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MOLECULAR MARKER-BASED CHARACTERIZATION OF ECUADORIAN DRY FOREST TAMARIND PLUS TREES

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ABSTRACT

To improve the potential of tamarind as an economically valued domesticated species it is important to characterize its variability in Ecuador for breeding purposes. Our aim was to investigate the genetic diversity of 32 tamarind plus trees using inter-simple sequence repeat (ISSR) markers. Eighty four loci were examined using 12 markers, with a mean number of 4.42 loci per primer; 8 loci (9.52 %) were monomorphic and 76 (90.48 %) polymorphic, revealing genetic variability among the individuals. Polymorphic information content (PIC) values varied from 0.29 (ISSR_808) to 0.93 (ISSR_HB12), whereas the marker index ranged from to 26.4 (ISSR_814) to 62.5 (ISSR_17899A). Primers ISSR_HB11, ISSR_836, ISSR_842, ISSR_848, ISSR_860, ISSR_17899A and ISSR_17899B were useful to discriminate the grouping of the accessions according to their PIC values. Ward cluster analysis grouped accessions into two major groups with five subgroups with 46 % similarity according to Jaccard distance. The genotypes from Loja, Manabí and Guayas provinces were grouped in the first cluster; while only individuals from Manabí located in the other group, indicating major diversity in the latter province. Genotypes T1-ECUM-001 and T1-ECUM-002 presented 76 % similarity, while T1-ECUM-008, T1-ECUM-010, T1-ECUM-012, T1-ECUM-017 and T1-ECUM-018 shared 60 %. All materials from Loja grouped with 65 % similarity. Other genotypes clustered with similarity of 54 %. The cophenetic correlation coefficient (0.634) showed a good fit between the data matrix and the dendrogram results. A reasonable degree of diversity was found among tamarind genotypes potentially useful to select plus trees for clonal propagation as well as to identify diverse parents for hybridization programs.
Additional key words: Fruit tree breeding, ISSR, molecular markers, *Tamarindus indica*

RESUMEN

Caracterización molecular de árboles élite de tamarindo del bosque seco ecuatoriano

Para aumentar el potencial del tamarindo como especie domesticada con valor económico es importante caracterizar la variabilidad en Ecuador con propósitos de mejoramiento. Nuestro objetivo fue investigar la diversidad genética de 32 árboles élite de tamarindo utilizando marcadores de secuencia inter-simple repetida (ISSR). Se examinaron 84 loci con 12 marcadores, con un número promedio de 4,42 loci per primer; 8 loci (9,52 %) fueron monomórficos y 76 (90,48 %) polimórficos, revelando variabilidad genética entre individuos. El contenido de información polimórfica (PIC) osciló entre 0,29 (ISSR_808) y 0,93 (ISSR_HB12), mientras que el índice de marcador fluctuó entre 26,4 (ISSR_814) y 62,5 (ISSR_17899A). ISSR_HB11, ISSR_836, ISSR_842, ISSR_848, ISSR_860, ISSR_17899A e ISSR_17899B fueron útiles para discriminar accesiones según sus CIPs. El análisis de conglomerados de Ward formó dos grupos principales y cinco subgrupos con 46 % de similitud según la distancia de Jaccard. Genotipos de Loja, Manabí y Guayas se aglomeraron en un grupo; mientras que sólo accesiones de Manabí quedaron en el otro, indicando mayor diversidad en la última provincia. Los genotipos T1-ECUM-001 y T1-ECUM-002 presentaron 76 % similitud, mientras T1-ECUM-008, T1-ECUM-010, T1-ECUM-012, T1-ECUM-017 y T1-ECUM-018 compartieron 60 %. Todos los materiales de Loja se agruparon con 65 % de similitud. Otros genotipos se concentraron con similitud de 54 %. El coeficiente de correlación cofenética (0,634) mostró buen ajuste entre la matriz de datos y los resultados del dendrograma. Se encontró un grado razonable de diversidad entre los genotipos de tamarindo potencialmente útil para seleccionar árboles élite para propagación clonal así como para identificar progenitores diversos para programas de hibridación.
Palabras clave adicionales: ISSR, marcadores moleculares, mejoramiento de frutales, *Tamarindus indica*

INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a dicotyledonous perennial tree with a wide geographical distribution in the subtropics and semi-arid tropics. It is native to areas throughout Africa and

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Southern Asia (Tapia et al., 2012), although the precise origin of this species is a subject of controversy (Diallo et al., 2008). It was introduced into America during the 16th century and now grows widely in tropical and subtropical areas. In Ecuador, this tree species is cultivated in areas where production systems include scattered trees, mainly for local consumption.

