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GENETIC DIVERSITY AMONG ETHIOPIAN COFFEE (*COFFEA ARABICA* L.) COLLECTIONS AVAILABLE IN INDIAN GENE BANK USING SEQUENCE RELATED AMPLIFIED POLYMORPHISM MARKERS

ABSTRACT

The South-Western highlands of Ethiopia are considered to be the centre of origin and diversity of the arabica coffee, *Coffea arabica*. More than 80 accessions of arabica coffee collected from Ethiopia are available in Indian gene bank. However, the genetic diversity of these accessions is not studied in detail. In the present study, genetic diversity analysis of 48 accessions collected from eight provinces of Ethiopia was carried out using Sequence-related amplified Polymorphism (SRAP) marker. Among the thirty two SRAP primer combinations tested, 14 primer pairs were polymorphic and generated 203 distinct fragments. The number of fragments ranged from 7 to 21 with a mean of 14.5 fragments per primer combination. Of the total 203 amplified fragments, 182 (89.65%) were polymorphic and the percent of polymorphism ranged from 53.84% to a maximum of 100% using different primers. The average resolving power (Rp) and average polymorphism information content (PIC) of the 14 SRAP primer combinations was 14.31 and 0.648 respectively. A total of 13 rare alleles were obtained from SRAP assays, of which six rare alleles were obtained from the accessions collected from Shoa province.

The UPGMA clustering algorithm from SRAP analysis grouped the 48 coffee accessions into two major clusters. The accessions collected from particular province clustered together which could be attributed to the substantial gene flow between adjacent population and the influence of geographical origin on genetic diversity. The study demonstrated the existence of substantial genetic variation in Ethiopian germplasm which could be utilized in coffee germplasm conservation and improvement program.

Key words: *Coffea arabica* L., Ethiopian germplasm, Fingerprinting, Genetic diversity, SRAP marker.

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INTRODUCTION

The genus *Coffea* belongs to the family Rubiaceae and contains more than 100 species (Davis *et al.*, 2006) of which only two, *Coffea arabica* (known as arabica coffee) and *C. canephora* (known as robusta coffee) are commercially cultivated. Arabica contributes about 65% of total world coffee production and preferred for its superior beverage quality compared to robusta coffee. However, arabica coffee is susceptible to a number of pests and diseases. During the last 90 years, several elite arabica cultivars with improved agronomic traits have been released for commercial cultivation using conventional breeding techniques (Mishra and Slater, 2012). However, development of arabica cultivars with durable resistance for majority of pests and diseases remain as a challenging task. Conventional arabica coffee breeding usually involves intermating of superior individual plants in a population. However, selections of genetically diverse parental lines based on morphological, agronomic and quality traits are often difficult because of the high degree of genetic uniformity of commercial arabica cultivars. This is because of the narrow genetic origin of commercially cultivated varieties as well as the self fertile nature of the species (Van der Vossen 1985; Lashermes *et al.*, 1996).

The generations of new and improved arabica cultivars can be facilitated by incorporating new sources of genetic variation from diverse germplasm sources. South Western highlands of Ethiopia is considered to be the place of origin of *C. arabica* and several land races of this species are known from this region (Meyer, 1965). Several Ethiopian Arabica collections were characterized by employing different molecular markers by various authors (Lashermes *et al.*, 1996; Anthony *et al.*, 2001; Aga *et al.*, 2003; Aga *et al.*, 2005; Tornincasa *et al.*, 2007). In India, about 400 arabica accessions have been collected from Ethiopia during 1960s under FAO collection. These arabica accessions constituted the core germplasm and continued to be used in coffee breeding programs. However, the genetic variability among these Ethiopian arabica accessions preserved in Indian coffee gene bank has not been studied using molecular markers. Among various molecular markers currently employed, Sequence related amplified polymorphism (SRAP) is a new class of molecular markers developed by Li and Quiros during 2001. SRAP markers are PCR-based markers Primers that are used to amplify open-reading frames(ORFs). These primers consist of a 14 bp-core sequence in which the first 10 bp from 5'end are a filler sequences which is followed by CCGG in the forward-primer and AATT in the reverse primer. In both primers, three selective nucleotides exist at the 3'-end. (Li and Quiros 2001). 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 a PCR based marker system that preferentially and randomly targets coding sequences dis-

