Inheritance of RAPD and ISSR markers in hybrid derivatives of inter-specific coffee hybrid (*Coffea congensis* Froehner x *Coffea canephora* Pierre): Implications on genetic improvement and plant variety protection

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Abstract

An inter-specific hybrid involving *Coffea congensis x Coffea canephora* is popularly known as C x R coffee hybrid in India. This hybrid was developed with the primary objective of reducing the plant size for high density cultivation and also to improve the coffee beverage quality. Though this hybrid is popularly cultivated in India, segregation of this hybrid due to cross pollination either within the hybrid or with other diploid species of *Coffea* is one of the major problems to achieve seed purity and higher yield. To utilize DNA marker assisted selection (MAS), two dominant DNA markers (RAPD and ISSR) were analyzed for their utility. About 24 RAPD and 15 ISSR primers were tested for screening the effective primers and only three RAPD and five ISSR primers were found suitable for discriminating the parent. These selective RAPD and ISSR primers produced nine and 23 polymorphic bands respectively with size range of 400 bp – 1200 bp in RAPD and 100 bp – 2000 bp in ISSR. Species specific RAPD and ISSR markers were found in female (*Coffea congensis*) and male (*Coffea canephora*) parent of the hybrid and certain of these RAPD and ISSR markers and they are found to inherit into F1 and backcross progenies. The primers for suitability for marker assisted selection were evaluated with parameters such as Polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP). Importance of these markers in genetic improvement of this hybrid is discussed.

Introduction

Coffea canephora Pierre (2n = 2x = 22), popularly known as 'Robusta coffee' is one of the important species cultivated in the world, contributing 30% of coffee production in the international market (Orozco-Castillo et al. 1994; Ruas et al. 2003). *C. canephora* is the most suitable *Coffea* species for cultivation due to its durable resistance to major pest and disease (Prakash et al. 2005). However, this species produces inferior quality of coffee bean as compared to *C. arabica* (2n = 4x = 44) which contributes the remaining 70% of world coffee production (Lashermes et al. 1996; Berthaud and Charrier, 1988; Carvalho, 1988; Anthony et al. 2002; Steiger et al. 2002). Therefore, genetic improvement of *C. canephora* for enhancement of coffee quality is prioritized in many coffee producing countries (Leroy et al. 2006). *C. congensis*, originally found at native in Africa and predominantly occurring along the basin of river Congo. This species became the center of attraction as this is the only species produces superior quality of coffee bean among the diploid species of *Coffea* (Davis and Rakotonasolo, 2009). This species is highly compatible for inter-crossing with *C. canephora* and become one of the potential genetic resources for improving the coffee quality in *C. canephora* (Davis and Rakotonasolo, 2009). Therefore, inter specific hybridization between *C. congensis* and *C. canephora* was employed in India to incorporate better liquor quality in *C. canephora* followed by a number of backcrossing with either of the parents to stabilize beverage quality and yield (Jamsheed et al. 1996). Systematic breeding in India led to the development of Congensis × Robusta hybrid, popularly known as C × R hybrid (Jamsheed et al. 1998).

C × R hybrid is cultivated as mixed plantation in India with *Coffee canephora* (male parent of this hybrid) as an efficient pollen donor for improving the productivity. Under field condition, this hybrid is distinguishable morphologically from both the parents with an intermediate bush size an drooping

behavior of primary branches (Anonymous, 1988). However, upon the maturity of this hybrid, it tends to change its vegetative growth and resemble with the male parent. This hybrid shows wide range of polymorphism due to influence of different agro-climatic condition, leading to practical difficulties for the farmers and researchers to distinguish C x R hybrid from *Coffee canephora* (Jamsheed et al., 1996) A number of reports on the utility of RAPD (Orozco-Castillo et al., 1994; Prakash et al., 2005; Agwanda et al., 1997; Aga et al., 2003; Maluf et al. 2005) and ISSR (Carvalho, 1988; Paulo et al. 2003; Lin et al. 2019) makers for analyzing the genetic diversity of *Coffea* species and several coffee hybrids have been reported. Although most of the reports on DNA markers are relating to genetic diversity of *Coffea*, only a few works could demonstrate the applications of DNA marker in molecular assisted selection (MAS) breeding in coffee. For example, ISSR marker was used for determination of inter specific hybrid of Coffea (Paulo et al. 2003) and demonstrated the use of ISSR marker for determination of inter-specific hybrid of *Coffea*. A panel of SNPs for identification of twelve arabica coffee cultivars has been reported (Lin et al. 2016). Our study revealed a few unique RAPD and ISSR markers to distinct the parental species of a commercially important inter-specific hybrid of coffee and demonstrated that these markers transmit into F1 hybrid and its advanced progenies. Utilization of these markers in genetic improvement of this hybrid and other possible applications are discussed.

Materials and Methods

Plant material

Young leaves of *C. congensis*, *C. canephora*, *C. congensis* × *C. canephora* (F1 hybrid) and backcross progeny of C × R to *C. canephora* were collected from the germplasm block of Central Coffee Research Institute, Chikmagalur Distract, Karnataka State, India. Derivation this hybrid through conventional breeding (Fig. 1) and their characteristic features are described (Table 1). Whole leaves were washed with sterile distilled water, covered with polypropylene bags and stored at − 70°C freezer (Sanyo, Japan). Frozen leaf samples were used for this study.