The tree has an important role in local economies, supplements the local diet, it is used in traditional and modern therapies by 80 % of the world's population in Africa, Asia and Latin America, and it is used as a laxative and purgative with minimal side effects (El-Siddig et al., 2006). Furthermore, it also exhibits antibacterial, antifungal, and antioxidant properties (Graf et al., 2016), and is used as a construction material, and for fuel and fodder (Tapia et al., 2012). Pharmaceutical companies have invested money and time in developing natural products extracted from this tree to generate remedies that are affordable (Doughari, 2006).

Despite its commercial importance worldwide, this multi-purpose tree has been little investigated (Algabal et al., 2011), although it was identified as one of the top ten agroforestry tree species to be prioritized for crop diversification programs and development in sub-Saharan Africa in efforts to enhance the conservation and utilization of genetic species (Gunasena & Hughes, 2000).

Tamarind has a relatively long generation time and reproduces primarily by outcrossing, so any conventional breeding approaches would require considerable investment in time and money. Although it is one of the oldest domesticated crops, little is known about its genetic characteristics and population biology. Available knowledge focuses on developing efficient *in situ* conservation and genetic improvement strategies (Fandohan et al., 2010). Two key elements for cultivar development are the identification of ''plus trees'' in natural populations and their propagation by vegetative techniques (Leakey & Page, 2006). Tamarind is mostly self-sown or sown with seeds of unknown parentage, which results in wide variation among seedling progenies (El-Siddig et al., 2006).

Characterization of tamarind trees has been mainly limited to descriptions of morphological and agronomic traits, which are known to be deeply affected by environmental factors. Identification of cultivar and estimation of genetic diversity using phenotypic markers have several limitations, especially in perennial crops (Purushotham et al., 2008). However, molecular diversity using DNA and protein-based molecular markers are more reliable and unaffected by environmental factors (Dhanraj et al., 2002). Establishment of core collections based on field evaluation and molecular variation shown by accessions could be obviously advantageous. However a clear and detailed assessment of molecular diversity in tamarind is not currently available (Gangaprasad et al., 2013), although there have been some efforts to characterize several tamarind populations with molecular techniques such as RAPD (Diallo et al., 2007; Gangaprasad et al., 2013; Kumar et al., 2015) and AFLP (Algabal et al., 2011).

In the present study, ISSR have been used for a deeper molecular analysis of genotypes because of the advantages of this technique over SSR and RAPD (Reddy et al., 2002). The ISSR markers are highly polymorphic and represent a simple, reproducible, efficient and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). ISSR markers have high reproducibility possibly due to the use of longer primers (16-25mers) as compared to RAPD primers (10 mers) (Reddy et al., 2002).

This research was conducted to estimate genetic diversity and to assess relationships among 32 accessions of tamarind using ISSR markers for the basis of a breeding program in Ecuador.

MATERIALS AND METHODS

Plant material. The experimental material (Table 1) comprised 32 tamarind accessions (geographically distinct) that were collected from January to December 2015 in three provinces of Ecuador (Guayas, Manabi and Loja), in dry forest areas with less than 500 mm rain per year, temperatures from 20 to 25 °C, high luminosity, and low nutrient soils.

Young and healthy leaves were harvested individually in the field, tagged, submerged in a solution of polyvinylpyrrolidon (PVP) 1 %, placed in paper envelopes and transported to the

Biotechnology Department, Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estacion Experimental Litoral Sur, Guayas Province, for DNA extraction.

