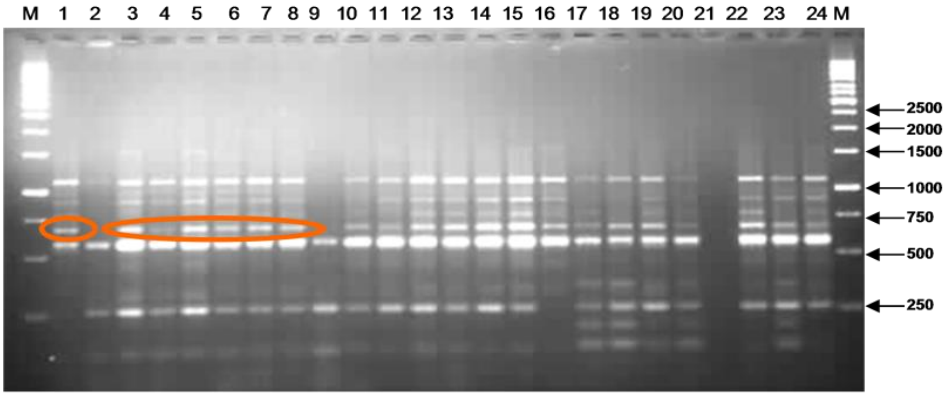
Table 7: Similarity matrix between parents, F_1 hybrids and F_2 progenies

Combination	Female parent (Cauvery)	Male parent (C x R)	F_1 Arabica type	F_1 Robusta type	F_2 Arabica type	F_2 Robusta type	F_2 Off-type	F_2 Intermediate
Female Cauvery	1	-	-	-	-	-	-	-
Male (C x R)	0.67	1	-	-	-	-	-	-
F_1 arabica	0.86	0.73	1	-	-	-	-	-
F_1 robusta type	0.82	0.78	0.91	1	-	-	-	-
F_2 arabica type	0.85	0.8	0.96	0.92	1	-	-	-
F_2 robusta type	0.83	0.8	0.93	0.93	0.94	1	-	-
F_2 off-type	0.82	0.791	0.90	0.90	0.91	0.93	1	-
F_2 intermediate	0.81	0.81	0.91	0.93	0.93	0.93	0.93	1



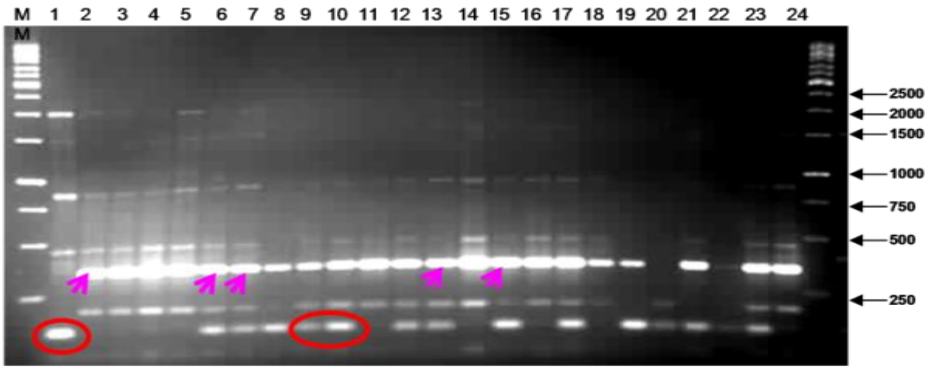
Me2+ Em6

Fig.1. Confirmation of Hybrid status in Cauvery x CxR interspecific F1 hybrid plants using SRAP primer pairs me2 + em6. Arrow marks in lane 1,3 and 7 indicates the integration of paternal specific band and circle in lane number 2, 3,4,5 and 6 indicate the integration of maternal specific bands in F1 hybrids



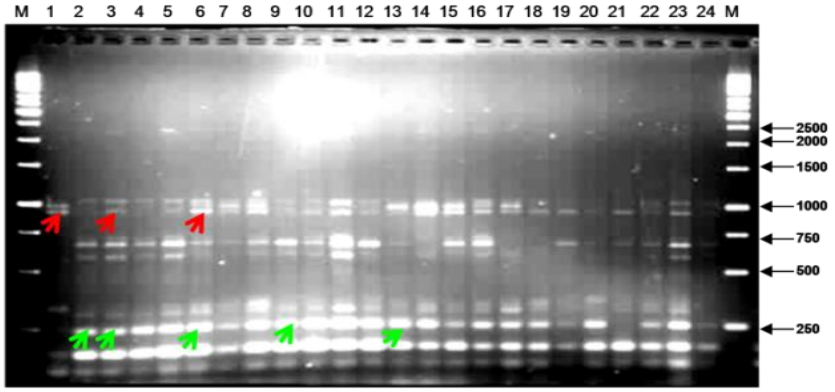
Me4+ Em16

Fig. 2. Confirmation of Hybrid status in Cauvery x CxR interspecific F_1 hybrid plants using primer me4 + em16. Circle in lane 1 indicates the paternal specific fragment and fragments within the circles of lane 3-8 indicates the integration of paternal specific fragments in both F_1 hybrids.