Isolation of genomic DNA

About 0.5 g of frozen leaf tissue of coffee was ground into fine powder under liquid N_2 using sterile/chilled mortar and pestle. The powder samples were added into 1 ml of extraction buffer (100 mM Tris-pH8.0, 1.4M NaCl, 20mM EDTA- pH 8.0, 2% CTAB, 0.3% β mercaptoethanol and 1% PVP) in a centrifuge tube and incubated at 60°C for 60 min in heating block (Thermo Fisher Scientific, Mumbai, India). Samples were allowed to attain normal temperature and equal volumes of (24:1) Chloroform and isoamyl alcohol (HiMedia Laboratories, Mumbai, India) were added and gently mixed to form emulsion. Samples were centrifuged at 12,000 rpm for 10 min (Kubota, Japan). After centrifugation, supernatant was gently recovered without disturbing the pellet. The supernatant was washed once again with chloroform and isoamyl alcohol and subjected to next round of centrifugation at 12,000 rpm for 10 min. Clear supernatant was recovered and added 2/3 volume of the isopropanol (HiMedia Laboratories,

Mumbai, India) and incubated at − 80°C (Cryo Scientific Systems Private Limited, Chennai, India) for 60 min. Samples were once again centrifuged at 12,000 rpm for 12 min and the supernatant was carefully recovered without disturbing DNA pellet and washed with 20 µl of 70% ethanol before centrifugation at 12,000 rpm for 5 min. Ethanol was removed by micropipette and the final DNA pellet was vacuum dried for 15 min. DNA pellet was re-suspended in 50 µl of 0.1 × TE buffer-pH8 (10 mM Tris, 1 mM EDTA) and stored at − 20°C. DNA was run in 0.8% agarose gel (Sigma Aldrich, Mumbai, India) to determine the quality of DNA. Quantification of DNA was carried out using UV-Vis spectrophotometer (Bio Rad, India) purity of DNA was determined by OD_{260}/OD_{280} ratio.

Screening of RAPD and ISSR primers

Template DNA of *Coffea congenis* was initially used for screening with 25 RAPD and 15 ISSR primers procured from Sigma Aldrich, India (Table 2). The polymerase chain reaction mixture and conditions for amplification of genomic DNA are described (Table 3). The RAPD primer series includes 11 primers of OPA series, two primers each from OPB, OPI and OPO series and one primer each from OPC, OPF, OPK, OPL, OPN, OPP, OPS and OPU series. RAPD and ISSR primers were procured based on the published work of Mishra et al., (2011) and Ruas et al., (2003) respectively. These primers were diluted as per the manufacturer protocol and stored at -20^{o}C (Blue Star, India). PCR amplification was done with a Thermal Cycler (Himedia, India) and PCR products were run in 1.5% agarose gel and RAPD and ISSR fingerprints were scored for number of scorable and polymorphic bands and documented.

Development of DNA fingerprints of parents and hybrids

Upon the screening, three RAPD (OPAB3, OPAL12, OPAL15) and five ISSR (ISSR1, ISSR2, ISSR6, ISSR9, ISSR13) primers were used further to develop species specific DNA markers for *C. congensis* (female parent) and *Coffea canephora* (male parent). To determine the inheritance of RAPD and ISSR markers to F1 hybrid (S.885) and its backcross progeny to *C. canephora* (S.2568) and advanced progeny (S.2568), parental samples were included for PCR amplification with F1 and backcross progeny. Fingerprints were carefully analyzed by comparing between parent and its derivatives. Reproducible and scorable nature of bands observed in the hybrid derivatives were compared with either of the parent and repeated the amplification atleast five times for reproducibility. Both RAPD and ISSR marker inherited from parents to F1 hybrid and its backcross and advanced progenies were analysed for marker inheritance and documented.

DNA Marker Analysis

RAPD and ISSR marker analysis was done based on the assumption that each band represented the phenotype at a single biallelic locus. Consistent and scorable bands were selected for data generation. To detect the molecular weight accurately for unique and species specific DNA bands corresponding to C. congensis and C. canephora and their derivatives, fingerprints were photographed using Biorad Gel Documentation system and each row of the gel was analysed using EgyGene GelAnalyzer4 Program based on the guidelines (https://egygenes.blogspot.com). By comparing the banding pattern of female (*C. congensis*) and male (*C. canephora*) parent and their F1 and backcross progenies, species specific

bands were detected and molecular weight was determined. Binary data was used to calculate number of scored bands (NSB), number of polymorphic band (NPB), and percentage of polymorphic bands (PPB). To determine the efficiency of RAPD and ISSR markers, four parameters such as polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP) were analyzed.

Polymorphic information content (PIC)

PIC value of each DNA band was calculated based on the formula $PIC_{i\ =}$ 2fi (1-f_i) (Roldan et al. 2000). Where PIC_i is the PIC of the band i, fi is the frequency of the amplified fragment (band present) and 1-f_i is the frequency of non-amplified fragment (band absent). The frequency was calculated as the ratio between the number of amplified bands at each locus and the total number of progenies .The PIC of each primer was calculated using the average PIC value from all loci of each primer.