tributed throughout the genome. Zaefizadeh and Golieb, (2009) reported that SRAP markers possess multiloci and multi-allelic features, which make them potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting of genotypes. Recently, SRAP markers were also used for identification of cultivars and species in a number of crop plant species including coffee (Ferriol *et al.* 2003; Esposito *et al.*, 2007; Hao *et al.*, 2008; Merotto *et al.*, 2009; Mishra *et al.*, 2011., Mishra *et al.*, 2012). SRAP is highly reproducible and comparatively less expensive than other types of markers (Cravero *et al.*, 2007). In the present study, we have undertaken a detailed analysis of the genetic variability among Ethiopian arabica coffee germplasm by using SRAP markers.

MATERIALS AND METHODS

Plant Materials

Fresh young leaves were collected from 10 individuals of 48 Ethiopian arabica germplasm accessions (Table.1) planted in coffee gene bank at Central Coffee Research Institute, Chikmagalur, Karnataka, India and used for DNA isolation.

Table 1

List of 48 *Ethiopian arabica* coffee germplasm with place of collection and year of introduction to gene bank

No	Code	Collection area / source	Year of introduction
1	AB-S.2438	INERA* (Abyssinia)	1963
2	AB-S.2440	INERA (Abyssinia)	1963
3	AB-S.2443	INERA (Abyssinia)	1963
4	AB-S. 2445	INERA (Abyssinia)	1963
5	AB-S.2447	INERA (Abyssinia)	1963
6	AB-S. 2450	INERA (Abyssinia)	1963
7	AB-S.2454	INERA (Abyssinia)	1963
8	AB-S.2457	INERA (Abyssinia)	1963
9	AB-S.2459	INERA (Abyssinia)	1963
10	AB-S.2461	INERA (Abyssinia)	1963
11	HA-S.2600	Kombolcha (Harar)	1964
12	HA-S.2601	Dire Dawa (Harar)	1964

Table 1

Continued

No	Code	Collection area / source	Year of introduction
13	SH-S.2602	Bishoftu (Shoa)	1964
14	SH- S. 2605	Sheshamanne (Shoa)	1964
15	SH-S.2612	Wolkite (Shoa)	1964
16	SH-S.2613	Wolkite (Shoa)	1964
17	SH-S.2615	Wolkite (Shoa)	1964
18	SI-S. 2604	Onega (Sidamo)	1964
19	SI-S. 2606	Rift valley (Sidamo)	1964
20	SI-S. 2607	Konga (Sidamo)	1964
21	SI-S. 2608	Yirga Chefe (Sidamo)	1964
22	SI-S. 2609	Rift valley (Sidamo)	1964
23	I-S. 2647	Tepi (Illubabor)	1964
24	I-S. 2649	Arira (Illubabor)	1964
25	I- S. 2650	Daremmo (Illubabor)	1964
26	I -S. 2652	Teppi (Illubabor)	1964
27	I- S. 2653	Teppi (Illubabor)	1964
28	I- S. 2675	Gore (Illubabor)	1965
29	K-S. 2614	Sapa forest (Kaffa)	1964
30	K-S. 2616	Sapa forest (Kaffa)	1964
31	K-S. 2620	Doyo, (Kaffa)	1964
32	K- S. 2624	Chera (Kaffa)	1964
33	K -S. 2625	Afillo (Kaffa)	1964
34	K- S. 2634	Agaro (Kaffa)	1964
35	K- S. 2642	Bonga (Kaffa)	1964
36	K -S. 2656	Ainaamba burial place (kaffa)	1965
37	K- S. 2672	2 km from Ainaamba burial place (kaffa)	1965

Table 1

Continued

No	Code	Collection area / source	Year of introduction
38	K- S. 2680	Tui (kaffa)	1965
39	K-S. 2629	Limu (Kaffa)	1964
40	K-S.2644	Bonga (Kaffa)	1964
41	K-S.2657	Mizan (Kaffa)	1965
42	Agaro-1	Agaro (Kaffa)	1964
43	Agaro-2	Agaro (Kaffa)	1964
44	Agaro-3	Agaro (Kaffa)	1964
45	Agaro-4	Agaro (Kaffa)	1964
46	E-S.2708	Faghena Experiment station, Eritria	1965
47	G-S. 2707	Zeghie (Gojam)	1965
48	G-S.2709	Bahir dar (Gojam)	1965