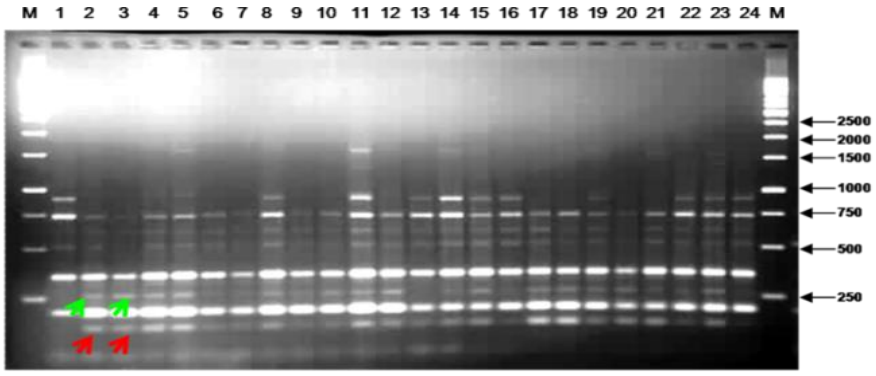
Me3+Em3

Fig. 3. Inheritance of maternal and paternal specific fragments in F1 and F2 interspecific hybrids using Me3 + Em3 SRAP primer pairs. Arrow mark in lane 2 indicates the maternal specific fragment and in lane number 6,7 and 13, 15 indicates inheritance of maternal fragment in F1 robusta type and F2 robusta type respectively. Fragment encircled in lane number 1 indicates the paternal specific fragment and its integration in F2 arabica type hybrids in lane number s 9 and 10.



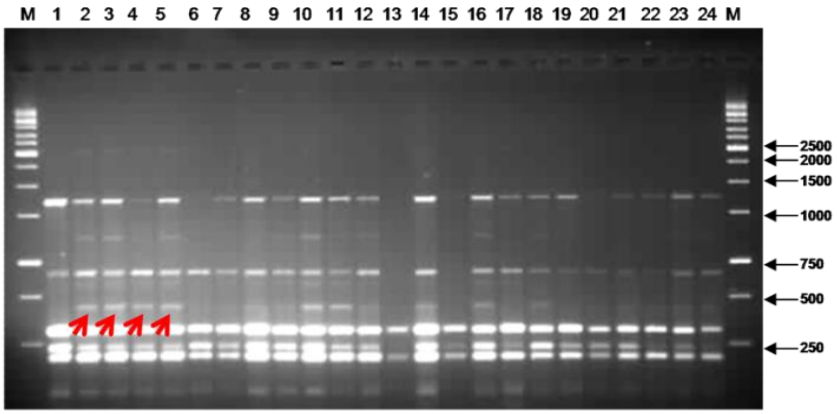
Me12+Em16

Fig. 4. Integration of maternal and paternal specific fragments in F_1 and F_2 interspecific hybrids of Cauvery x CxR using Me12 + Em16 SRAP primer pairs. Red arrow in lane number 1 indicates the paternal specific fragment and its integration in F_1 arabica type (lane number 3) and F_1 robusta type (lane number 6) hybrids. Green arrow in lane number 2 indicates the maternal specific fragment and its integration in F_1 arabica (lane no. 3) F_1 robusta type (lane no. 6), F_2 arabica type (lane no. 9) and 2 robusta type (lane no. 13) hybrid plants.



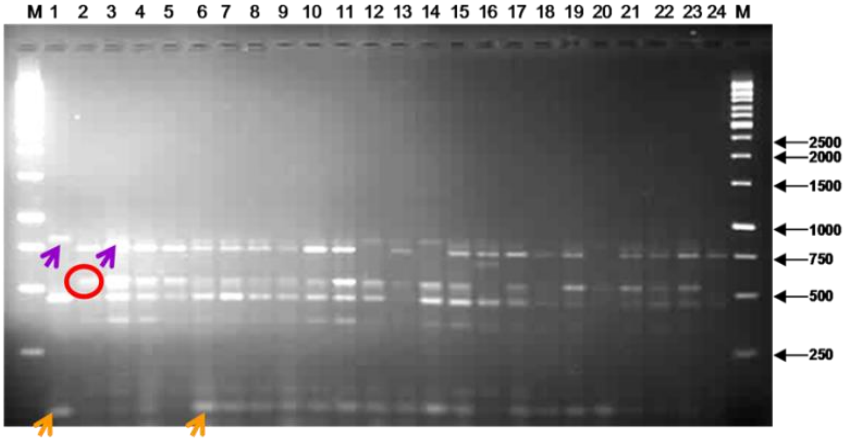
Me11+Em16

Fig. 5. Integration of maternal specific fragments in F1 and F2 interspecific hybrids of Cauvery x CxR using Me11 + Em16 SRAP primer pairs. Green and red arrows in lane number 2 indicate maternal specific fragments.



