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Genetic diversity, yield, and fruit quality of persimmon in the tropics

Abstract – The objective of this work was to determine the genetic diversity, yield, and fruit quality of persimmon genotypes grown in the tropics, in order to select promising genetic materials. DNA extraction was performed on young leaves of 19 persimmon genotypes. For pomological characterization, 15 genotypes were selected. From each genotype, 50 fruit at the physiological maturity stage were harvested in the morning, in order to determine the following parameters: physicochemical characteristics; and the productive variables number of fruit per plant, average fruit fresh mass, average yield, and estimated average yield in two seasons. Twenty SSR markers were tested, out of which 12 were selected to evaluate genetic similarity, which allowed of the identification of distinct groups. The mean genetic diversity value found was 0.41, which is an indicative of low diversity among the analyzed persimmon genotypes. The 'Guiombo', 'Iapar 125', 'Kakimel', 'Mikado RJ', 'Rama Forte Tardio', and 'Taubaté' genotypes show a high yield. The genotypes classified as pollination-constant astringent ('Pomelo', 'Regina', 'Rubi', and 'Taubaté') and those classified as pollination-variant astringent ('Rama Forte', 'Guiombo', and 'Cereja') are potential materials for selection and genetic breeding programs due to their excellent fruit physicochemical characteristics. The investigation through molecular markers is an efficient approach to study the genetic diversity of persimmon genotypes grown in the tropics.

Index terms: *Diospyros kaki*, molecular markers, phenology.

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Diversidade genética, produtividade e qualidade de frutos de caqui nos trópicos

Resumo – O objetivo deste trabalho foi estimar a diversidade genética, o rendimento e a qualidade de frutos de genótipos de caquizeiros cultivados nos trópicos, para selecionar materiais genéticos promissores. A extração de DNA foi realizada em folhas jovens de 19 genótipos de caqui. Para a caracterização pomológica, foram selecionados 15 genótipos. Para cada genótipo, 50 frutos foram colhidos pela manhã, no estágio de maturidade fisiológica, para determinar os seguintes parâmetros: características físico-químicas; e as variáveis produtivas número de frutos por planta, massa de matéria fresca média dos frutos, produtividade média e produtividade média estimada, em duas safras. Vinte marcadores SSR foram testados, dos quais 12 foram selecionados para avaliar a similaridade genética, o que permitiu a identificação de grupos distintos. O valor médio de diversidade genética encontrado foi 0,41, o que é indicativo de baixa diversidade entre os genótipos de caqui analisados. Os genótipos 'Guiombo', 'Iapar 125', 'Kakimel', 'Mikado RJ', 'Rama Forte Tardio' e 'Taubaté' apresentam alta produtividade. Os genótipos classificados como sendo de polinização constante adstringente ('Pomelo', 'Regina', 'Rubi' e 'Taubaté') e os classificados como sendo de polinização variante adstringente ('Rama Forte', 'Guiombo' e 'Cereja') são materiais com potencial para uso em

programas de seleção e melhoramento genético, devido às suas excelentes características físico-químicas de fruta. A investigação por meio de marcadores moleculares é uma abordagem eficiente para estudar a diversidade genética de genótipos de caquizeiro cultivados nos trópicos.

Termos para indexação: *Diospyros kaki*, marcadores moleculares, fenologia.

Introduction

Persimmon (*Diospyros kaki* L.f.) fruit tree is traditionally grown in temperate or subtropical regions (Martínez-Calvo et al., 2013). Persimmon was introduced in Brazil in the late 19th century. However, its cultivation only expanded around 1920 with the arrival of Japanese immigrants, who provided new cultivars and production techniques (Blood et al., 2020). In Brazil, programs for the conservation of persimmon genetic materials are located at the IAC (Instituto Agronômico, in the municipality of Campinas, in the state of São Paulo, Brazil), which was the institution responsible for disseminating this fruit in Brazilian subtropical regions (Peche et al., 2016a, 2016b). However, the last genotype launched by IAC is the persimmon 'Fuyuhana' – in 1983.

One of the main problems related to the diversity management of persimmon genetic resources is the assignment of genotype identity, due to the existence of synonyms and homonyms among local varieties, misleading Japanese translations, and incorrect labeling in the past.

The main characteristics used as references for the classification of persimmon genotypes are the fruit astringency losses related to cross-pollination that resulted in four genotypes groups (Yonemori et al., 2008b), as follows: pollination constant nonastringent (PCNA), pollination variant nonastringent (PVNA), pollination constant astringent (PCA), and pollination variant astringent (PVA).

The germplasm banks are the first step for implementing a program of selection. One of the most important requirements of germplasm banks is the ability to identify the accessions, for which techniques of molecular genetics have become a set of tools for the identification, characterization, and genetic study of species. Molecular markers are important to detect the variability, since they can be used to indicate polymorphisms at the DNA level, and to establish links between the presence or absence of genes that control a particular characteristic (Badenes et al., 2016).