Marker Index (MI)

Marker index (MI) was calculated to characterize the capability of each primer to detect polymorphic loci between the parents, hybrid and its backcross derivatives as demonstrated previously (Varshney et al. 2007). MI = EMR × PIC; Where, EMR (effective multiplex ratio) = $n \times \beta$, where n is the average number of fragments amplified in each accession/genotypes and β is estimated from the number of polymorphic loci (PB) and the number of non-polymorphic loci (MB). Therefore, β = PB/ (PB + MB) as described (Kumar et al. 2014).

Resolving power (RP)

Resolving power of each primer was calculated based on the following formula (Prevost and Wilkinson, 1999). RP = ΣI_b, Where I_b represents the informative fragments. The I_b can be represented on a scale of 0/1 by the following formula; $I_b = 1 - [2 \times (0.5 - p_i)]$, Where p_i is the proportion of accessions containing the i^{th} band.

Inheritance of RAPD and ISSR markers

Parental specific DNA marker corresponding to *C. congensis* (female parent) and *C. canephora* (male parent) were scored and their molecular weights were determined using EgyGene GelAnalyzer4 Program. Subsequently, these unique markers were analyzed critically for their inheritance into the hybrid and its backcross progenies.

Results

Screening of RAPD and ISSR primers

A total of 25 RAPD and 15 ISSR primers were selected for screening based on the published report for RAPD primers (Mishra et al. 2011) and ISSR primers (Ruas et al. 2003) .These primers were screened once again in our lab primarily to select most suitable primers to ensure better reproducibility for molecular characterization of C x R hybrid and its progenies. The number of amplicons produced by each

primer was varying from one to fourteen for RAPD and three to nine for ISSR and size of the amplicon was ranging from 250 bp – 2800 bp for RAPD and 200 bp to 1300 bp for ISSR (Fig. 2,3). Based on the number of reproducible bands, three RAPD primers (OPAB-03, OPAL-12, OPAG-15) and five ISSR primers (ISSR-1, ISSR-2, ISSR-6, ISSR-9, ISSR 13) were selected for developing DNA markers for precise identification of C x R hybrid. Genomic DNA of selected parental samples (*C. congensis* and *C. canephora*) and their hybrid derivatives produced unique fingerprints upon the PCR amplification using the selective RAPD and ISSR primers.

Comparativeness of RAPD and ISSR markers

A total of 10 loci were obtained in case of RAPD and 26 loci in ISSR from C x R coffee hybrid and its derivatives. Most of the PCR products were in the size range of 250–2800 bp in case of RAPD and 200–1300 bp in case of ISSR with a mean value of 3.3 and 8.8 amplicons per primer. From the above date, nine and 23 loci of RAPD and ISSR respectively were found polymorphic (either present or absent in less than 95% of selected genotypes). We found that one and three loci from RAPD and ISSR were found to be monomorphic with an average of 5.33 and 7 per primer respectively. Based on the RAPD data, the frequency of polymorphism was varied from primer to primer. One of the primers (OPAB3) had produced 100% polymorphic loci. A similar result was observed in case of ISSR experiment with a frequency of 80–85% polymorphic loci (Table 4).

Performance of RAPD and ISSR markers

The information on genetic profile of *C. congensis*, *C. canephora* and their hybrid derivatives obtained using the three RAPD primers and five ISSR primers were used to assess the marker performance through evaluation of four parameters: PIC, EMR, MI and RP (Table 5). To determine the PIC values for all loci of each primer, we have taken the mean of PIC values for all the loci of each primer. The range of PIC for 3 polymorphic loci of RAPD markers was 0.25–0.55 and for ISSR markers 0.28 to 0.40 averaging 0.38 and 0.33 respectively. Three of the polymorphic loci were highly informative (PIC > 0.45) in case of RAPD markers while in case of ISSR primer 20 of the polymorphic loci were highly informative (PIC > 0.45). When the average polymorphic loci were correlated with PIC value data for individual loci, it was found that fragments falling within the 3.5 -7.0 were highly informative.

The ISSR effective multiplex ratio (EMR) depends on the number of polymorphic loci. In this study, the highest effective multiplex ratio was observed with the RAPD primer OPAB-03 (EMR 7) and ISSR primers ISSR 1 and ISSR 2 (7.3) with mean value of 4.77 and 6.12 respectively. To determine the usefulness of markers, we calculated the marker index (MI) for each RAPD and ISSR primer. The highest MI was observed in RAPD in OPAG-15 (3.30) and lowest in OPAL-12. In case of, ISSR markers, highest MI value was obtained with ISSR 1 (2.48) and lowest with ISSR 6(1.66).

The resolving power (RP) is a parameter that indicates the discriminatory potential of the primer chosen. The average RP was 3.81 for RAPD and 4.48 for ISSR markers and the highest with RAPD primer OPAB3 (5.2) and lowest with OPAL12 (1.50). In case of ISSR, primer ISSR 1 shown the highest RP (5.6) and ISSR

6 with lowest RP (3.6). We have validated some of the primers for their efficiency for developing the genetic profile using RAPD and ISSR in C x R coffee hybrid.