* Institut National pour l'Etude et la Recherche Agronomique (Presently in Congo)

DNA extraction

Genomic DNA was extracted using a CTAB method of Murray and Thomson (1980) with slight modifications as described earlier (Mishra *et al.*, 2011). The quality and concentration of DNA were determined by UV/visible spectro photometer at 260 nm and by electrophoresis using 0.8% agarose gel stained with ethidium bromide. The resuspended DNA was then diluted in sterile distilled water to 10 ng/ µl concentration for use in amplification reactions.

SRAP analysis

A total of 32 SRAP primer pairs involving seven forward and nine reverse primers were initially screened of which 14 primer pairs were found to be polymorphic and therefore used in further analysis. SRAP analysis was performed by adapting the procedure described by Li and Quiros (2001) with minor modifications as described earlier (Mishra *et al.*, 2011). Each 20 µl PCR reaction mixture consisted of 30 ng template DNA, 2 µL of 10× reaction buffer (75mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2$ SO_4, 0.01% Tween 20), 200 µM dNTP mixture, 2.5 mM $MgCl_2$, 3 µM each of forward and reverse primer, and 1.0 U *Taq* DNA polymerase. The PCR amplification program was 4 min initial denaturation at 96ºC; 5 cycles consisting of 1 min denaturation at 94ºC, 1.15 min primer annealing at 35ºC; and 2 min extension at 72ºC followed by 30 cycles consist-

ing of 1 min denaturation at 94ºC, 1.15 min primer annealing at 50ºC; and 2 min extension at 72ºC; and a final extension of 15 min at 72ºC.

PCR products of SRAP were run on 2.0% (w/w) agarose gels containing 0.5 µg ethidium bromide/ml in 1X TAE buffer and then visualized and photographed using the UV-transilluminator (SYNGENE) and documented using the Gene Snap software program.

Data analysis

SRAP amplified bands were scored for presence (1) or absence (0). The total number of bands, distribution of bands across all the accessions, polymorphic bands, and average number bands per primer were calculated. The value of each primer was assessed using two indices; PIC, which is the same as the diversity index (Botstein *et al.*, 1980; Milbourne *et al.*, 1997) and Resolving power (Rp) (Prevost and Wilkinson, 1999). PIC or DI was estimated as PIC= Σ $(1-p^2_i)/n$, where n is the number of band positions analyzed in all the cultivars, p_i is the frequency of the ith banding pattern. The resolving power of a primer is Rp = Σ I_b where I_b (band informative ness) takes the value of 1- [2x (0.5-p)] and p is the ratio of forty eight collections sharing the band. A pair wise similarity matrix was constructed using the Jaccard similarity coefficient (Sneath and Sokal, 1973). The relationship between various germplasm collections was displayed as a dendrogram constructed using NTSYS –PC 2.1 software (Rohlf, 1995) based on unweighted pair group method using arithmetic averages (UPGMA). Statistical support of the clusters was assessed by means of 1000-bootstrap replicates.

RESULTS

In the preliminary assays, fourteen of the 32 SRAP primer combinations (Table 2) produced clear and unambiguous amplification pattern and therefore, these primer pairs were subsequently used to assess the polymorphism among 48 Ethiopian germplasm accessions. A total of 203 fragments were amplified from the 48 accessions using 14 SRAP primer combinations. The number of fragments detected by individual primer combination was specific and ranged from 7 (ME3+ EM7) to 21 (ME1+ EM12, ME2+EM4 and ME3+EM12) with an average of 14.5 fragments per primer combinations (Table 2 and 3). The size of the amplified fragments ranged from 80bp to 3000 bp. All primer combinations detected polymorphism in the whole 48 accessions. Of the total 203 amplified fragments, 182 (89.65%) were polymorphic, with a mean of 13 polymorphic fragments per primer combination. Percent of polymorphism ranged from 53.84% (Me3-Em3) to a maximum of 100%, with a mean of 87.38%. Of the 14 SRAP primer combinations used, five showed 100% polymorphism (Table 3).

