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Research Article

Molecular profiling of selected semi- dwarf genotypes of Coffee (*Coffea arabica* L.) using Sequence Related Amplified Polymorphism (SRAP) markers

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Abstract: Commercial coffee production relies on two species, *Coffea arabica* and *Coffeea canephora*. Popularly known as arabica and robusta coffees respectively, the two species differ in their ploidy level (tetraploid and diploid), breeding behaviour (self and cross compatible), adaptability (high and low elevations) and agronomic performance. Transfer of desirable genes, in particular for disease resistance from diploid robusta into tetraploid arabica cultivars without affecting quality has been the main objective of arabica breeding. Occurrence of spontaneous tetraploid interspecific hybrids between robusta and arabica facilitated for arabica coffee improvement through introgressive breeding in India and elsewhere in the world. Catimor and Sarchimor were two popular semi-dwarf varieties of arabica developed by introgressive breeding. A new semi-dwarf line, S.4634 was developed from reciprocal crosses of Catimor and Sarchimor with an objective of developing a vigorous semi-dwarf coupled with durable resistance. The hybrid populations were subjected to selection and progenies were established for on-farm evaluation of agronomic performance. The present study involved molecular profiling of selected plants from these hybrid progenies along with the parental lines using SRAP marker approach. The SRAP assays proved efficient as 18 variety/genotype specific fragments were identified, 15 fragments in Sarchimor and three fragments in Catimor. Analysis of introgression revealed that the extent of Sarchimor introgression varied from 13.33% to 86.67% in hybrid plants analysed, which was also reflected in variable agronomic performance of these progenies especially with respect to field tolerance to leaf rust and bean size. The results clearly established the genomic status of the S.4634 derivatives in comparison with Sarchimor and Catimor lines.
Keywords: Coffee, Molecular Profiling, Introgression; Sequence Related Amplified Polymorphism

INTRODUCTION

Coffee is an important plantation crop of the tropics and a popular beverage of choice in most of the developed countries across the globe. The genus *Coffea* comprises of over 100 species of which only two species, *Coffea arabica* L popularly referred as arabica coffee and *Coffea canephora* Pierre ex A. Froehner known as robusta coffee are commercially cultivated. The two species constitute 99 per cent of global coffee production. *C. arabica* is suited for elevations above 1500 mts (3000 feet) MSL while robusta coffee is suited for lower elevations ranging from 600 to 900 mts. These two commercially grown species also differ in their genetic makeup and breeding behaviour. *C. arabica* is the only tetraploid (2n = 4x = 44) species of the genus and self-fertile while *C. canephora* and also all other species of the genus *Coffea* are diploids (2n = 22) and generally self-incompatible [1]. Further, the coffee produced form arabica coffee is of superior quality compared to robusta and fetches more premiums. But, arabica plants are more susceptible to diseases and pests compared to robusta coffee, which as the name suggests, is more robust in growth. Thus robusta is an important donor for genes conferring disease resistance, especially the coffee leaf rust, the major disease of concern for arabica coffee.

Thus, Arabica improvement programmes have been focused on transfer of resistant genes from Robusta as well as other diploid species to Arabica. However, inter-specific gene transfer through conventional breeding technique is a slow and challenging process due to the variation in policy level of the donor and recipient species (diploid vs. tetraploid). In this context, introgressive breeding using the spontaneous tetraploid inter-specific hybrids assumes greater significance. Two spontaneous hybrids of Robusta and Arabica known as 'Devamachy' identified in India and Hybrido-de-Timor (HDT) spotted in Timor island of Indonesia, formed the major sources for resistance genes in breeding programmes not only in India but also in other coffee growing countries [2]. Among these two hybrids, HDT has been extensively used in Arabica breeding especially for developing high yielding and disease tolerant hybrids of compact stature by crossing HDT with high yielding dwarf mutants of Arabica such as Caturra, Villasarchi, San Ramon etc. These semi-dwarf genotypes

predominantly the derivatives of the crosses, Caturra x HDT and Villasarchi x HDT, known as Catimor and Sarchimor, respectively, are cultivated commercially in different coffee growing countries. In India, these varieties were released for commercial cultivation as Cauvery (Catimor) and Chandragiri (Sarchimor) during 1985 and 2007, respectively.