Inheritance of unique RAPD and ISSR marker

We also found that RAPD and ISSR markers found in either of the parents could inherit into F1 hybrid. Based on our results, OPAB-03 and OPAG12 had amplified unique DNA bands with the molecular weight of 1100 bp and 1300 bp in *C. congensis* (female parent) and *C. canephora* (male parent) respectively. These markers were constantly inherited to C x R hybrid and its progenies. OPAG- 15 had amplified one unique band in *C. canephora* (male parent) with 500 bp, found to inherit into F1 and backcross progenies (Fig. 4). ISSR markers namely, ISSR 1, ISSR2, ISSR6 had amplified unique bands with 900bp, 1000bp, 500bp in female parent (*C. congesnis*) with constant inheritance into F1 and backcross progenies. ISSR9 which amplified unique DNA band with 600 bp in male parent (*C. canephora*) constantly inherited to F1 and its backcross progeny. ISSR13 produced a faint DNA bands with 700bp in F1 and backcross progeny but not found in either of the parent (Fig. 5). All the unique bands scored in this study were constantly inherited to F1 and its backcross progenies.

Discussion

C. congensis is a smaller bush and known for superior coffee flavor among the diploid species of *Coffea.* But *C. canephora*, an another diploid origin is known for higher productivity but inferior in aroma quality (Koshiro et al. 2007) Genetic improvement in diploid species of *Coffea* for enhancement of aroma quality is one of the breeding activities in India and other coffee growing countries. Crosses were made between *C. congensis* and *C. canephora* during 1942 and their progenies were evaluated for more than 17 years to release a hybrid, popularly known as C x R hybrid (Anonymous, 1998). The F1 progeny was intermediate in plant stature with improved aroma than *C. canephora* but with lesser productivity (Anonymous, 1998) .To improve the productivity, backcross was performed with *C. canephora*. This hybrid is clearly distinguishable from either of the parents during the early stage of growth under field condition. However, after 15–20 years of cultivation, the vegetative features often resembles to *C. canephora*.

Mixed cultivation of C x R hybrid and *C. canephora* (one of the parents of C x R hybrid) was recommended in commercial plantation in India as the latter one was realized as an effective pollen donor to C x R hybrid for higher fruit set and productivity (Jamsheed et al. 1996). However, due to varying behavior of vegetative growth of C x R hybrid under different environmental conditions, distinction of C × R hybrid from *C. canephora* was one of the constraints during plant selection process for seed production and cloning. To support this, different bush sizes of C x R hybrid, namely *C. congensis* type (compact bush with smaller leaves) intermediate type (bush size is intermediate to *C. congensis* and *C. canephora*) and *C. canephora* type (buses are similar to *C. canephora* with broader leaves) were documented (Jamsheed et al. 1996). Unauthentic sources of clones of C x R hybrid with a close resemblance to *C. canephora* often became difficult in precise identification C x R hybrid in a routine selection process for planting and breeding exercise.

Genetic compatibility among different species of *Coffea* for development of inter-specific hybrids has been well established by surpassing the ploidy barriers between diploid and tetraploid species of *Coffea* (Louarn, 1993; Charrier, 1978). For example, Hibrido de Timor popularly known as HdeT (a natural hybrid of *C. arabica* and *C. canephora*) and S-26 (a natural hybrid of C. liberica ×*C. arabica*) were identified in the chikmagalur province of southern part of India (Anonymous, 1988). HdeT (Hibdido de Timor) and its derivatives became the core genepool for breeding several arabica varieties of coffee for leaf rust resistance (*Hamelia vastatrix*) as this is a only source of genes in arabica coffee breeding programme (Charrier and Eskes, 1997). In India, S.26 was the first inter-specific hybrid which offered durable resistance to coffee leaf rust disease (*Hemileia vastatrix*) owing to genetic introgression between *C. liberica*, a diploid origin offering resistance to coffee leaf rust disease and *C. arabica*, tetraploid origin predominantly susceptible to coffee leaf rust disease (Surya Prakash et al. 2002). However, genetic improvement of diploid species of *Coffea* has not been paid much attention than tetraploid species as diploid species of *Coffea* are highly tolerant to major pests and diseases (Charrier ,1978; Filho et al. 1999; Luis Fernando and Matthew, 2022). Nevertheless, breeding among diploid species of *Coffea* is one of the priorities in many robusta coffee growing countries to improve the aroma (Carvalho, 1988; Van der Vossen, 1985).