Table 1. Codes and collecting site of the tamarind plus accessions analyzed in this research

Accession code	Owner´s name	Location			Coordinates		Elevation (masl)[1]
		Site	County	Province	Longitude	Latitude	
TI-ECUM-001	Sócrates Quimis	Joa	Jipijapa	Manabí	26°36´2.0"	01°05´49.1"	
TI-ECUM-002					26°36´2.0"	01°05´48.7"	79.25
TI-ECUM-003					26°36´1.6"	01°05´48.3"	
TI-ECUM-004	Marco Zambrano	Cantagallo		Manabí	80°48´27.3"	01°17´01.2"	105.77
TI-ECUM-005					80°43´27.8"	01°17´00.7"	
TI-ECUM-006	Viterbo Navarrete Macías	El Cady		Manabí	80°24´19.6"	01°07´04.5"	62.79
TI-ECUM-007	Fradil Parraga Macías	Maconta	Portoviejo	Manabí	80°21´16.6"	01°02´17.8"	83.21
TI-ECUM-008					80°21´16.8"	01°02´19.4"	
TI-ECUM-009	Vicenta Ramírez Macías	Tabacales	Rocafuerte	Manabí	80°26´43.2"	00°56´32.2"	36.58
TI-ECUM-010	Calixto Ruiz	Valdez		Manabí	80°26´31.8"	00°56´52.6"	45.42
TI-ECUM-011	Bosco Giler Parraga	El Cardón		Manabí	80°23´62.5"	00°54´55.7"	44.20
TI-ECUM-012	Honorato Navia Navia	La Balsita		Manabí	80°23´38.5"	01°00´25.8"	44.81
TI-ECUM-013	José Roque Cevallos	La Horma		Manabí	80°23´44.9"	00°54´41.2"	78.33
TI-ECUM-014	José Zamora Arteaga	Las Flores		Manabí	80°21´22.4"	00°55´37.5"	104.85
TI-ECUM-015	Ulbio Muentes Zambrano	Zapatón		Manabí	80°28´22.9"	00°53´14.7"	21.03
TI-ECUM-016					80°28´22.5"	00°53´14.3"	24.99
TI-ECUM-017	Manuel Zambrano Figueroa	Cristo Rey	Sucre	Manabí	80°29´39.1"	00°49´05.4"	35.97
TI-ECUM-018	Geravides Lucas Herrera	El Blanco		Manabí	80°29´45.9"	00°49´02.1"	26.52
TI-ECUM-019	Eduardo Castro Choez	Costa Rica	Portoviejo	Manabí	80°27´43.0"	00°59´54.8"	28.35
TI-ECUM-020	Verónica Pinargote Vergara	El Retiro		Manabí	80°28´37.5"	00°59´31.6"	39.62
TI-ECUM-021	INIAP-E.E. Portoviejo	Lodana	Santa Ana	Manabí	80°23´16.8"	01°10´13.6"	73.15
TI-ECUM-022	Diocles Pico Barrezueta			Manabí	80°38´3.99"	01°19´96.6"	65.53
TI-ECUM-023					80°38´3.54"	01°19´96.2"	
TI-ECUM-024	Gloria Coloma Garofalo	Mate		Manabí	80°33´23.0"	01°22´87.5"	96.01
TI-ECUM-025	Marcela Ortega Zambrano	Los Tillales	24 de Mayo	Manabí	80°25´3.39"	01°15´0.86"	113.69
TI-ECUM-026	José Delgado Varela	El Guarango	Rocafuerte	Manabí	80°24´14.3"	00°53´4.39"	43.28
TI-ECUG-027	Ana Decimaviya	Valle de la Virgen	Pedro Carbo	Guayas	80°11´46.2"	01°44´33.9"	77.42
TI-ECUM-028	Domingo Moran Macías	Guale	Paján	Manabí	80°12´29.3"	01°40´50.4"	110.34
TI-ECUL-029	INIAP	Garza Real	Zapotillo	Loja	80°13´58.3"	04°18´24.3"	236
TI-ECUL-030	INIAP	Garza Real	Zapotillo	Loja	80°13´58.0"	04°18´24.0"	236
TI-ECUL-031	INIAP	Garza Real	Zapotillo	Loja	80°13´57.7"	04°17´58.5	233
TI-ECUL-032	INIAP	Garza Real	Zapotillo	Loja	80°13´17.1"	04°17´59.6"	232

