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CROP SCIENCE

Old but still good: genetic diversity of ancient pecan genotypes from southern Brazil

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Abstract: Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a crop fruit native to the USA and Mexico currently cultivated in several countries, including Brazil, Uruguay, Argentina, Chile, Peru, China, South Africa, and Australia. Supported by the increasing consumption and market prices, the interest in the cultivation of this fruit crop is strongly growing around the world. In this study, AFLP and S-SAP markers were employed to characterize the genetic diversity of ancient accessions of pecan from southern Brazil. The evaluated plants were selected and preserved by the farmers and are remnants of the first introduction of seedlings from the U.S.A into southern Brazil aiming at developing research towards establishing commercial orchards. High levels of genetic diversity were estimated, suggesting that these plants have an important genetic background for the establishment of a germplasm collection with a wide genetic basis, for the development of breeding programs for this fruit crop. Cluster analysis of the genetic datasets revealed some correlation between the nuts' morphometric traits and genetic markers. Such correlation should be further exploited. These ancient genotypes must be evaluated for other agronomic traits of interest and included in core collections of pecans.

Key words: AFLP, breeding, *Carya illinoinensis*, molecular markers, pecaniculture, S-SAP.

INTRODUCTION

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch, Jungladaceae] is a crop fruit predominantly cultivated in the northern hemisphere's temperate regions (Sparks 2005, Rivera-Rangel et al. 2018). Native to the U.S.A. and Mexico, the emergent market of the crop encouraged its cultivation in several countries in the southern hemisphere, such as Brazil, Uruguay, Argentina, Chile, Peru, South Africa, and Australia (Sparks 2005, Wells 2017, Nagel et al. 2020).

The structuring of the Brazilian supply chain, the cooperation with international researchers, the larger mechanization of production (such as pruning, harvesting, and irrigation), the establishment of new processing industries, and the recent access to the international market promoted the expansion of pecan cultivation and the recovery of abandoned orchards in the country (Poletto et al. 2014). Furthermore, the incorporation of new technologies and scientific information has assisted and improved the production process, adding value and quality to the final product, and increasing the interest in the cultivation of this crop in southern Brazil.

However, the advances in the production processes, management, and processing of the fruit must be connected to cultivars with high production potential. The maximum yield conceivable of a crop is significantly determined by its genetic potential. Thus, genetic breeding largely determines the production potential and quality of the orchards. Similarly, genetic

resistance to diseases is a quite desired trait in any orchard.

In the United States, for many years the breeding program of pecan selects and develops cultivars that are more productive, resistant to diseases, and adapted to each region of the country. Recently, this breeding program launched the cultivar "Avalon" with high productivity and resistance to the main diseases of the crop (Conner 2018). Genetic breeding programs are dynamic also in other countries where pecan is commercially cultivated. Mexico launched the cultivar "Norteña" adapted to arid climates (Pérez et al. 2015), Argentina developed the "Delta 1" and "Delta 2" cultivars, adapted to warmer and wetter regions (Madero 2007), and Spain launched the cultivars "Churriana 2" and "Churriana 5" adapted to the Mediterranean climate (Domínguez et al. 2018).

In Brazil, pecan farming started through the introduction of some cultivars from the United States in the 1870s (de Oliveira et al. 2021), which were propagated directly to the field. This practice led to the abandonment of many commercial orchards, as several North American cultivars were not adapted to Brazilian ecological conditions. In the 1950s, pecan seedlings from the U.S.A. were introduced in the Rio Grande do Sul State, Southern Brazil, where the climatic conditions are somewhat closer to the North American ones. This introduction aimed at developing systematic investigations towards the establishment of commercial orchards in this region of the country. Some of the introduced genotypes and seedlings from open pollination across these introduced plants still survive (Figure 1) and represent an important pool of genetic diversity, including genotypes highly adapted to the climatic conditions of Southern Brazil.