SSR markers provide an ideal tool for this purpose, since they have desirable molecular marker properties, mainly because they are highly polymorphic, reproducible, abundant, and codominant (Soriano et al., 2006). The development of techniques that use codominant markers as simple sequence repeats (SSRs) for persimmon has provided reliable markers for persimmon genetic and diversity studies (Naval et al., 2010; Gil-Muñoz et al., 2018). The characterization of pomological traits is not sufficient for the estimation of available genetic variability, but it can be used in an additive way for the identification by molecular markers.

The objective of this work was to determine the genetic diversity, yield, and fruit quality of persimmon genotypes grown in the tropics, in order to select promising genetic materials.

Materials and Methods

The plant materials came from the germplasm bank located in the municipality of São Bento do Sapucaí, in the state of São Paulo, Brazil (22°41'S, 45°43'W, at 886 m altitude), with mesothermal climate – Cwa, according to the Köppen-Geiger's classification, with dry winters and concentrated rains from October to March, with greater intensity between the months of December and February (Alvares et al., 2013).

The soil is classified as a Latossolo Vermelho-Amarelo distrófico, according to the Brazilian Soil Classification System (Santos et al., 2018). The 22 genotypes used in the present study were: the PCA types 'Costata' (COST), 'Paraguai' (PAR), 'Pomelo' (POMB), 'Regina' (REG), 'Rubi' (RUBI), 'Taubaté' (TAUB), and 'Trakoukaki' (TRAK); the PCNA type 'Fuyu' (FUYB); the PVA types 'Cereja' (CER), 'Chocolate' (CHOC), 'Erma Rideo' (ERRI), 'Guiombo' (GUIOMB), 'Iapar 125' (IAPAR), 'Kakimel' (MEL), 'Mikado' (MIKB), 'Rama Forte' (RF), 'Rama Forte Tardio' (RFT), and 'Rojo Brillante' (RB); additionally, PVNA types 'Kyoto' (KYOTO) and 'Vaniglia' (VAN). Two species used as rootstocks served as the following outgroups: *Diospyrus lotus* (LOTUS) and *Diospyrus virginiana* (VIRG).

The DNA extraction was performed on young new leaves of 19 persimmon genotypes – 'Cereja',

'Chocolate', 'Erma Rideo', 'Fuyu', 'Guiombo', 'Kyoto', 'Kakimel', 'Mikado', 'Paraguai', 'Pomelo', 'Rama Forte', 'Regina', 'Rojo Brillante', 'Rubi', 'Taubaté', 'Trakoukaki', and 'Vaniglia' –, as well as on the species *D. lotus* and *D. virginiana* (Soriano et al., 2006), and quantified in the spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 260 and 280 nm wavelengths. The DNA quality was evaluated by agarose gel electrophoresis (0.8%, m/v) and visualized in L-pix HE photographic documentation (Loccus Biotecnologia - São Paulo, SP, Brazil). The quantified samples were diluted in TE [10 mmol L^{-1} Tris-HCl, pH 8.0, 1 mmol L^{-1} EDTA] at 25 ng μL^{-1}, and stored at -20°C for subsequent use in SSR reactions. Each polymerase chain reaction (PCR) was performed with three primers: the specific primer from each microsatellite with the M13 tail (-21) at its 5' end, the sequence-specific reverse primer, and the universal fluorescent-labeled M13(-21) primer. The PCRs were performed in a 20 µL volume containing 2 µL of the genomic DNA solution, 2 µL of each primer, and a mixture composed of 2 µL of 10X PCR buffer, 0.4 µL dNTP (10 mmol L^{-1}), 0.6 µL $MgCl_2$ (50 mmol L^{-1}), 0.2 µL of Invitrogen Taq DNA polymerase (5 U μL^{-1}), and 12.8 µL ultrapure water. The amplifications were performed in a thermal cycler programmed for a 5 min denaturation cycle at 95°C, followed by 45 denaturation cycles of 15 s at 94°C, with 1 min annealing, 1 min extension at 68°C, a final extension at 72°C for 5 min, and a final extension at 4°C.

A total of 2 µL of loading buffer (0.02% bromophenol blue, 40% glycerol) was added to 20 µL of each PCR volume after amplification; 17 µL were loaded into 2% agarose gel (dissolved in 1X TBE - 89 mmol L^{-1} Tris, 89 m mol L^{-1} boric acid, 2.5 m mol L^{-1} EDTA, pH 8.3) and subjected to horizontal electrophoresis at 200 V, 200 mA, and 100 W for approximately 95 min. The gels were stained in a solution containing ethidium bromide (0.02 µL water) for 60 min for visualization under ultraviolet light. The 1 kb molecular weight marker (Promega Corporation, Madison, WI, USA) was used as the standard molecular-weight size marker.