Table 2

Sequences of SRAP forward and reverse primer and primer combinations used in hybrid analysis

Forward primer		Reverse primer		Polymorphic Primer combinations	
Name	Sequence	Name	Sequence	Forward	Reverse
Me1	TGAGTCCAAACCGGATA	Em3	GACTGCGTACGAATTGAC	Me1	EM6/EM7/EM12
Me2	TGAGTCCAAACCGGAGC	Em4	GACTGCGTACGAATTTGA	Me2	Em3/Em4/Em6/Em12
Me3	TGAGTCCAAACCGGAAT	Em5	GACTGCGTACGAATTAAC	Me3	Em3/Em4/Em7/ Em11/Em12
Me4	TGAGTCCAAACCGGACC	Em6	GACTGCGTACGAATTGCA	Me6	Em5
Me6	TGAGTCCAAACCGGACA	Em8	GACTGCGTACGAATTCAC	Me10	Em13
Me8	TGAGTCCAAACCGGACT	Em9	GACTGCGTACGAATTCAG		
Me9	TGAGTCCAAACCGGAGG	Em10	GACTGCGTACGAATTCAT		
Me10	TGAGTCCAAACCGGAAA	Em11	GACTGCGTACGAATTCTA		
		Em12	GACTGCGTACGAATTCTC		
		Em13	GACTGCGTACGAATTCTG		

Table 3

Polymorphism obtained by SRAP markers in Ethiopian germplasm

Sl no	Primer	Total bands	Size range (bp)	No of Polymor-phic bands	Percentage of Polymorphism	RP	PIC
1	Me1-Em12	21	80-2400	20	95.24	20.91	0.702
2	Me2-Em12	20	180-2900	19	95.0	20.33	0.687
3	Me3-Em12	21	100-2000	20	95.24	13.87	0.840
4	Me3-Em11	10	110-475	9	90.0	8.50	0.742
5	Me6-Em5	15	110-475	15	100	11.70	0.797
6	Me10-Em13	12	80-490	12	100	7.62	0.848
7	Me1-Em6	11	390-2200	11	100	11.70	0.688
8	Me1-Em7	13	400-2000	13	100	10.20	0.831
9	Me2-Em3	19	150-2000	17	89.47	20.25	0.632
10	Me2-Em4	21	190-2900	21	100	18.50	0.725
11	Me2-Em6	9	250-3000	5	55.55	12.45	0.403
12	Me3-Em3	13	400-3000	7	53.85	20.50	0.320
13	Me3-Em4	11	90-1500	9	81.81	12.87	0.571
14	Me3-Em7	7	210-2000	4	57.14	11.0	0.290
	Total	203		182	1255.1	200.34	9.07
	Average				89.65	14.31	0.648

The resolving power (RP) of the 14 SRAP primer combinations ranged from 7.62 (Me10-Em13) to 20.91 (Me1-Em12), with a mean of 14.31. Similarly, the average polymorphism information content (PIC) or the genetic diversity of 14 SRAP primer combinations ranged from 0.290 (Me3-Em7) to 0.848 (Me10-Em13), with a mean of 0.648. Among the 14 SRAP primer pairs, nine primer combinations (78.57%) produced PIC values higher than 0.50 or more.

Genetic Relationship among the Germplasm

The average similarity coefficients among various Ethiopian arabica genotypes varied considerably and ranged from 0.29 to 0.99, with an average of 0.75 (data not shown). The lowest similarity (0.29) was between SH - S. 2612 (genotype from Shoa province) and KA-S. 2616 (genotype from Kaffa province) whereas, the highest similarity (0.99) was obtained between Ha - S. 2600 and HA - S. 2601 (both from Harar Province).