In the course of breeding for rust resistance, reciprocal crosses were also made between Catimor and Sarchimor and mixture of reciprocals was given for on farm field trials with accession no. S.4634. Some of the enthusiastic coffee planters exercised selection in the population of S.4634 and progenies are being used for cultivation in the names of Hemavathi, NERI etc. In general, the selection was more towards Sarchimor types coupled with high yielding characteristic features of Catimor. The present study was undertaken with an objective of analysing the extent of genetic similarity of these genotypes in comparison with the parental lines, the Catimor and Sarchimor. For this purpose, randomly selected plants in base populations were subjected to molecular profiling by using Sequence Related Amplified polymorphism (SRAP), a PCR based dominant marker system first reported by Li and Quiros (2001) [3]. The SRAP Marker system was selected as it is simple, inexpensive, and effective for producing genome-wide fragments with high reproducibility and versatility [4].

MATERIALS AND METHODS

Plant material

The experimental material was 20 plants randomly selected from among the base populations of S.4634 used for exercising selection and also the commercial blocks of the same material established in planter's fields in Chikmagalur region of Karnataka. The selected plants though expressed high similarity in phenotype, differences were noticed in agronomic behaviour especially with respect to field tolerance to leaf rust and fruit size. Two plants each of Sarchimor and Catimor selected from parental populations available at Central Coffee Research Institute were included for comparative analysis.

SRAP primers

Keeping in view the low polymorphism among Arabica genotypes, preliminary screening was made with over 200 SRAP primer combinations to assess the parental polymorphism. Based on preliminary screening, four primer combinations that showed clear polymorphism between the two parental lines were used for introgression analysis of the hybrid derivatives. The details of the SRAP primers used for marker assays are furnished in Table 1.

Table-1: Primer combinations and sequences used for SRAP analysis

Primer	Forward	Reverse
O24	Me15: GTAGCACAAGCCGGACC	Em24: GACTGCGTACGAATTTAG
P6	Me16: CGAATCTTAGCCGGATA	Em6: GACTGCGTACGAATTGCA
S11	Me19: CGAATCTTAGCCGGAAT	Em11: GACTGCGTACGAATTCTA
N4	Me14: GTAGCACAAGCCGGAGC	Em4: GACTGCGTACGAATTTGA

Extraction and Quantification of DNA

Genomic DNA was extracted from leaf samples of the individual plants following the method of Krizman *et al.,* [5] with slight modifications [6] to suite coffee tissues. The DNA extracted was quantified using bio-photometer instrument and the quality of DNA was assessed by running 3µl of sample in 2% agarose gel.

PCR conditions

The PCR amplifications were performed as detailed earlier [3] with minor changes so as to suite coffee [6]. PCR was carried out in a 20 µl reaction mixture containing 1 x Taq buffer, 75mM Tris-HCL of pH 8.8, 20 mM $(NH_4)_2SO_4$, 30ng DNA, 200 µM dNTP mixture, 2.5 mM $MgCl_2$, 0.2 µM primer and 1 unit Taq DNA polymerase. The amplification cycles consisted of 5 min initial denaturation at 94°C followed by 5 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 35°C and 2 min extension at 72°C followed by 30 cycles with annealing temperature of 50°C; and a final extension of 10 min at 72°C.

PCR products were separated on a 2% (w/w) agarose gels containing 0.5 µg ethidium bromide/ml in 1X TAE buffer, visualised using UV-transilluminator (SYNGENE) and documented using the Gene Snap software program.

RESULTS AND DISCUSSION

The panel of plant samples consisted of two Sarchimor lines, two Catimor lines and 20 randomly collected plants in base populations of S.4634. Four SRAP primers (O24, P6, S11 and N4) that showed polymorphism between the two parental lines were used for molecular assays. All the four SRAP primers produced clear amplification profiles that could be easily scored in each of the genotype analysed. In all, the four SRAP primers generated a total of 58 bands that ranged from 13 (O24) to 16 (N4) per primer with an average of 14.5 bands per primer pair. In general, the number of bands amplified was higher in Sarchimor than in Catimor especially with primer combinations P6 and S11. In Sarchimor, on an average 12.13 amplified products were scored as against 8.38 bands in Catimor. The primer pair S11 amplified maximum number of genotype specific bands (10) followed by 5 bands with

P6. Among hybrid samples, the average number of bands amplified ranges from 7.5 (P6) to 12 (N4) per primer pair (Table 2). Out of the total number of bands amplified, 66% (38 bands) were polymorphic and 34% (20 bands) were monomorphic (Table2).