The results were visualized on Locophy L-pix HE photographic documentation equipment (Loccus Biotecnologia, São Paulo, SP, Brazil). The electrophoretic profile of each SSR primer was transformed into a binary matrix. The presence of a fragment was represented by 1, and the absence of fragment was represented by 0. Binary data were used to perform all subsequent analyses.

To evaluate the information of the microsatellites used, the polymorphism information content (PIC) was calculated from allele frequencies for all accessions. The matrix of genetic similarities in pairs was made with the software GeneAlEx v. 6.41. The resulting matrix was used to generate a principal coordinate analysis (PCoA). A phylogenetic tree was constructed by applying the distance matrix computed with the software Phylogenetic Computer Tools v. 1.3. To perform a neighbor-joining (NJ) analysis the software Phylip v. 3.695 was used. Tree stability was tested with 1000 bootstrap data arrays.

For the pomological characterization, 15 persimmon genotypes were selected – 'Cereja', 'Costata', 'Fuyu', 'Guiombo', 'Iapar 125', 'Kakimel', 'Kyoto', 'Mikado', 'Paraguai', 'Pomelo', 'Rama Forte', 'Regina', 'Rama Forte Tardio', 'Rubi', and 'Taubaté' –, which were collected in São Bento do Sapucaí, in the state of São Paulo, Brazil. For each genotype, fifty fruit at the physiological maturity were harvested in the morning. The fruit were dried with a paper towel and left on a stainless-steel shelf to complete the ripening at room temperature (25°C). The following variables were analyzed: fresh fruit mass (g); the longitudinal (DL) and transversal (DT) diameters (mm), measured with a caliper; color (L*, a*, and b*); and fruit firmness. The color was determined using the colorimeter model CR-400 (Konica Minolta, Inc., Chiyoda-ku, Tokyo, Japan) with illuminant D65 and in the CIE system L*, a* and b*. Fruit firmness (expressed in Newtons, N) was determined at three points on the fruit surface using a TAXT2i texture analyzer (Stable Micro Systems, Godalming, United Kingdom), and the P/3 N probe.

The following analyses were performed on the pulp of the 15 persimmon genotypes: titratable acidity (TA, in percentage of malic acid) was determined by titration with 0.1 N NaOH solution and 1% phenolphthalein as an indicator, with values expressed as the percentage of malic acid, and soluble solids (SS, °Brix) were measured using an Atago digital refractometer (Atago CO., Shiba-koen, Minato-ku, Tokyo, Japan).

The evaluated productive variables of the 15 persimmon genotypes were as follows: number of fruit per plant; fruit fresh mass (g); yield (kg per plant); and estimated average yield (Mg ha^{-1}) in two seasons. Fruit

were harvested weekly, counted, and weighed using a semi-analytical balance. Total fruit mass was used to determine plant yield. Subsequently, the estimated productivity was attained by multiplying the plant yield by the number of plants per hectare (410 plants ha^{-1}).

Data were subjected to the analysis of variance by the F-test, and the experimental design was completely randomized with 15 treatments (genotypes) and six replicates. The means were compared y the Scott-Knott's test, at 5% probability. The analyses were performed using the computer program for analysis of variance Sisvar, version 5.6. (Ferreira, 2014).

Results and Discussion

In the analysis of 17 persimmon genotypes, 141 alleles were identified by 12 SSR polymorphic markers. The allele sizes ranged from 134 to 365 bp. The average of 11.75 alleles per locus was obtained, ranging from five (ssrdk04) to 18 alleles (ssrdk03). Rare and unique alleles were also obtained. The number of rare alleles (frequency < 0.02) varied from zero (ssrdk04, ssrdk14, and ssrdk16) to four (ssrdk03), totaling 21 rare alleles averaging 1.75. Moreover, 20 unique alleles (that is, amplified in only one accession) were found in 10 marker loci (Table 1).

The PIC results for each marker confirmed their utility for identifying differences among the samples analyzed in the present study (Table 1). Moreover, they ranged from 0.6289 (ssrdk04) to 0.926 (ssrdk03), averaging 0.86755. All 12 markers except for ssrdk04 were highly polymorphic, having a PIC value equal to or higher than 0.78. The PIC values of a locus were associated with the number of detected alleles; for instance, the highest PIC value corresponded to ssrdk03 (18 alleles), and the lowest one corresponded

Table 1. Summary of microsatellite allele data revealed by 12 microsatellite loci in 17 genotypes of *Diospyros kaki*.