A number of DNA markers have been developed to characterize wide range of coffee genetic resources using RAPD (Mishra et al. 2011; Silvestrini et al. 2008; Kathurima et al. 2012; Achar et al. 2015; Ramadiana et al. 2021) and ISSR (Mishra et al. 2011; Tshilenge et al. 2009) markers to understand the genetic relations among the diploid and tetraploid species of *Coffea*, including the inter-specific origins. In addition to genetic diversity analysis, RAPD and ISSR markers are applied in breeding programs such as identification of inter-specific hybrid (*C. canesphora* x *Coffea arabica*), known as 'Arobasta hybrid' (Ruas et al. 2003; Gimase et al. 2019). In India, hybrid specific SNPs as DNA marker was developed for an inter-specific hybrid (*Coffea congensis* x *Coffea canephora*) using two DNA barcoding locus, namely *rbc*L and *mat*K (Bharatha Nandhini et al. 2013)A detailed studies on genetic diversity of various species of *Coffea* using RAPD and ISSR markers revealed that *Coffea congensis* and *C. canephora* are closer to each other than other species of *Coffea* (Mishra et al. 2011; Silvestrini et al. 2008) This gives an understanding that *C. congensis* and *C. canephora* are freely inter crossable in nature to develop fertile hybrids. Therefore, possibilities of genetic dilution in addition to new recombination among the diploid species of *Coffea* cannot be ruled out. Most importantly, there are substantial evidences that phenotypic characters are force to be changed under different environmental conditions (Nguyen et al. 2015) in addition to changes in coffee quality (Leroy et al. 2006; André et al. 2008). This is true in a perennial crop like coffee as the plantation is exposed to wide range of micro and macro environmental condition. To support this, the C x R hybrid of coffee that are under extensive cultivation in India often experiences the phenotypic variations (Raghu et al. 2003). In the above context, parental and hybrid specific RAPD and ISSR markers are expected to be highly useful as genetic markers for precise identification of C x R hybrid in a number of applications related genetic improvement.

India is one among the coffee growing countries, thriving to improve the bean quality of *C. canephora* involving the most compatible and better source of genes for coffee quality from diploid species of

Coffea. This effort has lead to development of a popular C x R variety with acceptable quality of coffee bean than *C. canephora* (https://www.indiacoffee.org). A similar effort involving a cross between C. Arabica x C. canephora lead to improvement of aramoa quality of coffee bean (Priolli et al. 2008). Our study demonstrated parental specific RAPD and ISSR markers linked to both the (*C. congensis* and *C. canephora* as female and male parent respectively) and their constant inheritance into advanced progenies. Upon the repeated PCR amplification using RAPD and ISSR primers, these markers are detectable with high degree of reproducibility in advanced progenies. Previously, we have identified specific SNPs in chloroplast genes of *C. congensis* and *C. canephora* in *rbc*L and *mat*K locus and found that these SNPs are detectable in F1 and backcross progenies of C x R hybrid and its progenies (Bharatha Nandhini et al. 2013). However, utilization of these SNPs as marker is time consuming process as this requires PCR amplification following the sequence analysis. Our present study has lead to development of alternative DNA markers (RAPD and ISSR) for precise identification of C x R hybrid to utilize in genetic improvement program. We also confirmed that these markers are tightly inheritable in advanced progenies of C x R hybrid for utilizing them as a potential DNA marker. As both RAPD and ISSR markers are dominant in nature and easier to detect directly from the fingerprints, they can be of greater practical utility than other DNA markers such as SSR (microsatellite), AFLP and SNPs which has limitations for screening large number of individual plants for commercial seed production and nursery screening. Propagation of C x R hybrid and other diploid varieties of coffee (*Coffea canephora*) are conventionally propagated through seeds and cloning of orthotropic shoots. Therefore, RAPD and ISSR markers are very handy to ensure seed purification/certification in addition to determine the genetic purity of clones in commercial nurseries.

Declarations

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Author contributions DK: Investigation, Methodology, BN: Experiments and data generation, SPR: material source, field data collection, YSK: experiments and data collection, TS: draft preparation, data analysis, GD: Writing, editing, overall supervision.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no known competing fnancial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval No ethics approval is required about this article.

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Tables

Tables 1-5 is available in the Supplementary Files section.