Table-2: Polymorphism obtained in the parental lines and hybrids using SRAP markers

Primers	Total bands	Size range (bp)	Average no. of bands amplified (Min-Max)			Polymorphic bands	% polymer-phism	Sarchimor specific bands	Catimor specific bands
			Sarchimor (Parent 1)	Catimor (Parent 2)	Hybrid progeny				
O24	13	250-2200	10	11	10.4 (7-13)	7	53.85	2	3
P6	14	220-1250	13	7.5 (7-8)	7.5 (5-14)	10	71.42	5	0
S11	15	150-1200	14	4	9.37 (6-14)	12	80	10	0
N4	16	280-1500	11.5 (11-12)	11 (10-12)	12 (6-15)	9	56.25	1	0

Based on the Sarchimor specific amplified products, the genome introgression from Sarchimor in each hybrid genotype was assessed to be at varying levels ranging from 13.33% to 86.67% while two of the hybrids did not show the presence of any Srachimor specific bands (Table 3). Depending on the extent of introgression, all the 20 hybrid plants analysed were classified into three groups (Table 4). The plants with above 50% introgression were included in Group 1 and between 25% to 50% introgression in Group 2 while plants with less than 25% introgression were included in Group 3. Thus, the Group 1 included only five plants that showed high level of introgression (above 50%) from Sarchimor and Group 2 comprises of seven plants with medium range of introgression (25% to 50%) while Group 3 consists of eight plants characterized by low level of introgression (less than 25%).

Table-3: Extent of Sarchimor introgression in hybrid plants

Sample No.	**Plant details**	**No. of Sarchimor specific bands**	**% introgression from Sarchimor**
1	Sarchimor (Plant $_1$)	15	-
2	Sarchimor (Plant $_2$)	15	-
3	Hybrid plant 1	10	66.67
4	Hybrid plant 2	3	20.00
5	Hybrid plant 3	9	60.00
6	Hybrid plant 4	4	26.67
7	Hybrid plant 5	11	73.33
8	Hybrid plant 6	7	46.67
9	Hybrid plant 7	3	20.00
10	Hybrid plant 8	2	13.33
11	Hybrid plant 9	3	20.00
12	Hybrid plant 10	13	86.67
13	Hybrid plant 11	5	33.33
14	Hybrid plant 12	5	33.33
15	Hybrid plant 13	4	26.67
16	Hybrid plant 14	4	26.67
17	Hybrid plant 15	9	60.00
18	Hybrid plant 16	3	20.00
19	Hybrid plant 17	3	20.00
20	Hybrid plant 18	5	33.33
21	Hybrid plant 19	0	0
22	Hybrid plant 20	0	0
23	Catimor (Plant $_1$)	0	-
24	Catimor (Plant $_2$)	0	-

The advances in DNA marker technologies provided new scope and opportunities for a wide variety of applications such as genotyping, cultivar identification, finger printing of germplasm, construction of linkage maps, and identification of Quantitative Trait Loci (QTL) and Marker Assisted Selection (MAS) for improving the efficiency of breeding. The great potential of DNA marker based technologies for genetic improvement is well demonstrated in several crops including the difficult species like coffee. DNA marker technologies were found equally useful in solving many evolutionary puzzles about origin, spread, and taxonomic relationships of many crop species.

The present study provided useful information on genome introgression in hybrid populations of *Coffea arabica*, by using Sequence Related Amplified Polymorphism (SRAP) markers. A relatively new PCR based marker approach, SRAP appears to be highly efficient in detecting the introgression of alien genetic material in arabica coffee (*C. arabica*), by combining a high Level of polymorphism and reproducibility Earlier, SRAP markers were efficiently used in coffee for understanding the genetic relationships among *Coffea* species [6], hybrid analysis [7] and confirming genome introgression in inter-specific hybrid progenies of *Coffea* [8]. This study was basically aimed at identifying the genotype/variety specific markers with respect to two commercially grown varieties, Cauvery (Catimor) and Sarchimor and to assess the extent of genome introgression in hybrid derivatives of these two varieties.