Marker	Repeat motif	Primers (5′-3′)	Allele size (bp)	No. of alleles	Rare alleles	Unique alleles	PIC
ssrdk01	(AG)19	F:GGCATGAAGGAATAAGGAA R:GCTCACATTCCAACCAATCA	155–184	13	1	1 ('Paraguay')	0.906
ssrdk02	(GA)17	F: TTAATTTGGACACAAGTTCT R: TCTCTTCAAGTCTTCTATCCT	196–224	11	2	2 ('Fuyu' and 'Paraguay')	0.863
ssrdk03	(AG)16	F: GGCTCTCGGTCAAATAGTAG R:GGAGGTTAGAAATCCAGCTA	158–198	18	4	2 ('Rama Forte')	0.926
ssrdk04	(GA)17	F:CATTTGAAAGCAGTCGTCCA R: GCGCCAAATCATTGCTATCT	336–365	5	0	1 ('Chocolate')	0.629
ssrdk06	(AG)19	F: CGGCATGAAGGAATAAGGAA R: GCTCACATTCCAACCAATCA	154–187	11	1	1 ('Paraguay')	0.894
ssrdk14	(AG)16	F: GTGAAGGAACCCCATAGAA R: CCATCATCAGGTAGGAGAGA	155–178	10	0	0	0.869
ssrdk16	(GA)12	F: ACTACAACGGCGGTGAGAAC R: GTCCTTCACTTCCCGCATT	134–173	9	0	0	0.841
ssrdk25	(CT)15	F: GGGGTAATATGAATTGAATC R: CTCAGAGAGGAGAAGAAATAG	219–283	15	2	2 ('Fuyu' and Vaniglia)	0.898
ssrdk26	(GA)15	F: GGGAAATTAAGAGGGAAGAA R: AGGAACTGGATCAGCATAAA	152–202	13	2	2 ('Fuyu' and 'Rama Forte')	0.894
ssrdk30	(TG)9(AG)17	F: TGGTGATCGTGGTAGTGGTT R: GGCCTAATCTCTGTCCATCC	137–275	9	2	2 ('Kyoto' and Vaniglia)	0.819
ssrdk36	(GA)16	F: GGAAGAACAAAGAGAACTG R: ACGAAGTTGTAATCCTGAGC	226–259	13	3	3 ('Fuyu', 'Kyoto', and 'Rubi')	0.888
ssrdk37	(CT)10	F: CAAAATGAAGCCCATAAGAC R: GTGAAAGTGTGGTTGGATTT	154–211	14	2	2 ('Fuyu')	0.901
Mean				11.75	1.75		0.866
Total				141	21	20	

Range of fragment size, allele number, rare alleles (freq. < 0.02), alleles exclusive of one accession (name between parentheses), and polymorphism information content (PIC).

to ssrdk04, with five alleles. These alleles can be considered markers of interest to identify genotypes separately.

Jing et al. (2013) evaluated the genetic diversity of 48 genotypes for seven species of the *Diospyros* genus, using 11 SRAP primers, and obtained 303 totally polymorphic fragments that allowed of the identification of all the species and the genotypes into species. Similarly, Naval et al. (2010) evaluated 71 persimmon genotypes, using SSR primers, and obtained 206 fragments with 100% polymorphism. A lower percentage (75%) was obtained by Yang et al. (2015), using 14 SSR primers in different species of *Diospyros*.

The genetic distance values determined by Nei (1972) varied from 0.32 (for 'Trakoukaki' compared with 'Erma Rideo') to 0.67 (for 'Trakoukaki' compared with 'Chocolate'). In the present study, the higher distances corresponded to the outgroups *D. lotus* and *D. virginiana* with the *D. kaki* genotypes, and the distance ranged between 0.78 and 0.97 (Table 2).

Nei's index (Nei, 1972) is a genetic diversity identifier ranging from 0 to 1, with 0 indicating no genetic diversity, and 1, showing the maximum genetic diversity (Giustina et al., 2014). The average genetic diversity value found in the present study was 0.41, which suggests low diversity among the analyzed genotypes. The greatest variation found between the Nei's genetic distance values occurred between the *D. lotus* and *D. virginiana* outgroups and the *D. kaki* genotypes.

The PIC value ranges from 0 to 0.25 for markers considered less informative, and it ranges from 0.25 to 0.5 for moderately informative markers, and above 0.5, for markers that show high information content. The PIC value allows of primer classification and indicates the primer efficiency in the detection of polymorphisms (Costa et al., 2015). Thus, in the present study, the primers were very informative (PIC=0.866). Markers that show lower PIC values can also be considered markers of interest, since they can identify genotypes separately. This is an important issue in non-Asian countries, where the introduction of persimmon varieties led to a high number of poorly identified genotypes (Naval et al., 2010). The SSRs used in the present study allowed of the identification of several persimmon genotypes, including 'Paraguai',

Table 2. Pairwise Nei's (1972) genetic distance among the different persimmon (*Diospyros kaki*) genotypes.