In this study, we aimed at characterizing the genetic diversity of a core of such ancient pecan

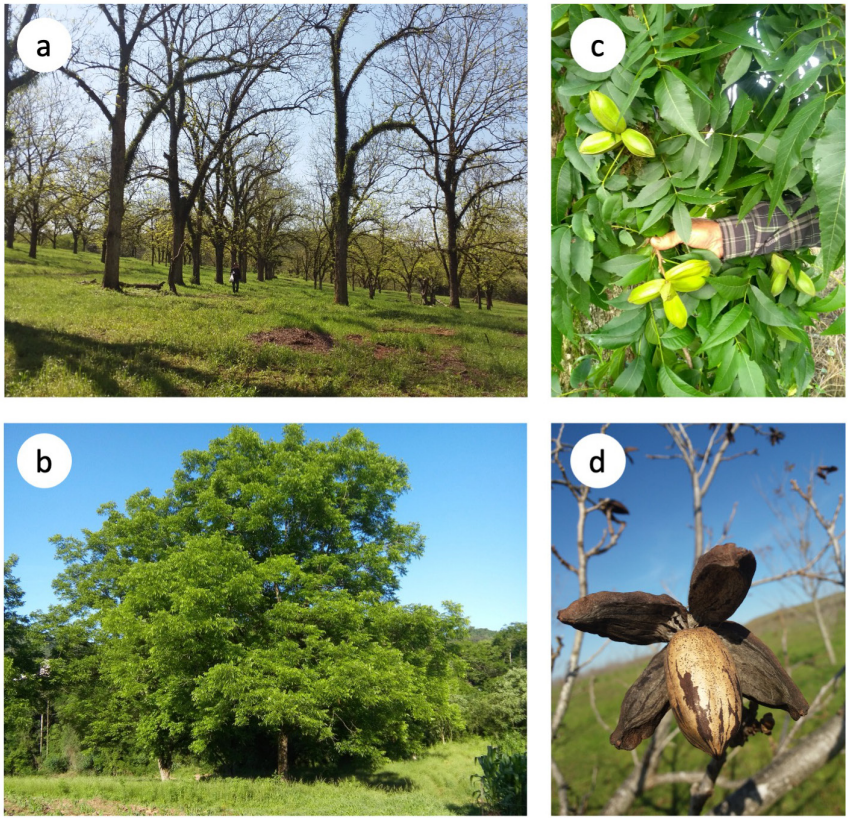

Figure 1. Pecan orchard in the Rio Grande do Sul State, southern Brazil. (a) Partial view of the ancient accessions in the orchard during the spring season. (b) Accession 29 during the summer season. (c – d) Fruits of ancient trees in the orchard.

trees without cultivar data recorded, using two different sets of molecular markers. In addition, we looked for correlations between molecular-genetic patterns and morphometric traits of the nuts. The results indicate that these old trees hold a rich pool of genetic diversity, with two genotypes presenting a high correlation between the S-SAP molecular pattern and nuts size. In addition, one of these genotypes also presents resistance to anthracnosis. Thus, the genetic diversity present in these ancient trees can be exploited in programs of genetic breeding, aiming at obtaining new commercial cultivars focusing on the Brazilian market and countries with similar environmental conditions.

MATERIALS AND METHODS

Sampling of plant material and DNA isolation

The 30 plants included in this study are ancient trees (older than 60 years) collected in a private orchard in the Rio Grande do Sul State, Brazil located at 28°58'02" S, 51°59'50" W, 420 m above sea level, with *Cfa* climate according to the Köppen classification (humid subtropical with the coldest month averaging above 0 °C, at least one month with the average temperature above 22 °C, no significant precipitation difference between seasons and no dry months in the summer; Kuinchtner & Buriol 2001). Healthy leaves from each tree were sampled and individually stored in paper bags filled with silica gel. Total genomic DNA was isolated from leaf samples from each plant using the CTAB protocol (Doyle & Doyle 1987). The isolated DNA was diluted in ultra-pure water and stored at -20 °C until use. DNA quality and quantity were evaluated by electrophoresis on a 0.8% agarose gel stained with GelRed® (Biotium) and visualized under UV light. A NanoVue™ spectrophotometer (GE Healthcare) was used to assess the DNA quality concerning the presence of phenolic contaminants, polysaccharides, and proteins.

Molecular genetic analyses

The genetic diversity and relatedness of the studied samples were assessed using AFLP (Vos et al. 1995) and S-SAP (Waugh et al. 1997) molecular markers. AFLPs are multilocus, dominant, and highly polymorphic markers based on the enzymatic restriction and PCR amplification of DNA fragments randomly distributed through the whole genome. The S-SAP technique combines the robustness of the classic AFLP procedure and the conservation of the termination RTL regions of retrotransposons, with the PCR amplification based on one AFLP primer combined with one primer complementary to an RTL sequence.