Table-4: Grouping of hybrid plants based on level of introgression

Group	% of introgression	No. of plants
1	Above 50%	5
2	25% to 50%	7
3	Less than 25%	8
	Total	20

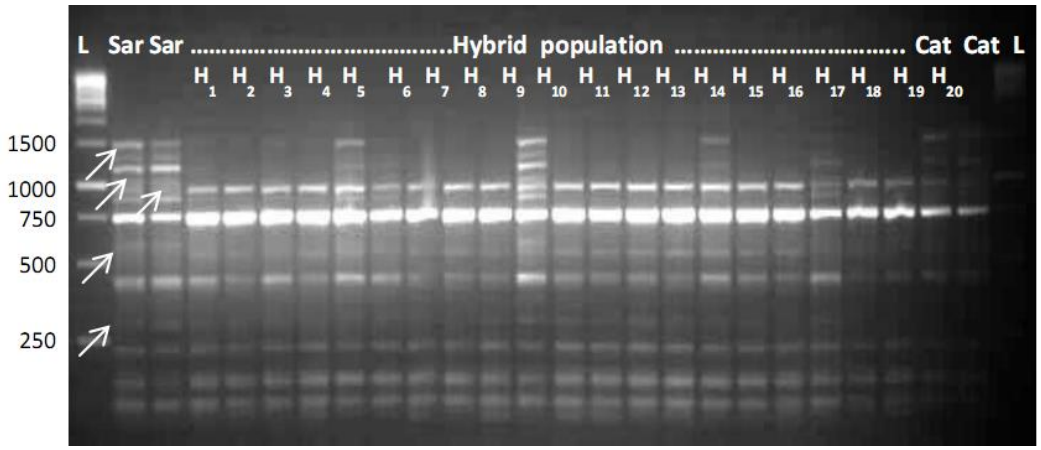

Fig-1: Amplification profile generated using SRAP primer P6, showing clear distinction of banding pattern in Sarchimor and Catimor genotypes and hybrid plants

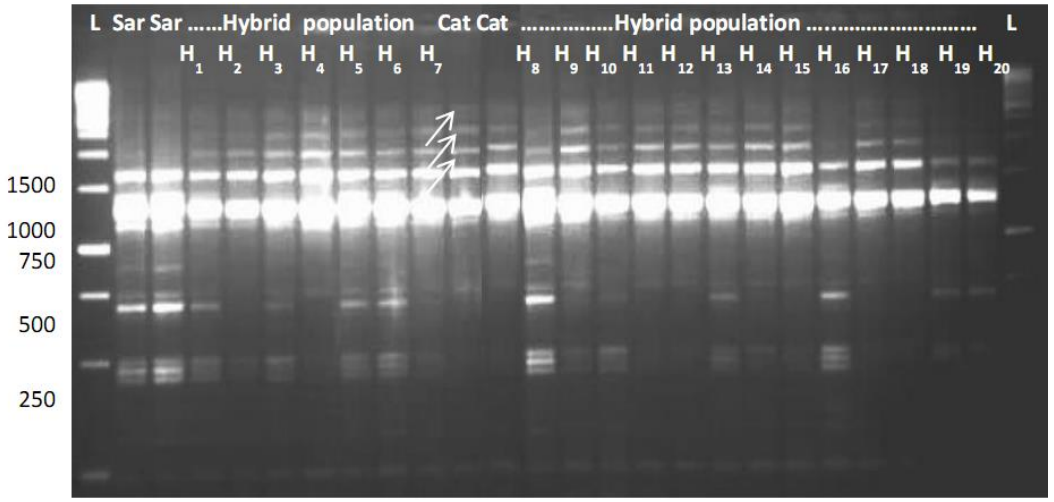

Fig- 2: Amplification profile generated using SRAP primer O24, showing Catimor specific bands