Genotype	PARA	POMB	REG	RUBI	TAUB	TRAK	CER	CHOC	GUIOMB	MEL	MIKB	RF	RFT	ERRI	KYOTO	FUYB	VAN	LOTUS	VIRG
PARA	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POMB	0.53	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REG	0.57	0.39	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RUBI	0.63	0.40	0.42	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TAUB	0.57	0.48	0.44	0.48	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TRAK	0.63	0.47	0.51	0.39	0.43	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-
CER	0.57	0.48	0.56	0.51	0.35	0.38	0.00	-	-	-	-	-	-	-	-	-	-	-	-
CHOC	0.54	0.53	0.63	0.65	0.54	0.67	0.53	0.00	-	-	-	-	-	-	-	-	-	-	-
GUIOMB	0.52	0.44	0.37	0.39	0.41	0.39	0.47	0.49	0.00	-	-	-	-	-	-	-	-	-	-
MEL	0.54	0.50	0.55	0.56	0.55	0.62	0.65	0.52	0.49	0.00	-	-	-	-	-	-	-	-	-
MIKB	0.54	0.46	0.57	0.62	0.58	0.56	0.55	0.37	0.52	0.41	0.00	-	-	-	-	-	-	-	-
RF	0.53	0.54	0.52	0.70	0.55	0.61	0.52	0.41	0.49	0.53	0.33	0.00	-	-	-	-	-	-	-
RFT	0.57	0.37	0.38	0.57	0.52	0.42	0.54	0.68	0.43	0.59	0.61	0.56	0.00	-	-	-	-	-	-
ERRI	0.60	0.51	0.47	0.49	0.40	0.32	0.37	0.60	0.38	0.50	0.57	0.56	0.41	0.00	-	-	-	-	-
KYOTO	0.49	0.56	0.56	0.59	0.65	0.70	0.72	0.39	0.49	0.43	0.45	0.51	0.56	0.54	0.00	-	-	-	-
FUYB	0.57	0.48	0.54	0.54	0.65	0.63	0.64	0.70	0.52	0.57	0.60	0.59	0.62	0.58	0.59	0.00	-	-	-
VAN	0.48	0.42	0.54	0.52	0.59	0.59	0.58	0.52	0.56	0.55	0.58	0.55	0.64	0.60	0.51	0.45	0.00	-	-
LOTUS	0.83	0.81	0.78	0.90	0.89	0.79	0.78	0.88	0.81	0.97	0.81	0.83	0.78	0.84	0.97	0.86	0.83	0.00	-
VIRG	0.87	0.88	0.91	0.90	0.91	0.86	0.85	0.93	0.85	0.97	0.91	0.94	0.82	0.88	0.97	0.91	0.91	0.42	0.00

'Paraguai' (PAR), 'Pomelo' (POMB), 'Regina' (REG), 'Rubi' (RUBI), 'Taubaté' (TAUB), 'Trakoukaki' (TRAK), 'Cereja' (CER), 'Chocolate' (CHOC), 'Guiombo' (GUIOMB), 'Kakimel' (MEL), 'Mikado' (MIKB), 'Rama Forte' (RF), 'Rama Forte Tardio' (RFT), 'Erma Rideo' (ERRI), 'Kyoto' (KYOTO), 'Fuyu' (FUYB); 'Vaniglia' (VAN), *Diospyrus lotus* (LOTUS), and *Diospyrus virginiana* (VIRG).

'Fuyu', 'Rama Forte', 'Chocolate', Vaniglia, 'Kyoto', and 'Rubi' with unique alleles.

Based on microsatellite data, the genetic distances among persimmon accessions were used to generate a neighbor-joining cladogram (Figure 1). The cladogram showed two major groups. Group I comprises mainly PCA genotypes ('Pomelo', 'Regina', 'Rubi', 'Trakoukaki' and 'Taubaté'). Group II, with 63.5% bootstraps, included mostly PVA type genotypes ('Kakimel', 'Chocolate', 'Mikado', and 'Rojo Brillante'), except for the genotypes 'Kyoto' and 'Vaniglia', both of which are PVNA type genotypes; within this group, the genotypes 'Rojo Brillante' and 'Mikado' grouped together with 56.9% probability. Moreover, the 'Fuyu' PCNA genotype was grouped with a probability of 64.3%, and the genotype 'Paraguai' was isolated from the others.