[1]Meters above sea level

DNA extraction. DNA was extracted following the method reported by Khanuja et al. (1999) with modifications. Briefly, 120 mg fresh leaf tissue were ground in a mortar, transferred to a 2 mL microcentrifuge tube containing 1 mL extraction buffer (100 mM Tris, HCl pH 8.0; 1.5 M NaCl; 25 mM EDTA pH 8.0; 2.5 % CTAB; 1 % PVP; 0.2 % 2β-mercaptoetanol), mixing by inversion. The sample was incubated at 60 °C for 1.5 h and shaken. Following cell lysis, 1 mL chloroform:isoamyl alcohol (CIA) (24:1) was added mixing by inversion for 15 min and centrifuged at 5900 g for 10 min. The supernatant was transferred to a fresh 1.5 mL tube, adding 500 µL 5M NaCl and 0.6 volume cold isopropanol, mixed by inversion and placed at -20 °C for 1 h. Samples were centrifuged at 15616 g for 10 min, pellet washed with ethanol at 80 %, dried for 20 min and resuspended in 170 µL high salt TE (Tris HCl 10 mM, EDTA 1 mM, NaCl 1 M), followed by RNase treatment, adding 2 µL RNase A (10 $mg \cdot \mu L^{-1}$) and keeping at 37 °C for 30 min. After

that, 100 µL CIA was added, mixing well and then centrifuged at 15,000 g. Supernatant was transferred to a tube where 100 µL of cold ethanol at 100 % was added and centrifuged at 15616 g for 10 min. The pellet was washed with 80 % ethanol, dried for 20 min and dissolved in 50 µL of sterile double-distilled water.

DNA quantification and quality. DNA quantification was estimated in a Quantus Fluorometer (Promega) using the Quant-iT assay kit developed by ThermoFisher Scientific. The quality was determined by running a 1 % agarose gel.

PCR amplification. A total of 19 ISSR primers (Table 2) were selected to characterize the tamarind accessions.

Table 2. ISSR primers used for the comparative study of genetic variability of 32 plus trees of *Tamarindus indica* L.

PRIMER	Oligonucleotide sequence 5'to 3`	Range (bp)	Tm (°C)	TBN	PBN	MBN	POL	PIC	MI	SE
HB12	CACCACCACGC	750-1400	48.9	3	1	2	33.33	0.93	30.9	8.1
HB11 *	GTGTGTGTGTGTCC	500-2800	44.3	8	4	4	50.00	0.86	42.9	6.3
17899A *	CACACACACACAAG	650-1600	52.9	4	4	0	100.00	0.63	62.5	11.8
815	CTC TCT CTC TCT CTC TG	1000-1600	50.3	2	2	0	100.00	0.59	58.6	11.3
842 *	GAG AGA GAG AGA GAG AYG	400-1200	47.6	4	4	0	100.00	0.59	58.6	11.3
17899B *	CACACACACACAGG	600-2200	50.3	6	6	0	100.00	0.58	58.3	9.6
860 *	TGT GTG TGT GTG TGT GRA	700-2800	44.3	8	7	1	87.50	0.56	48.9	8.5
17898A	CACACACACACAAC	400-1650	50.3	6	6	0	100.00	0.55	54.7	19.1
836 *	AGA GAG AGA GAG AGA GYA	500-1400	44.3	3	3	0	100.00	0.53	53.1	18.2
848 *	CAC ACA CAC ACA CAC ARG	160-2000	51.7	3	3	0	100.00	0.51	51.0	21.3
812	GAG AGA GAG AGA GAG AA	800-1750	44.3	4	4	0	100.00	0.43	43.0	21.1
844[a] *	CTCTCTCTCTCTCTCTAC	1500-4000	45.8	4	4	0	100.00	0.42	42.2	15.4
HB9	GTGTGTGTGTGTGG	1150-1500	47.6	2	2	0	100.00	0.41	40.6	27.6
873	GAC AGA CAG ACA GAC A	650-850	52.9	2	2	0	100.00	0.36	35.9	27.3
814 *	CTC TCT CTC TCT CTC TA	350-1750	44.6	4	3	1	75.00	0.35	26.4	10.0
835 *	AGA GAG AGA GAG AGA GYC	300-1500	52.9	4	4	0	100.00	0.34	33.6	12.9
807 *	AGA GAG AGA GAG AGA GT	800-2800	44.6	6	6	0	100.00	0.33	32.8	5.9
844B	CTCTCTCTCTCTCTCTGC	1200-2000	51.7	3	3	0	100.00	0.32	32.3	17.3
808 *	AGA GAG AGA GAG AGA GC	600-2500	48.9	8	8	0	100.00	0.29	28.9	8.5
Total		350 -4000		84	76	8				
Mean				4.42				0.50	44	