Catimor and Sarchimor were two semi-dwarf varieties of Arabica cultivated on different names across the coffee growing countries. Catimor known as Cauvery in India was a derivative of the cross between a high yielding dwarf mutant and Hibrido de Timor (HDT) a spontaneous tetraploid interspecific hybrid of Robusta x Arabica coffee types. HDT manifest high resistance to coffee leaf rust caused by an obligate parasitic fungus, *Hemileia vastatrix*. Therefore HDT has been extensively used as donor for rust resistance in arabica coffee breeding programmes. Cauvery (Catimor) the high yielding and rust tolerant variety given for commercial cultivation in India during 1985 recorded promising performance initially but later breakdown in resistance was noticed due to appearance of new races of coffee leaf rust pathogen [9]. Similarly, `Sarchimor` was a derivative of the cross between a dwarf mutant Villa Sarchi and HDT. The yield potential of Sarchimor lines is slightly less compared to Catimor lines. On the other hand Sarchimor lines manifest high field tolerance to leaf rust and also to root knot nematode (*Meloidogyne spp*.) whereas Catimor lines show susceptibility to these pests and diseases. Further, the beans are elongated and large in size in Sarchimor lines enabling to realize high percentage of 'AA' (~20%) and 'A' grade beans (~50%) that stands on 7 mm and 6.6 mm sieves, respectively which is a value addition in Sarchimor over Catimor lines. Hence, a derivative of Sarchimor line, named as 'Chandragiri' was given for commercial cultivation in India during 2007. In order to improve the vigour, rust resistance and bean quality in Catimor, reciprocal hybrids were developed between Catimor and Sarchimor and the mixture of reciprocals were established in onfarm trials under the accession no. S.4634. These hybrid populations showed semi-dwarf phenotype with vigorous growth and improved rust resistance as well as bean size compared to Catimor. Hence, some of the coffee planters exercised selection in the populations of S.4634 and their progenies have been used for cultivation. As the selection was more towards Sarchimor type of plants with vigour and yielding characteristics of Catimor, progenies of selected populations popularly known as 'Hemavathi' and NERI among coffee planting community, resemble 'Chandragiri' variety in plant phenotype. However, the field tolerance to leaf rust and bean size were not on par with 'Chandragiri'. In the present study, SRAP profiling of randomly selected plants clearly established the genomic constitution of the populations derived from S.4634. Although the plant phenotype of these plants resembled Sarchimor/Chandragiri, the marker assays revealed substantial variation at genomic level. Analysis of introgression revealed that the extent of Sarchimor introgression varied from 13.33% to 86.67% in different plants, which might be the reason for variable agronomic performance of these progenies compared to original Sarchimor/Chandragiri especially with respect to field tolerance to leaf rust and bean size. The study provided scope to identity/select the elite plants based on genotype, plants with high levels of Sarchimor introgression coupled with good agronomic performance of both Sarchimor and Catimor for commercial cultivation.

It is apparent from the results that the preliminary screening of over 200 SRAP primers for parental polymorphism was helpful in identifying polymorphic primers. Although the number of polymorphic SRAP primers used for screening the hybrid population was limited (four primers), the SRAP assays were proved efficient and 18 variety/genotype specific fragments were identified, 15 fragments in Sarchimor and three fragment in Catimor. Identification of genotype specific SRAP markers has helped in analysis of introgression which was otherwise difficult with phenotypic traits/markers. Similar findings were reported earlier by Mishra et al. [7] and Anilkumar et al. [8] based on SRAP analysis of certain inter-varietal and interspecific hybrid derivatives of arabica coffee (*Coffea arabica*). It was inferred that 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 and marker inheritance in coffee. Relatively more number of amplified fragments (58 bands from four primer combinations) and the high polymorphism rate (66%) among the coffee plant samples analysed in the present study indicated that SRAP is an extremely efficient technique for DNA marker generation in coffee especially the *C. arabica* populations known for low genetic diversity.

CONCLUSION

Our results clearly established the genomic status of the S.4634 derivatives in comparison with Sarchimor and Catimor lines of arabica coffee by molecular profiling and suggest that SRAP may well offer an efficient way of distinguishing the genotype and genome introgression.

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