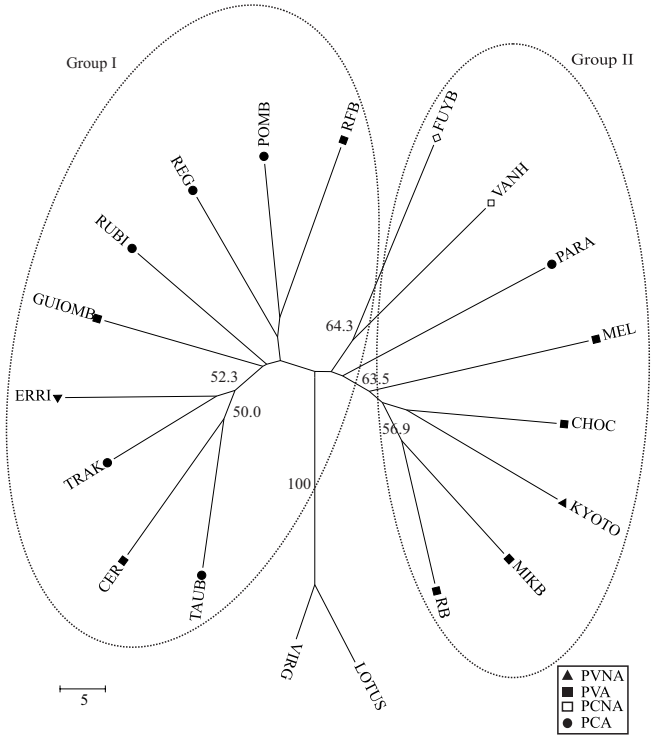

Figure 1. Cladogram obtained by neighbor-joining analysis for 19 persimmon (*Diospyros kaki*) genotypes based on SSRs markers. Bootstrap values >50% are shown in the tree. Pollination variant nonastringent (PVNA), pollination variant astringent (PVA), pollination constant nonastringent (PCNA), and pollination constant astringent (PCA). Genotypes: 'Paraguai' (PARA), 'Pomelo' (POMB), 'Regina' (REG), 'Rubi' (RUBI), 'Taubaté' (TAUB), 'Trakoukaki' (TRAK), 'Cereja' (CER), 'Chocolate' (CHOC), 'Guiombo' (GUIOMB), 'Kakimel' (MEL), 'Mikado' (MIKB), 'Rama Forte' (RF), 'Rama Forte Tardio' (RFT), 'Erma Rideo' (ERRI), 'Kyoto' (KYOTO), 'Fuyu' (FUYB); 'Vaniglia' (VANH), *Diospyrus lotus* (LOTUS), and *Diospyrus virginiana* (VIRG).

Microsatellite data were subjected to PCA to obtain an alternative view of the relationships among the accessions (Figure 2). As expected, the results of this analysis agreed with the neighbor-joining cladogram. PC1 accounted for 16.25% of the variability, while PC2 accounted for 13.8% of the variability. The plot of the genotypes in the space of PC1 and PC2 resulted in groups similar to those in the cladogram.

The pomological traits showed significant differences ($p<0.05$) among the genotypes (Table 3). At least two groups were formed for the evaluated characters of color (L*, a*, and b*) and firmness. 'Regina', 'Guiombo', 'Iapar 125', 'Kyoto', 'Pomelo', 'Rama Forte Tardio', and 'Taubaté' showed higher values of L* and b* (that is, a lighter-colored fruit with a more intense yellow color). For the color parameter a*, the fruit of 'Costata', 'Guiombo', 'Iapar 125', 'Kakimel', 'Regina', 'Rama Forte Tardio', and 'Taubaté' stood out due to their greater intensity of red color than that of the other genotypes. 'Pomelo', 'Guiombo', 'Iapar 125', 'Kyoto', 'Regina', 'Rama Forte

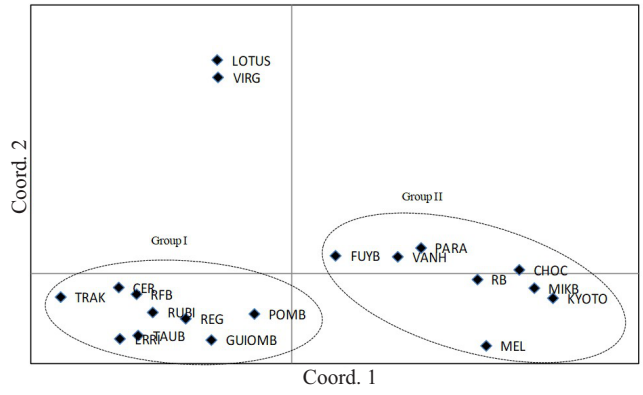

Figure 2. Scatter plot of 19 persimmon (*Diospyros kaki*) genotypes estimated with 12 SSR markers, using the genetic similarity matrix based on first and second components of principal coordinates analysis (PCoA). Genotypes: 'Paraguai' (PARA), 'Pomelo' (POMB), 'Regina' (REG), 'Rubi' (RUBI), 'Taubaté' (TAUB), 'Trakoukaki' (TRAK), 'Cereja' (CER), 'Chocolate' (CHOC), 'Guiombo' (GUIOMB), 'Kakimel' (MEL), 'Mikado' (MIKB), 'Rama Forte' (RF), 'Rama Forte Tardio' (RFT), 'Erma Rideo' (ERRI), 'Kyoto' (KYOTO), 'Fuyu' (FUYB); 'Vaniglia' (VANH), *Diospyrus lotus* (LOTUS), and *Diospyrus virginiana* (VIRG).