Tm = annealing temperature; TBN = Total band number; PBN = Polymorphic band number; MBN = Monomorphic band number; POL = Polymorphism percentage; PIC = Polymorphic information content; MI = Marker Index; SE = Standard Error; IUPAC 1-letter code abbreviations for mixed oligo bases: R= A + G; Y = C + T.
Primers with asterisks were used for statistical analysis.

Polymerase chain reactions were carried out in a 20 µL volume, in a tube containing 2 µL 10X buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl); 50 mM $MgCl_2$ 1 µL; 10 mM dNTPs 0.8 µL; 0.4 µL 0.2 µM ISSR primer; 0.12 µL Taq polymerase 5U· μL^{-1} (Invitrogen), and 3.2 µL of template DNA (5ng·μL^{-1}). PCR amplifications were performed using a thermocycler (Eppendorf Master Cycler 230 AG model). The amplification profile was kept for initial denaturing at 94° C for 5 min followed by 40 cycles of denaturation at 94 °C for 30 s; primer annealing at recommended temperature for 1 min; extension at 72 °C for 2 min; and a final extension at 72 °C for 7 min.

The amplification products were mixed with 2.5 µL of 10X loading dye (0.25 %) bromophenol blue. PCR products were resolved by electrophoresis on 1.5 % (w/v) agarose gels using 1X TAE buffer (40 mM Tris–acetate, pH 8, 1 M

EDTA), at 100V for 85 min, followed by staining with 15 ppm ethidium bromide and photographed. The molecular marker (1 kb, Invitrogen) was loaded in the last lane.

Scoring of bands and statistical analysis. Gel electrophoresis DNA profiles of tamarind accessions, amplified by each ISSR primer, were used to generate a band presence (1) and absence (0) matrix. Each marker was reviewed by assessing its banding pattern i.e. the number of yielded bands and presence or absence in the studied accessions. The number of polymorphic or monomorphic loci, their percentage, standard deviation and experimental error were calculated.

The polymorphic information content (PIC) was calculated according to Roldan-Ruiz et al. (2000) where PIC_i (polymorphic information content of marker '*i*') = $2fi\ (1-fi)$; *fi* is the frequency of the amplified allele (band present), and $1-fi$ is the frequency of the null allele. The PIC value ranges from zero for monomorphic markers to 0.5 for markers that are present in 50 % of the plants and absent in the other 50 %. This content provides an estimate of the discriminatory power or whether a locus or loci is informative, taking into account not only the expressed number of alleles but their relative frequencies.

The value of each marker represents the probability of finding this marker in one of two different states (present or absent) in two plants drawn at random from the population. Marker index (MI), calculated as the product of the polymorphism percentage and the PIC, is used to estimate the overall utility of each marker system and was calculated according to Sorkheh et al. (2007).

The Jaccard genetic similarity coefficient was calculated for the data matrix using InfoStat version 2011 (Universidad Nacional de Córdoba, Argentina). The generated data was used to estimate genetic similarity for pairwise accessions based on Jaccard similarity coefficient. A similarity matrix was constructed and subjected to cluster analysis following Ward's method (Ward, 1963) to develop the dendrogram. To estimate congruency between the dendrogram and the data, a cophenetic correlation coefficient was calculated.