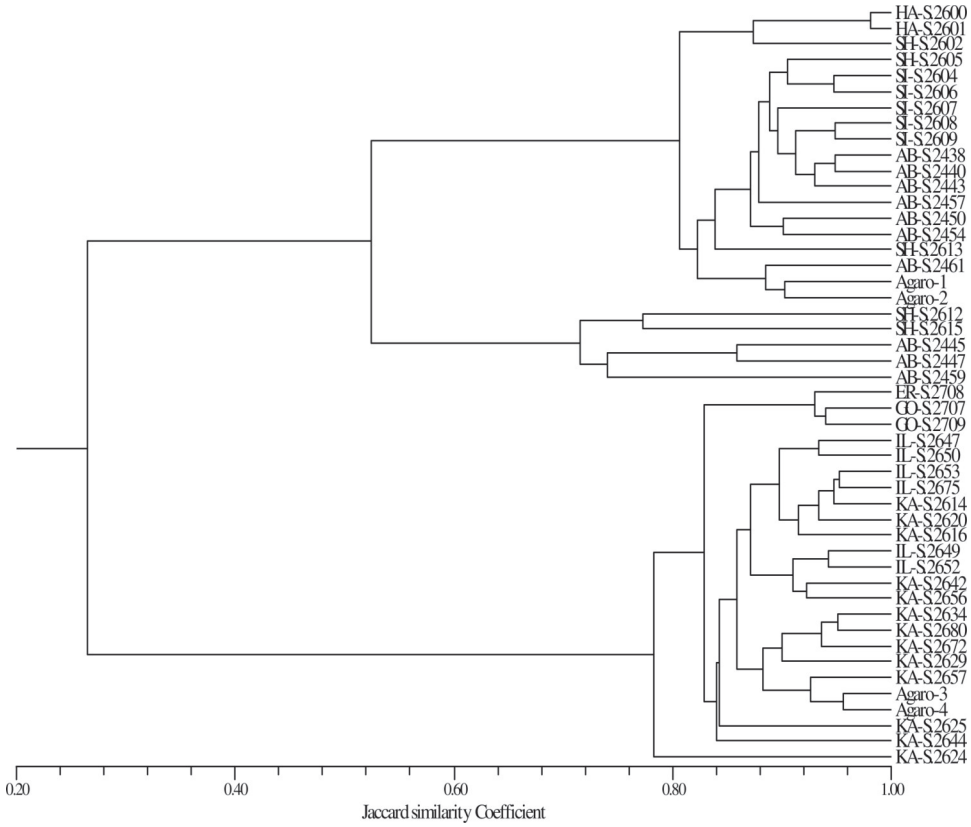

Fig. 1 Similarity coefficients among various Ethiopian arabica genotypes

The UPGMA clustering algorithm from SRAP analysis grouped the 48 coffee genotypes into two major clusters (Fig. 1). Among the two major clusters, the

first one comprised of two minor clusters. The first minor cluster included a total of 19 coffee genotypes collected from Harar (2), Shoa (3), Sidamo (5), Abyssinia (7) and Agaro (2) provinces. The second minor cluster included a total of 5 coffee genotypes collected from Shoa (2) and Abyssinia (3) provinces. The second major cluster also divided into two minor clusters. The first one represented by a single genotype from kaffa province whereas the second minor cluster comprised of a total of 23 genotypes from Eritria (1), Gojjam (2), Illubabor (6), Kaffa (12) and Agaro (2) provinces

The SRAP marker analysis, detected a total of 13 rare alleles, with a frequency of less than 5%, in the Ethiopian arabica germplasm. Maximum rare alleles were observed in the genotypes collected from Shoa (6) followed by the Kaffa (5) province.

DISCUSSION

Assessment of genetic diversity is an important component of plant breeding programs. Genetic assessment of germplasm helps in identifying parents with different agronomic traits for effective recombination in hybridization program. In the present study, genetic diversity was assessed in 48 Ethiopian arabica germplasm collections using SRAP markers. The SRAP assay has detected higher percentage of polymorphism among the Ethiopian germplasm collections indicating rich genetic diversity. In an earlier study, Mishra *et al.* (2012) analyzed the genetic diversity in 24 commercially grown Indian coffee cultivars using 43 SRAP primer combinations and obtained a mean of 9.23 fragments and mean of 6.77 polymorphic fragments per primer combination which is much lower compared to the Ethiopian germplasm collection analyzed in the present study. Ethiopia is considered to be the centre of origin of wild Arabica coffee and the the higher genetic diversity obtained clearly lends support to the linkage between centre of origin and high genetic diversity.