Tardio', and 'Taubaté' have firmer fruit, which exhibit greater resistance (firmness) and possibly greater postharvest durability than the other genotypes. The highest average values of fruit length were observed in the genotypes 'Regina' and 'Paraguai' (79.3 and 79.1 mm, respectively). 'Fuyu', 'Kakimel', 'Mikado', 'Paraguai', 'Pomelo', 'Regina', 'Rama Forte Tardio', and 'Rubi' had the largest fruit diameters (Table 3). The largest fruit weight were observed for 'Costata', 'Paraguai', and 'Regina' genotypes (205.8, 215.4, and 224.6 g, respectively).

Regarding the chemical compounds (Table 3), 'Iapar 125', 'Kyoto', and 'Rama Forte' genotypes showed the highest concentration of soluble solids (18.4, 17.6, and 18.6 °Brix, respectively). The studied genotypes were divided into five groups in accordance with the total sugar contents, for which 'Mikado' showed the highest value (21.9 g 100 g^{-1}). 'Rubi' showed the highest value for phenolic content (301.9 mg 100 g^{-1}).

'Guiombo', 'Iapar 125', 'Kakimel', 'Mikado RJ', 'Rama Forte Tardio', and 'Taubaté' showed higher productivity

Table 3. Color (L*, a*, and b*), average fruit length (FL), average fruit diameter (FD), average mass of fruit fresh matter (FM), firmness, soluble solids content (SS), titratable acidity (TA), total sugar, phenolics, number of fruit (NF), yield (kg per plant), and estimated yield (AY) of persimmon (*Diospyros kaki*) genotypes[(1)].

Genotype	L*	a*	b*	FL (mm)	FD (mm)	FM (g)	Firmness (N)
Cereja	36.7b	11.8b	20.8b	45.4f	55.7c	82.7d	3.4b
Costata	44.5b	33.2a	32.8b	70.8b	49.6d	205.8a	39.1b
Fuyu	48.1a	18.3b	34.0b	52.5e	70.7a	146.7c	25.1b
Guiombo	53.0a	24.3a	46.1a	60.2d	57.0c	110.7d	61.3a
Iapar 125	54.7a	29.1a	52.3a	59.2d	57.1c	139.1c	14.7b
Kakimel	40.5b	22.7a	31.0b	56.8d	66.5a	138.5c	51.1a
Kyoto	57.9a	18.6b	51.7a	65.8c	61.8b	142.0c	1.6b
Mikado	39.8b	13.0b	29.8b	67.0c	68.7a	182.8b	27.7b
Paraguai	40.2b	16.5b	35.8b	79.1a	68.2a	215.4a	13.2b
Pomelo	52.1a	20.0b	36.1a	58.8d	67.3a	151.1c	95.7a
Rama Forte	50.0a	21.4b	33.7b	45.4f	58.9c	84.1d	84.1a
Regina	60.7a	29.2a	61.0a	79.3a	71.4a	224.6a	89.1a
Rama Forte Tardio	54.8a	25.6a	50.3a	49.6e	67.9a	130.0c	67.0a
Rubi	38.9b	16.7b	22.4b	59.3d	74.3a	183.9b	46.3b
Taubaté	56.1a	24.7a	52.9a	60.9d	62.4b	143.9c	40.4b
Average	48.52	21.67	39.36	60.66	63.84	152.07	43.98
CV (%)	10.48	31.93	25.86	4.11	5.54	13.15	55.47
Genotype	SS (ºBrix)	TA (% malic acid)	Total sugar (g 100 g^{-1})	Phenolics (mg 100g^{-1})	NF	Yield (kg per plant)	AY (Mg ha^{-1})
Cereja	15.3b	1.0b	18.3c	145.1e	94.8b	7.8b	3.2b
Costata	16.3b	0.09b	19.0b	147.2e	20.0b	4.1b	1.7b
Fuyu	14.2b	0.04c	11.6d	208.6b	56.5b	8.6b	3.5b
Guiombo	16.6b	0.08b	20.2b	153.1e	260.0a	29.7a	12.2a
Iapar 125	18.4a	0.11b	15.9c	48.3j	194.3a	26.6a	10.9a
Kakimel	15.5b	0.06c	10.0d	114.4g	299.5a	38.4a	15.7a
Kyoto	17.6a	0.06c	16.0c	133.8f	28.8b	4.2b	1.7b
Mikado RJ	15.7b	0.08b	21.9a	53.1j	152.3a	27.8a	11.4a
Paraguai	15.3b	0.08b	16.9c	176.3d	77.5b	16.8b	6.9b
Pomelo	16.7b	0.16a	17.3c	76.7i	11.0b	1.7b	0.7b
Rama Forte	18.6a	0.08b	16.7c	142.0f	127.3b	10.9b	4.5b
Regina	15.7b	0.08b	16.5c	104.3h	77.0b	17.5b	7.2b
Rama Forte Tardio	16.3b	0.11b	20.0b	139.4f	278.3a	36.5a	15.0a
Rubi	16.0b	0.09b	15.8c	301.9a	56.3b	9.8b	4.0b
Taubaté	16.7b	0.09b	8.2e	187.7c	236.3a	33.5a	13.7a
Average	16.31	0.09	16.29	142.12	131.3	18.26	7.49
CV (%)	9.15	27.52	7.78	3.69	78.01	73.02	73.01