For the restriction/ligation reaction, about 100 ng of total genomic DNA were incubated with the restriction enzymes *Pst*I and *Mse*I at 37°C for 15 h followed by 72 °C for 15 min in a single reaction (2.5× of *T4* DNA ligase buffer, 5 U of *Mse*I, 5 U of *Pst*I, 50 ng μL^{-1} of BSA). The product of the restriction reaction was used as the template for the ligation of the adapters (10.0 pmol of *Mse*-adapter, 5.0 pmol of *Pst*-adapter, 1.0 U of *T4* DNA ligase) at 37°C for 8h. The reaction mixture was four-fold diluted and used as a template for the next steps of the AFLP and S-SAP reactions.

For the AFLP technique, the diluted DNA of the restriction-ligation reactions was amplified with the primer combinations *Pst*-GAG/*Mse*-GAA and *Pst*-GAG/*Mse*-GCC (Table I) in a 20 µL reaction (5.0 µL of template DNA, 0.25 pmol of fluorescently labeled *Pst* primer, 0.50 pmol of *Mse* primer, 1.0 U of *Taq* DNA polymerase, 1× of PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP). The *Pst*-GAG primer was fluorescently labeled with AlexaFluor® 680 nm (Invitrogen). The PCR conditions for the selective reaction were an initial denaturation step at 95°C for 2 min followed by 26 cycles at 95°C, 56°C,

Table I. AFLP and LTRs primer sequences employed for genotyping the ancient accessions of pecan.

AFLP primers	Primer sequence (5'- 3')
Pst-GAG	^{AlexaFluor680}GACTGCGTACAT GCAGACGAG
Mse-GAA	GATGAGTCCTGAGTAAGAA
Mse-GCC	GATGAGTCCTGAGTAAGCC
Mse-GCG	GATGAGTCCTGAGTAAGCG
Mse-GGA	GATGAGTCCTGAGTAAGGA
LTR primers	
CRM	ACCCAACGGGTGCACATC
Galadriel	GTGCGAGAAAAATAAATCGACCCTCTTTCA
Reina	AACCCCAGTAGATCTACTACGTAGCTAACA
Del	ACACTTTGGGACTTAAAAGCGTATTTTGAT

and 72°C for 1 min each and a final extension step at 72°C for 5 min. All PCR reactions were carried out in a BioRad C1000 Touch™ Thermo Cycler. AFLP fragments were separated through polyacrylamide gel electrophoresis using a Li-Cor 4300S automated DNA Sequencer® (Li-Cor Inc.) and automatically scored for the presence or absence of each fragment with aid of the Saga^{GT} Software® (Supplementary Material - Figure S1). An additional visual check of each gel was made to correct mismarked bands (Figure S1). The presence/absence of AFLP fragments was binary coded (1, 0) for posterior analyses.

For the S-SAP markers, diluted DNA of the restriction-ligation reactions was used as the template for PCR amplification with the combination of primers *Mse*-GGA/LTR and *Mse*-GCG/LTR, in a reaction containing 5 µL of template DNA, 0.25 pmol of LTR primer, 0.25 pmol of *Mse* primer, 1.0 U of *Taq* DNA polymerase, 2× of PCR buffer, 3.0 mM of $MgCl_2$, 0.2 mM of each dNTP. Primers for the retrotransposon regions were designed from conserved LTR regions, based on the DNA sequences of these elements, deposited in the GyDB database (Llorens et al. 2011). Primers for retrotransposons from four different elements of the *Ty3/Gypsy* LTR retrotransposon cluster (CRM, Galadriel, Reina, and Del) were designed (Table I).

PCR conditions were an initial denaturation step at 95°C for 2 min, followed by 26 cycles at 95°C, 56°C, and 72°C for 1 min each, and a final extension cycle at 72°C for 5 min. All PCR reactions were performed in a BioRad C1000 Touch™ thermal cycler. The S-SAP fragments were separated by electrophoresis on a 3% agarose gel, under 100 volts for about 90 minutes, using a 100 bp ladder as a reference. PCR products were stained with a solution of GelRed® and visualized under UV light. Gels were photographed and the images were analyzed using the TotalLab TL 120 1D v software 2009 (Nonlinear Dynamics Ltd., UK) for sizing the amplified fragments (Figure S1). The presence/absence of DNA fragments was coded as a binary matrix (1/0, respectively) for further data analysis.