RESULTS

The banding pattern of each marker was examined visually. It was considered that primers ISSR_873, ISSR_815 and ISSR_17898A gave little information; ISSR_812, ISSR_HB12, ISSR_844B and ISSR_HB9 were only just informative; but ISSR_HB11 showed to be an excellent primer due to its banding pattern across the population. The rest of the markers yielded intermediate information according to the evaluation criteria established for this research.

Only 12 of 19 primers (Table 2) were considered informative for the statistical analysis because of their consistency giving reproducible and good quality banding patterns; those were ISSR_807; ISSR_814; ISSR_836; ISSR_860; ISSR_HB11; ISSR_808; ISSR_844A; ISSR_835; ISSR_17899A; ISSR_17899B; ISSR_848; and ISSR_842.

This set of ISSR primers generated 84 loci, with a mean number of 4.42 loci per primer, ranging from 8 (ISSR_HB11, ISSR_808 and ISSR_860) to 1 (ISSR_HB12) (Table 2). Of the observed loci, 8 (9.52 %) were monomorphic and 76 (90.48 %) polymorphic, revealing high genetic variability between the individuals. PIC values varied from 0.29 (ISSR_808) to 0.93 (ISSR_HB12), with an average of 0.50, whereas MI ranged from 26.4 (ISSR_814) to 62.5 (ISSR_17899A). Primers ISSR_HB11, ISSR_836, ISSR_842, ISSR_848, ISSR_860, ISSR_17899A and ISSR_17899B were useful to discriminate the grouping of the accessions according to their PIC value above mean (0.50).

A typical polymorphic ISSR fingerprint, using the ISSR_808 marker, is shown in Figure 1. Genotype TI-ECUM-016 amplified only with a few primers, therefore it was not considered for further statistical analysis, and the study was maintained with 31 genotypes.

A moderate degree of genetic diversity was obtained with the Jaccard similarity coefficient. The plus trees formed five groups (Figure 2) with an average of 46 % similarity, although two subgroups clustered together and the other three subgroups shared another division. Genotypes T1-ECUM-001 and T1-ECUM-002 presented 76 % similarity, while T1-ECUM-008, T1-ECUM-010,

T1-ECUM-012, T1-ECUM-017 and T1-ECUM-018 shared 60 %. All materials selected at Loja Province grouped together with 65 % similarity. Genotypes T1-ECUM-015, T1-ECUM-019, T1-ECUM-020, T1-ECUM-021, T1-ECUM-022, T1-ECUM-023, T1-ECUM-024, T1-ECUM-025, T1-ECUM-026, T1-ECUG-027 and T1-ECUM-028, clustered in the largest group with an average degree of similarity of 54 %. Other genotypes grouped with a similarity of 65 %.

Using the Jaccard distance method, the cophenetic correlation coefficient (0.634) was obtained and showed a reasonable fit between the data matrix and the dendrogram results. No distortion was caused by the conglomerate method.