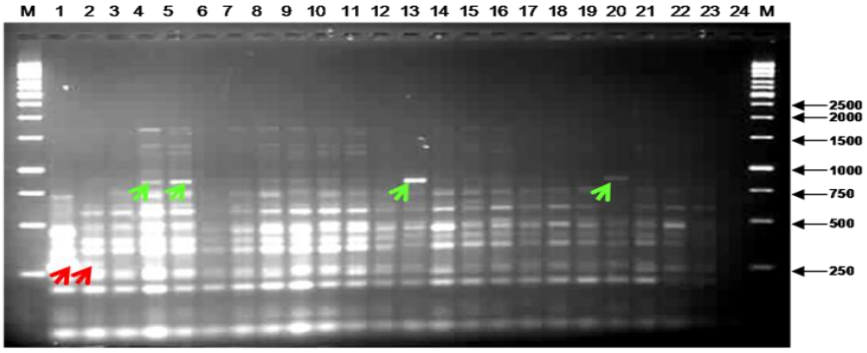
Me10+Em13

Fig. 6. Inheritance of maternal specific fragments in F_1 arabica type interspecific hybrids of Cauvery x CxR using Me10 + Em13 SRAP primer pairs. Red arrows in lane number 1 indicates the maternal specific fragments. This maternal specific fragment is integrated in F_1 arabica type hybrid (lane 3,4,5) but absent in robusta type F_1 hybrid.



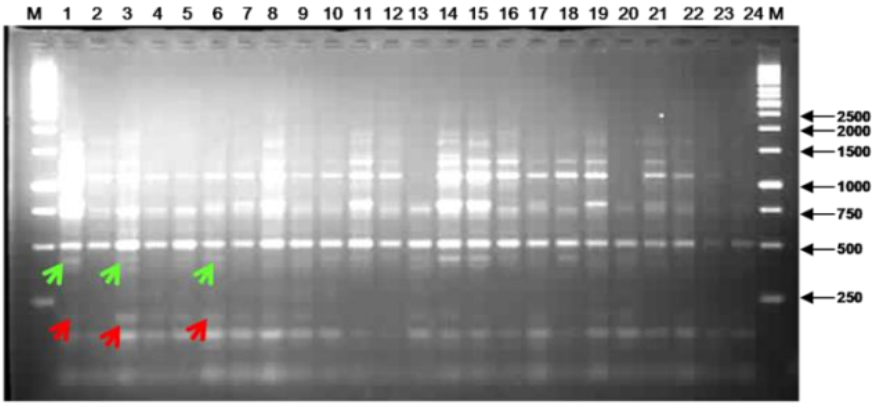
Me9+Em10

Fig. 7. Integration of paternal and maternal specific fragments in Cauvery x CxR F_1 and F_2 interspecific hybrids of using Me9 + Em10 SRAP primer pairs. Violet and brown arrows in lane number 1 indicate paternal specific fragments and red circle in lane number 2 indicates the maternal specific fragment.



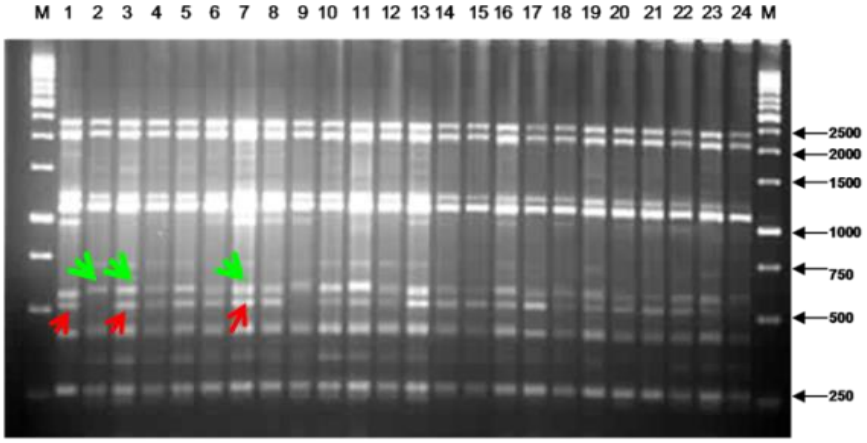
Me2+Em12

Fig. 8. Formation of hybrid specific fragments and non inheritance of maternal and paternal fragments in Cauvery x CxR F_1 and F_2 interspecific hybrids of using Me2 + Em12 SRAP primer pairs. Green arrow in lane number 4, 5, 13 and 20 indicates the hybrid specific fragments not found in any of the parents and red arrow in lane number 1 and 2 represents the parental fragments not inherited in hybrids.