Data Analysis

For both AFLP e S-SAP markers, the effective number of alleles (A_e) was estimated for each primer combination and overall. Assuming the absence of inbreeding (since the species is dioecious, thus reproducing primarily through cross-fertilization), gene diversity was estimated as H=2xpxq where $q=\sqrt{f_0}$ (with f_0 being the frequency of the fragment absence) and p=1-q . For markers whose information is based only on the presence/absence of the fragment, and assuming the existence of only two allelic states (presence or absence) the estimate of gene diversity ranges from H = 0.0 (all individuals have the same "allele", that is, presence or absence of the fragment) to H = 0.5 (same frequency for both "alleles"). The Shannon diversity index was computed as $I=-\sum\left[\left(p\times\ln_p\right)+\left(q\times\ln_q\right)\right]$, where p and q are the frequencies of 1 and 0, respectively. This index is independent of the Hardy-Weinberg

principle and holds I = 0.0 when all samples are monomorphic, independent of the "allele". The maximal estimate of this index is reached when both "alleles" have the same frequency in the evaluated group of samples and its value depends on the number of markers evaluated. All diversity parameters were estimated using the GenAlEx 6.5 software (Peakall & Smouse 2012).

Past 4.03 software (Hammer et al. 2001) was employed to estimate the pairwise genetic identity of the genotypes, based on the Jaccard index. The Jaccard index of similarity was computed as $J_{AB}=\frac{M_{11}}{M_{11}+M_{10}+M_{01}}$, where M_{11} is the number of fragments present in A and B, M_{10} is the number of fragments present in A and absent in B, and M_{01} is the number of fragments absent in A and present in B. A UPGMA dendrogram based on the identity matrix was built using the same software and the support of the clusters was determined using 1000 bootstrap replicates.

The estimations of the genetic diversity indexes and the pairwise genetic similarity were computed for each marker type individually and the combined dataset of AFLP and S-SAP markers.

Morphometric analyses of the nuts

A total of 600 fruits were collected (20 fruits from 30 trees) and had nine morphometric traits determined, focusing on two main characteristics: nut size [measuring the length, lateral diameter (the shortest axis), and longitudinal diameter (the longest axis) of the nuts] and kernel yield (measuring the total weight of the nut, kernel weight, shell weight, the ratio between the kernel and the shell weights, percentage of the kernel, and the number of nuts per kg). The length and diameter were obtained employing a calypter (0.01 mm) and weights were measured using a digital scale (0.001 g).

A UPGMA dendrogram based on the Morisita similarity index was built using the software Past 4.03 (Hammer et al. 2001) to evaluate the correlation among the sampled trees concerning the morphometric traits assessed. This index has been recommended whenever possible to avoid complex dealings with the effects of sample size and diversity (Wolda 1981).

RESULTS

Genetic diversity estimations

A total of 265 polymorphic AFLP fragments were evaluated, revealing a moderate multilocus genetic diversity estimation (Table II) for the effective number of alleles (A_e = 1.55, ranging from A_e = 1.43 to A_e = 1.64), gene diversity (H = 0.33, ranging from H = 0.27 to H = 0.37), and the Shannon index (I = 0.49, ranging from I = 0.41 to I = 0.55).

For S-SAP markers, 240 fragments were evaluated, resulting in 92 reliable and reproducible polymorphic markers (38.33% of polymorphism). In comparison to AFLP, S-SAP markers revealed a higher multilocus genetic diversity (Table II): A_e = 1.86 (ranging from A_e = 1.82 to A_e = 1.95), H = 0.46 (ranging from H = 0.45 to H = 0.49) and I = 0.65 (ranging from I = 0.63 to I = 0.68). The combined dataset comprised 357 fragments and the multilocus estimations of the genetic diversity indexes equaled A_e = 1.63, H = 0.37, and I = 0.53 (Table II).

Pairwise genetic relationship

The pairwise genetic identity of accessions had an average value of J = 0.35 based on the AFLP markers, J = 0.46 for S-SAP markers, and J = 0.39 for the combined dataset (Figure 2). The median was very similar to the media for all three datasets, while the highest variance and standard deviation were estimated for AFLP markers (Figure 2). The highest pairwise

Table II. Estimates of the effective number of alleles (A_e), gene diversity (*H*), and Shannon index (*I*) for 30 ancient accessions of pecan estimated with S-SAP markers, AFLP markers, and the combination of AFLP + S-SAP markers.