[(1)]Means followed by equal letters, in the columns, do not differ among themselves by Scott-Knott's test, at 5% probability. CV, coefficient of variation.

and yield (Table 3) than the other genotypes. In Japan, in temperate regions, Tetsumura et al. (2015) recorded the production of 22.6 kg per plant in 'Fuyu', which is 14 kg per plant larger than the 8.6 kg per plant of the 'Fuyu' genotype quantified in the present conditions of subtropical climate. Persimmon shows variation in its productive performance in accordance with the climatic conditions of cultivation, although it is a species of low-chilling requirements (Pio et al., 2019).

Cluster and principal component analysis grouped the genotypes by astringency type, showing a high level of genetic relatedness. Similar results were obtained by Yonemori et al. (2008a) and Gil-Muñoz et al. (2018). In both studies, the PCNA genotypes grouped together.

However, in another study, Yonemori et al. (2008b) analyzed a large number of persimmon accessions, including Japanese, Korean, and Chinese accessions, using AFLP markers; their findings showed a unique clade of PCNA Japanese genotypes, which suggests an independent evolution, although the authors did not report the bootstrap value that supported the clade groupings.

Naval et al. (2010) studied 71 persimmon cultivars, and Liang et al. (2015) analyzed 133 ones. They used SSR markers and obtained similar results, for which PCNA genotypes grouped together. This can be explained by the fact that the PCNA type is a recessive mutation that arose in Japan. The short history of the mutation and its low spread resulted in low genetic variability among genotypes of the same type (Yonemori et al., 2008b).

For the other groups, the results were similar to those found by Naval et al. (2010), Liang et al. (2015) and Gil-Muñoz et al. (2018), in which groups of PCA and PVA types were not separated as clearly as the PCNA type. The selection of characters of interest related to the pomological and chemical properties of fruit, phenology, and yield that are associated with genetic diversity data is important for the selection of promising genotypes.

Curi et al. (2017) analyzed the characteristics and environmental effects on persimmon genotypes, in a subtropical region, and determined different parameters for fruit size. We found similar results to those reported by Martínez-Calvo et al. (2013), who reported fruit weights between 150 and 160 g and average diameters ranging from 65 to 70 mm in different persimmon genotypes.

Based on the genetic markers, 'Rama Forte', 'Pomelo', 'Regina', 'Rubi', 'Guiombo', 'Cereja', and 'Taubaté' genotypes, classified as PCA and PVA, formed the group I (Figures 1 and 2). These genotypes were characterized by firmer fruit and uniform and reddish maturation; however their fruit weighed less, hence, these genotypes were less productive than those from group II. Nevertheless, these genotypes show fruit with high contents of soluble solids, total sugar, and phenolics (Table 3).

The groups formed by the cladogram and the principal component analysis suggest a correlation between the genetic characterization and the quality attributes of persimmon fruit, indicating a relationship between the fruit characteristics and the genetic origin of the genotypes. This evidence underscores the importance of characterization at both genetic and pomological levels, for the germplasm management and the selection of promising genotypes for later use in genetic improvement programs.

Conclusions

1. The average genetic diversity value found was 0.41, which suggests low diversity among the analyzed persimmon (*Diospyrus kaki*) genotypes.

2. Microsatellite markers grouped the persimmon genotypes into two major groups: group I comprising mainly the pollination constant astringent (PCA) genotypes 'Pomelo', 'Regina', 'Rubi', 'Trakoukaki', and 'Taubaté'; and group II that is composed mostly of pollination variant astringent (PVA) type genotypes ('Kakimel', 'Chocolate', 'Mikado', and 'Rojo Brillante'.

3. 'Guiombo', 'Iapar 125', 'Kakimel', 'Mikado RJ', 'Rama Forte Tardio', and 'Taubaté' show higher yield than the other studied genotypes.

4. The genotypes classified as PCA – 'Pomelo', 'Regina', 'Rubi', and 'Taubaté', and those classified as PVA –'Rama Forte', 'Guiombo', and 'Cereja' – are potential materials for the selection and genetic breeding programs because they excel for their physicochemical characteristics of fruit.

5. The investigation of molecular markers is efficient approach to study of the genetic diversity of Brazilian persimmon genotypes grown in the tropics.

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