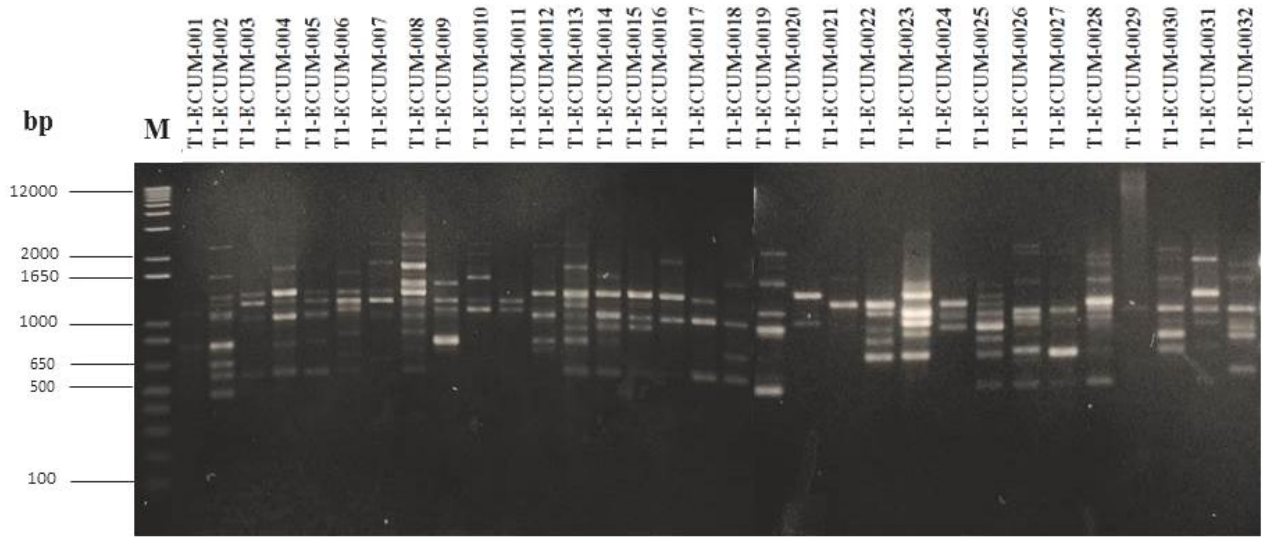

Figure 1. ISSR 808 marker showing typical polymorphic fingerprint amplification of 32 *Tamarindus indica* genotypes in an agarose 1.5 % gel. M =1kb Invitrogen ladder

DISCUSSION

In this research, the analyzed tamarind accessions showed intermediate genetic variability, with an average of 46 % similarity among selected individuals, and moderate level of polymorphism indicating that a wide and diverse genetic base existed among the tamarind plus trees genotypes of the three Provinces of Ecuador, which could be explained due to the cross pollinating nature of the species. We hypothesized that there is genetic flux among the studied tamarind populations because they are conformed by dispersed individuals (each one genetically different), thus their cross-pollination generates greater variability and genetic recombination. In addition, the distance among the majority of sampling sites is relatively short (about 200 km between Guayas and Manabí), making possible pollen exchange by vectors (insects) in close distances and seed transported by farmers in long distances, these two factors being important for the genetic variability process (Zetina et al., 2012). The relatively high percentage of polymorphism observed could also be related to the natural pollination method of this species which expands the genetic base. The tamarind populations analyzed could be considered as isolated, which favors genetic variability.

Figure 2 shows the formation of two major groups. The tamarind trees from Loja, Manabí and Guayas were grouped in the first cluster, while only individuals from Manabí were located in the other group, indicating that there is major diversity of this fruit tree in this Province.

Extending the analysis, five subgroups were observed for the molecular characterization of the 31 accessions of tamarind, with an ultrametric distance of 2.06 units, whereas six groups were formed using the Ward algorithm with the phenotypic information from the morphological characterization (data not shown). Therefore, morphological analyses yielded clusters that did not completely account for the genetic similarity

found among the accessions when using molecular markers. Morphological descriptions may be confounded with environmental variation and be prone to subjective evaluations. These results are comparative with those from Cervera et al. (2001) who reported that molecular and morphological characterization cannot be directly related, i.e. physical characteristics and molecular data are not usually associated. This is evidenced by the fact that individuals within groups are different for both cases. The markers used in this study are dominant, semi-random and cannot determine heterozygosity, inferring more specific differences for the formation of groups. It seems that molecular characterization is related mainly to the origin of the material, thus with their geographical distribution, while the grouping by morphological traits could be influenced by external factors (agronomic and environmental) that affect the phenotypic expression of the plant. However, different morphotypes were noted within phenotypic characterizations, and thus it is recommended to carry out crosses among individuals which showed contrasting traits related to pulp percentage, number of fruit per bunch, number of seeds per seedcase, and fruit weight, characters that are important for breeders of this fruit tree according to Diallo et al. (2008), since fruit from all the 31 evaluated accessions are harvested for human consumption.