Primer combination	N.° of fragments	A_e	*I*	*H*
AFLP				
Pst-GAG/Mse-GAA	155	1.64	0.55	0.37
Pst-GAG/Mse-GCC	110	1.43	0.41	0.27
Multilocus AFLP	265	1.55	0.49	0.33
S-SAP				
Mse-GCG-CRM	11	1.89	0.66	0.48
Mse-GCG-DEL	11	1.84	0.65	0.46
Mse-GCG-GALADRIEL	11	1.82	0.63	0.45
Mse-GCG-REINA	11	1.95	0.68	0.49
Mse-GGA-CRM	12	1.86	0.65	0.47
Mse-GGA-DEL	12	1.87	0.65	0.47
Mse-GGA-GALADRIEL	12	1.83	0.64	0.46
Mse-GGA-REINA	12	1.83	0.64	0.46
Multilocus S-SAP	92	1.86	0.65	0.46
AFLP + S-SAP				
Multilocus AFLP+S-SAP	357	1.63	0.53	0.37

similarity was observed for S-SAP markers (*J* = 0.69 between trees 29 and 30) and the smallest for AFLPs (*J* = 0.15) for four pairs of trees.

Based on the combined dataset (Figure 3a), the dendrogram presented subclusters holding the genotypes with the largest nuts, but no clear trend concerning such a structuring based on the morphometric traits measured. On the other hand, the dendrogram based on the S-SAP dataset (Figure S2) performed slightly better than the dendrogram based on the AFLP dataset (Figure S3), regarding the clustering of genotypes related to the measured morphometric traits.

Morphometric analysis

The morphometric measurements revealed a relatively high variation in the size and shape of the fruits of the sampled trees (Figures 3b, 4). The largest fruits were observed in genotypes 29 and 30, while genotypes 7 and 28 presented the smallest fruits (Figures 3b, 4). These genotypes presented an average proportion of kernel ranging from 48.5% (genotype 28) to 57.5% (genotype 7). Genotypes 29 and 30 clustered together in both UPGMA dendrograms, based on molecular (Figure 3a) and morphometric (Figure 3b) data.

Based on the number of nuts per kg as a measurement of nuts size (as higher the number of nuts kg^{-1}, as smaller the nuts), the dendrogram based on morphometric traits (Figure 3b) revealed two main clusters. One cluster holds 11 trees which have a mean number of nuts kg^{-1} ranging from 179.0 to 316.3 nuts kg^{-1} (the smallest nuts). The second cluster contains 16 trees with a mean number of nuts kg^{-1} ranging from 96.4 to 166.2 nuts kg^{-1} (the largest nuts). The highest rate of kernel/shell was observed in genotype 9, meaning a higher proportion of kernel in the nut.

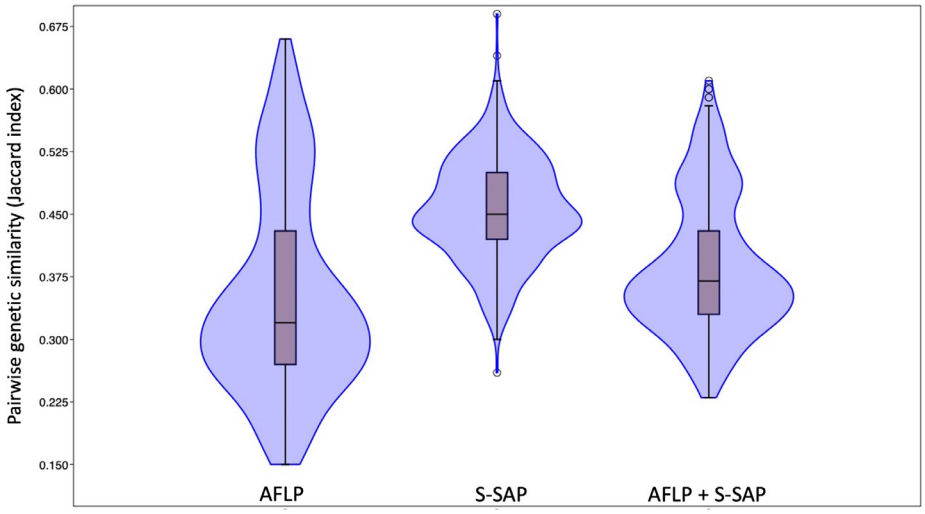

Figure 2. Pairwise genetic similarity between genotypes, based on the Jaccard index for AFLP, S-SAP, and AFLP + S-SAP markers. The curves around the boxplot represent the probability density function estimated. Wider curves represent a higher frequency of points. Outliers are represented by empty circles.