Figures

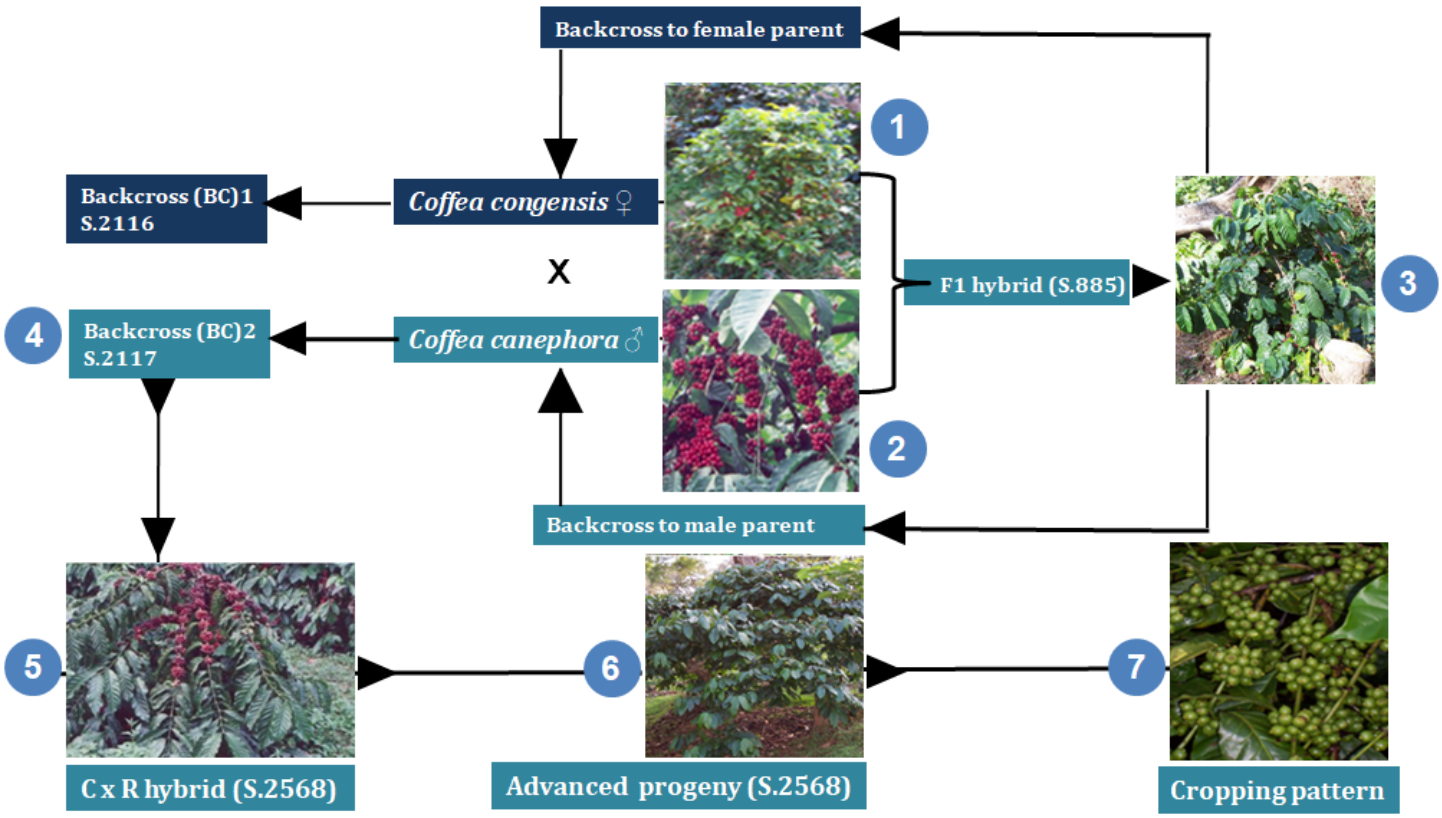


Figure 1

Derivation of C x R coffee hybrid: 1, *Coffea congensis*- female parent with small bush six and with poor cropping 2, *Coffea canephora* – male parent with larger bush size and with higher crop 3, C x R F1 hybrid with intermediate bush size and with broader leaves 5, C xR hybrid derived from the backcross of F1 with *C. canephora* (Accession S.2568) 6 &7, old plants of advanced progeny showing larger bush size similar to *C. canephora*with high cropping

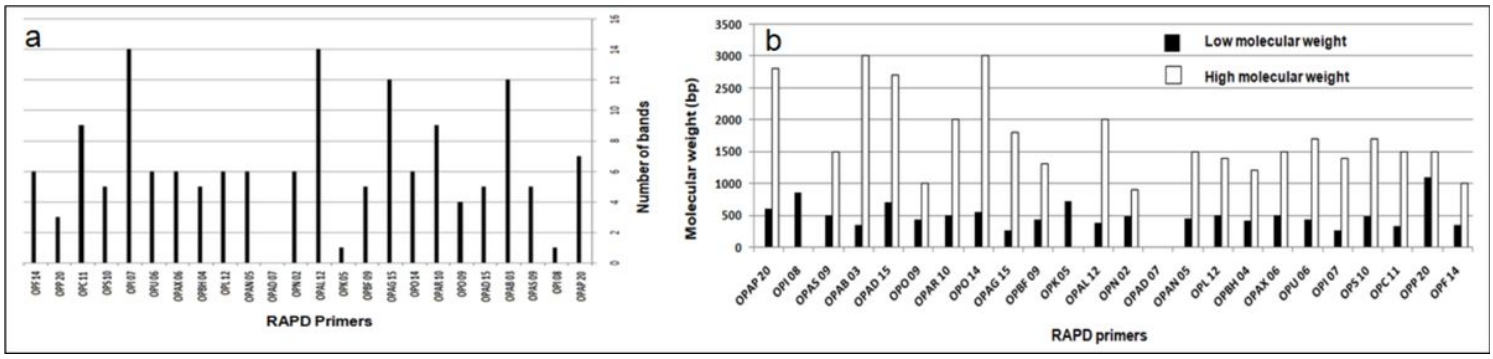


Figure 2

Screening of RAPD primers: Twenty four RAPD Primers showing the number of amplicons (a) and low and high molecular weight amplicons from the pooled genomic DNA of *Coffea congensis* and *Coffea canephora*