The PIC values, which were used as a reflection of allelelic diversity and frequency among the genotypes, varied from one locus to another. In fact, the PIC and RP values are estimators of usefulness of any marker system for cultivar distinction. Based on the polymorphism value, PIC was classified in to three different types high (PIC value higher than 0.5), medium (value between 0.25 and 0.5) or low (lower than 0.5) (Vaiman *et al.*, 1994; Xie *et al.*, 2010). In this study, 11 of the 14 SRAP primers have high PIC value (> 0.5) and 3 have medium (< 0.5 PIC > 0.25) value indicating that SRAP markers could be used to develop high loci polymorphism database in arabica coffee. Interestingly, the mean PIC and RP values of SRAP primer obtained the present study were higher compared to the earlier study involving commercial arabica cultivars (Mishra *et al.*, 2012). Baruah *et al.* (2003) reported very low PIC (0.27) values among different arabica genotypes using mostly dinucleotide repeat microsatellite markers and suggested that the narrow genetic base in arabica was responsi-

ble for the low level of polymorphism. Moncada and McCouch (2004) also observed low PIC value (0.30) in arabica cultivars using SSR markers. Mishra *et al.* (2012) obtained the mean PIC values (0.346) in Indian commercial coffee cultivars using polymorphic SRAP markers. The present mean PIC value of 0.648 is much higher than the previously reported values for arabica coffee using any other marker system. Thus the study not only demonstrated the efficiency of SRAP markers over other marker systems in detecting the polymorphism but also clearly demonstrated the existence of high genetic diversity among the Ethiopian arabica germplasm accessions. The existence of substantial genetic diversity among the wild arabica accessions has practical implications especially for exploitation of genetically diverse lines in breeding programs especially in India. In a previous study, Anthony *et al.* (2001) demonstrated high genetic diversity among wild arabica collections from South Western Ethiopia using RAPD markers. Tornincasa *et al.* (2006) evaluated genetic diversity among commercial arabica coffee cultivars from America, India and Ethiopian using SSR markers and demonstrated the existence of wide genetic diversity among Ethiopian population compared to the Indian and American commercial coffee cultivars and the present study lends support to their contention using SRAP marker system.

The average genetic similarity values obtained between different Ethiopian arabica accessions was comparatively low using SRAP markers. In a previous study, Steiger *et al.* (2002) obtained a genetic similarity value of 0.9 or more in 86% of pair-wise comparisons among 58 arabica cultivars using AFLP markers. Recently, Dessalegn *et al.* (2008) analyzed the Ethiopian arabica coffee genotypes using AFLP markers and obtained genetic similarity value of 0.851 to 0.982 with an average of 0.915. In the present study, 31.46 % of pair-wise comparisons displayed 0.9 or more similarity value with an average of 0.75 which clearly demonstrated the efficiency of SRAP marker system in detecting polymorphic loci even among the closely related Ethiopian arabica accessions. The differences in genetic similarities obtained by using different markers could be explained by the fact that while the SRAP marker system preferentially detect polymorphism in coding sequences, which are usually conserved among closely related cultivars with low mutation rate, the AFLP markers mostly detect the non-coding sequences known to cluster in low-recombination regions (Vuylsteke *et al.*1999).

The dendrogram generated through UPGMA revealed that accessions collected from same geographical origins are not clustered together tightly together indicating considerable differences among the accessions collected from different provinces as well as among the genotypes collected from same province viz. Kaffa, Agaro and Abyssinia using SRAP markers. Based on AFLP analysis of arabica coffee genotypes from Ethiopia, Dessalegn *et al.* (2008) reported that all the genotypes were not clustered together according to the collection regions and the preset study supports their contention.Among the Ethipian accessions,

maximum rare alleles were obtained in the accessions of Shoa and Kaffa provinces. In fact, both these provinces are adjacent to each other and particularly the Kaffa province which is considered to be the centre of origin and diversity of arabica coffee. The presence of rare alleles in accessions collected from these two provinces reflected their rich genetic diversity and offers great promise for coffee conservation and improvement programs.

CONCLUSION

This is the first study of the genetic diversity analysis of Ethiopian arabica germplasm using SRAP markers which normally targets the functional region of coffee genome.

The study has clearly demonstrated the usefulness of SRAP approach in determining the genetic variability among the arabica germplasm.

Identification of genetic variability among arabica coffee germplasm is critical to the conservation strategies as well as useful for designing appropriate breeding strategies for its genetic improvement.

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