	Mean	Standard deviation	Median	Maximum	Minimum	Variance
AFLP	0.35	0.12	0.32	0.66	0.15	0.014
S-SAP	0.46	0.06	0.45	0.69	0.26	0.004
AFLP + S-SAP	0.39	0.08	0.37	0.61	0.23	0.007

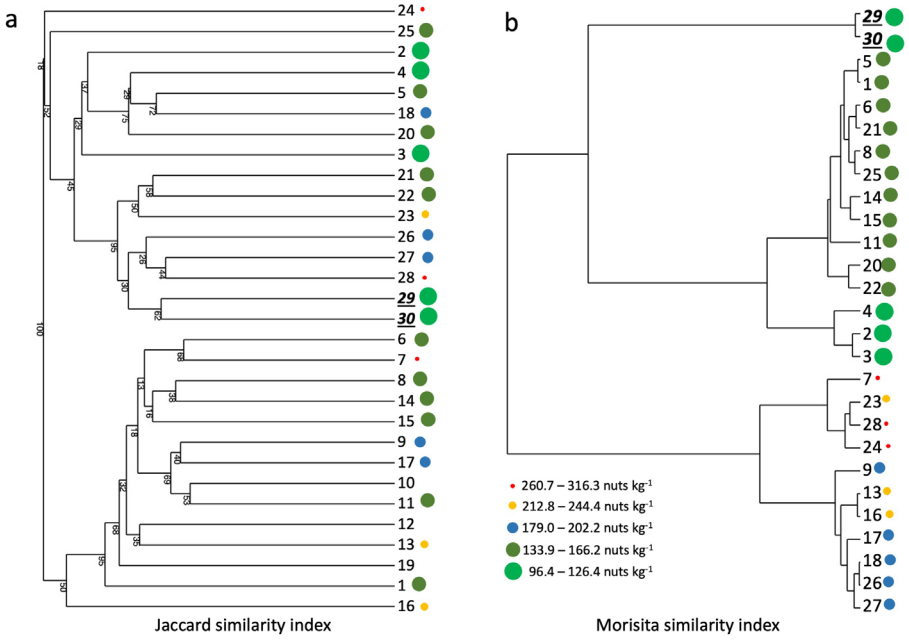

Figure 3. UPGMA dendrograms based on the Jaccard similarity index for AFLP + S-SAP markers (a) and the Morisita similarity index for morphometric traits (b). The circles in front of each number correspond to the mean size of the nuts, as estimated from the mean number of nuts per kg. The size intervals are listed in the inset in Figure b. Highlighted plants 29 and 30 present the largest nuts among all sampled trees.

DISCUSSION

Genetic variation is an important element in the breeding programs of pecan (Jia et al. 2011, de Oliveira et al. 2021), especially under a scenario of climatic changes. Independent of the dataset evaluated (AFLP, S-SAP, or AFLP + S-SAP), this study revealed high levels of genetic diversity in the sampled ancient pecan trees, supporting the expectation that this orchard holds a significant pool of genetic diversity. The effective number of alleles (A_e) is a diversity index that reflects the evenness of the allelic frequency and determines the number of alleles that likely will contribute to the next generation's gene pool in random crossing events, while estimates of the Shannon diversity index (I) and gene diversity index (H) represent the genetic multiplicity