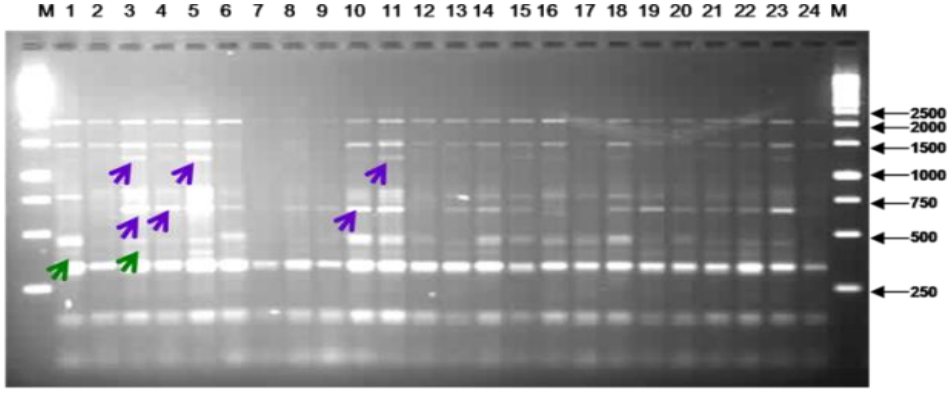
Me6+Em5

Fig. 9. Integration of paternal specific fragments in F_1 interspecific hybrids of Cauvery x CxR using Me6 + Em5 SRAP primer pairs. Green and red arrows in lane number 1 represent the paternal specific fragments. This fragment is inherited in F_1 arabica type (lane no.3) and F_1 robusta type (lane number 6) hybrids.



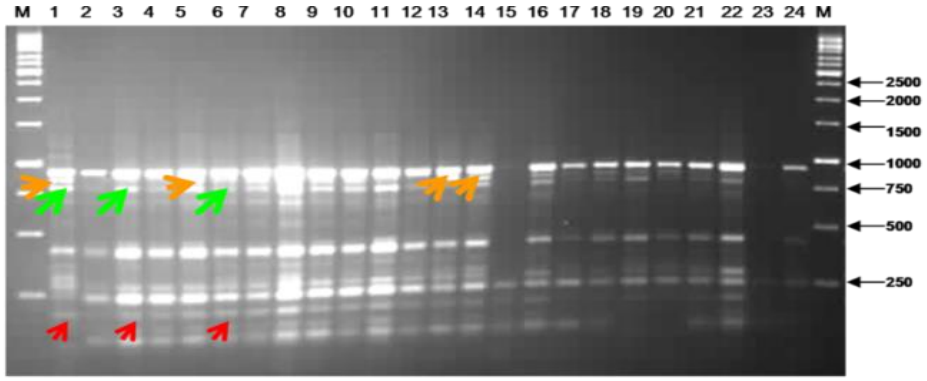
Me 4+ Em11

Fig. 10. Integration of paternal and maternal specific fragments in Cauvery x CxR F1 and F2 interspecific hybrids of using Me4 + Em11 SRAP primer pairs. Green and red arrows in lane number 1 and 2 indicate paternal and maternal specific fragments respectively. The integration of paternal and maternal specific fragments in F1 arabica (lane no.3) and F1 robusta (lane no.7) hybrids are shown.



Me1+Em4

Fig. 11. Formation of hybrid specific fragments in Cauvery x CxR F_1 and F_2 interspecific hybrids of using Me1 + Em4 SRAP primer pairs. Violet arrows in lane number 3, 4, 5, 10 and 11 indicates the hybrid specific fragments not found in any of the parents and green arrow in lane number 3 represents the integration of paternal fragment in CxR F_1 (arabica type) hybrid.



Me2+Em14

Fig. 12. Integration of paternal specific fragments in F_1 and F_2 interspecific hybrids of Cauvery x CxR using Me2 + Em14 SRAP primer pairs. Brown, green and red arrows in lane number 1 represent the paternal specific fragments. This fragment is inherited in F_1 arabica type (lane no.3 and 5) and F_1 robusta type (lane number 6) and F_2 (robusta type) hybrids.

CONCLUSION

Coffee being a perennial plant, it takes at least 5-7 years for attaining reproductive maturity for evaluation of new genotypes. Identification of suitable DNA markers for both vegetative and reproductive characters at an early stage of plant growth would be of much use in coffee breeding. In the present study, SRAP marker approach was found highly efficient and reproducible not only for identification and authentication of hybrid status but also for confirmation of alien genome introgression in coffee through molecular analysis.

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