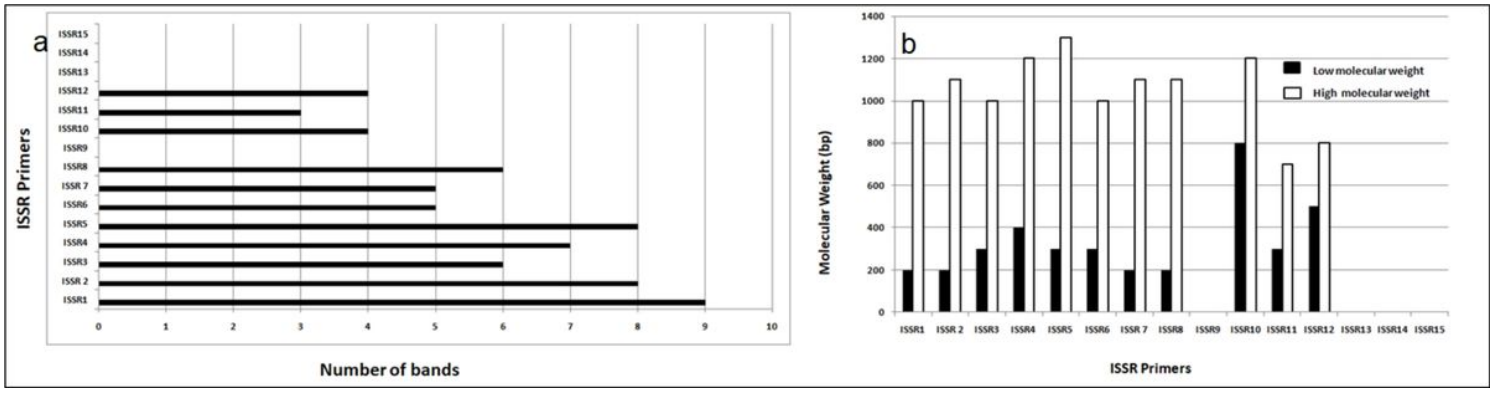
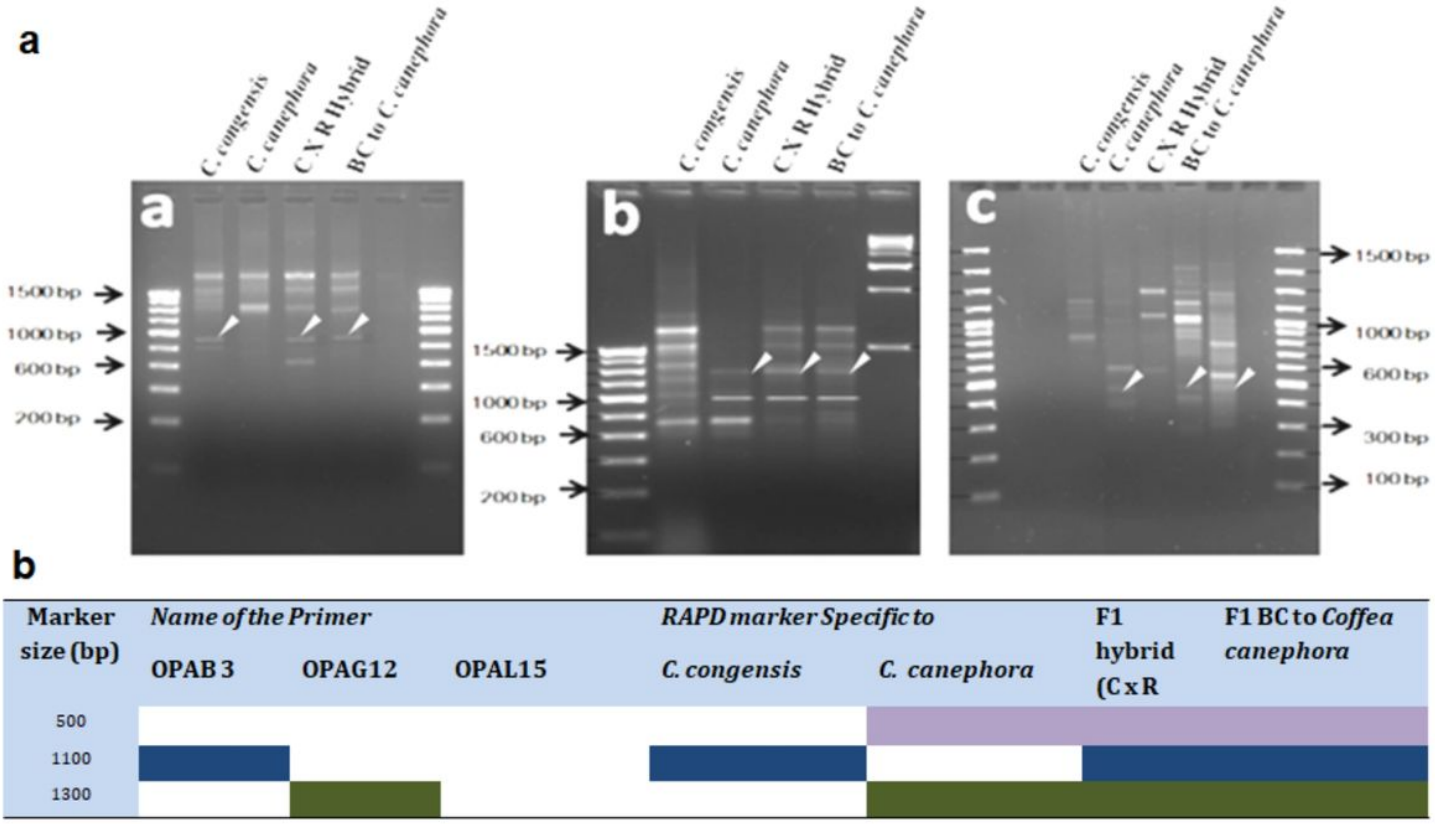