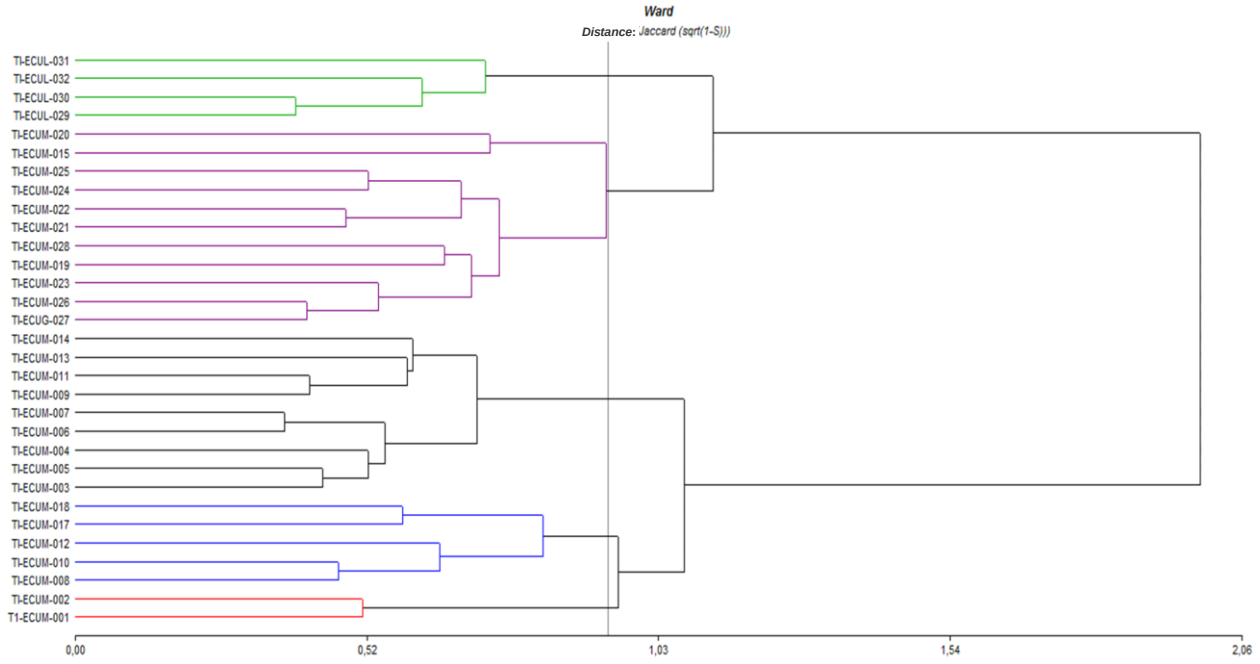

Figure 2. Dendrogram based on ISSR molecular data from 31 *Tamarindus indica* plus trees using Jaccard coefficient and Ward grouping. Cophenetic correlation coefficient = 0.634

The information generated can be used to suggest selected plus trees for clonal propagation as well as to identify diverse parents for hybridization programs.

As stated before, several studies using RAPD markers (Diallo et al., 2007; Gangaprasad et al., 2013; Kumar et al., 2015) and AFLP markers (Algabal et al., 2011) have characterized tamarind populations or genotypes and have reported genetic variability and diversity. However, ISSR markers are highly polymorphic and therefore useful for studies of genetic diversity (Reddy et al., 2002), and also variation between and within populations can be compared using this type of marker (Qian et al., 2001). In addition, higher polymorphism has been detected using ISSRs than any other technique (Virk et al., 2000).

In several cases genotypes did not cluster according to their site of collection, which was

attributed to their highly cross-pollinating nature, small distribution area and that most tamarind genotypes are grown from seed. Genotypes which were morphologically closely related were found to be unrelated at the molecular level (Kumar et al., 2015).

A breeding program should be based on the results of both morphological and molecular characterization; nonetheless, choosing parental plants based on the molecular results obtained in this study should be done by selecting individuals with higher genetic distances and corroborating the phenotypic traits of the parents in the field to avoid hybridizations between materials that genetically are different but express the same phenotype.

Tree breeding begins through the application of genetic principles basically directed towards modifying the heredity of tree populations to meet the needs of the farmers (Gunasena & Hughes, 2000). Determination of genetic variation is important to plant breeders for development of a high yielding variety (Kumar et al., 2015), and therefore this research contributes to generate knowledge about the diversity of tamarind trees in Ecuador in order to determine future advances in fruit breeding.

CONCLUSION

The tamarind genotypes assessed in this research showed a reasonable degree of genetic diversity that can be used as a basis for hybridization breeding programs.

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