Figure 3

Screening of ISSR primers: Fifteen ISSR Primers showing the number of amplicons (a) and low and high molecular weight amplicons from the pooled genomic DNA of *Coffea congensis* and *Coffea canephora*



b

Marker size (bp)	Name of the Primer			RAPD marker Specific to		F1 hybrid (C x R	F1 BC to *Coffea canephora*
	OPAB 3	OPAG12	OPAL15	*C. congensis*	*C. canephora*		
500							
1100							
1300							

Figure 4

Genetic fingerprints of female parent (*Coffea congensis*) and male parent (*Coffea canephora*), F1 hybrid and backcross progenies of C x R hybrids developed using three primers OPAB3 (a), OPAG 12 (b) and OPAL15 (c)

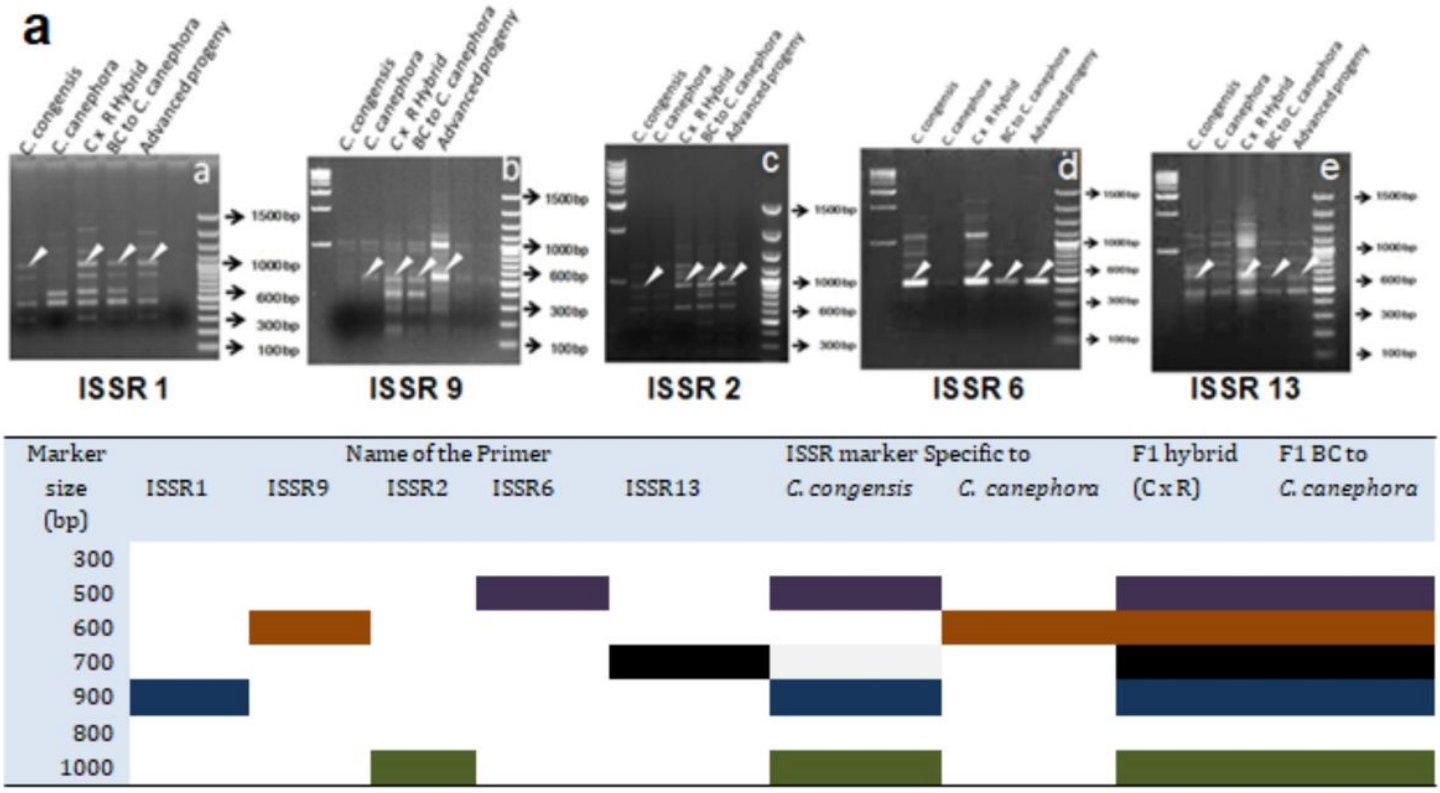


Figure 5

Genetic fingerprints of female parent (*Coffea congensis*) and male parent (*Coffea canephora*), F1 hybrid and backcross progenies of C x R hybrids developed using five ISSR primers ISSR1 (a), ISSR9 (b), ISSR2 (c), ISSR6 (d) and ISSR13 (e)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx
- Table4.docx
- Table5.doc