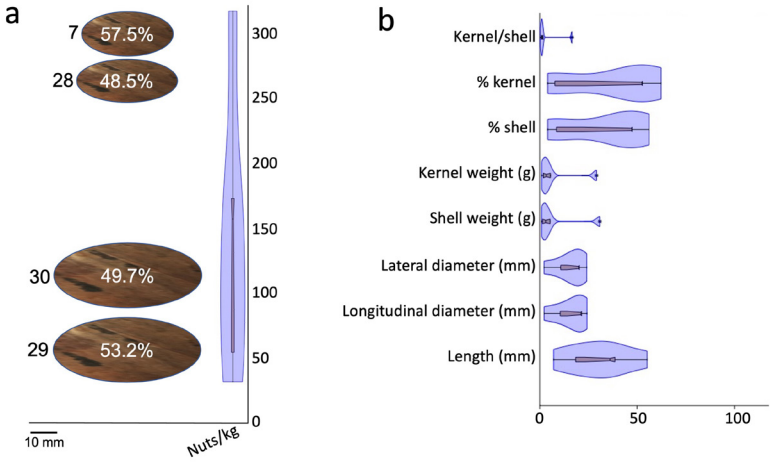

Figure 4. Average measures of morphometric traits of pecan nuts. (a) Schematic representation of the smallest (genotypes 7 and 28) and largest (genotypes 29 and 30) nuts, according to the mean number of nuts kg^{-1}. Values inside the nuts represent the average proportion of the kernel. The represented size of the nuts is based on the mean length and longitudinal diameters of 20 sampled nuts per plant. (b) Average values of morphometric traits. The curves around the boxplot represent the probability density function estimated. Wider curves represent a higher frequency of points.

of the evaluated samples. In comparison to pecan germplasm collections from different geographic origins evaluated with dominant molecular markers, our study revealed high estimates of the effective number of alleles (A_e = 1.63), Shannon index (I = 0.53), and gene diversity (H = 0.37) in the sampled genotypes (Table III).

ISSR markers were used to characterize a collection of pecan germplasm from China, including cultivars introduced from the U.S.A., Chinese seedling breeding cultivars, and some unrecorded accessions (Jia et al. 2011). The estimated effective number of alleles for ISSR was A_e = 1.46 and the gene diversity was H = 0.42. When genetic parameters were estimated only for North American cultivars, slightly lower values were obtained: A_e = 1.36 and H = 0.22 (Jia et al. 2011). Values of H = 0.37 and I = 0.54 for AFLP markers were reported in a sample of 60 pecan genotypes from southern Brazil (Poletto et al. 2020), including the 30 ancient plants from the present study (Table III).

Nuclear SSR markers were also employed to characterize the collection of Chinese and North American pecan germplasm (Jia et al. 2011). For this codominant marker, the estimated effective number of alleles was A_e = 1.42 and the gene diversity was H = 0.41 for the whole sample. For North American cultivars, lower values were obtained: A_e = 1.38 and H = 0.24 (Jia et al. 2011). The germplasm collection of the National Repository of Clonal Germplasm for Pecans and Hickories (NCGR-Carya) in the U.S.A. revealed estimations of A_e = 3.77, I = 1.20, and H = 0.52 for nuclear SSR markers and A_e = 1.55, I = 0.43, and H = 0.24 for plastid SSRs (Grauke et al. 2010). A sample including eight different cultivars and plants without defined cultivars collected in commercial Brazilian orchards and genotyped for 10 plastid SSR markers revealed high genetic diversity estimations (A_e = 2.80, H = 0.54, and I = 0.83; Nagel et al. 2022) in comparison to North American ones genotyped by Grauke et al. (2010).

Estimations of genetic parameters obtained using dominant molecular markers are not directly comparable with organellar markers (plastid and mitochondrial) and nuclear SSRs (nSSR). However, in comparison to other studies performed with dominant markers (ISSR and AFLP), the genotypes investigated in this study presented high levels of diversity (Table III).

Although there is no clear cluster that groups only trees with the largest nuts based on the genetic data (AFLP, S-SAP, or AFLPs + S-SAP), the combined dataset (Figure 3a) and the single datasets (Figure S2) suggest that there is an important correlation between morphometric traits and the genetic markers to be exploited.

Table III. Genetic diversity estimations for different collections of pecan genotypes, using different molecular markers. The estimations are not directly comparable for markers with different natures (dominant, codominant, or plastid markers).

Marker	*Ae*	*H*	*I*	Origin	Reference
Dominant markers					
AFLP	1.55	0.33	0.49	Brazil	This study
S-SAP	1.86	0.46	0.65	Brazil	This study
AFLP+S-SAP	1.63	0.37	0.53	Brazil	This study
ISSR	1.46	0.42	--	China + USA	Jia et al. (2011)
ISSR	1.36	0.22	--	USA	Jia et al. (2011)
AFLP	--	0.37	0.54	Brazil	Poletto et al. (2019)
Plastid markers					
cpSSR	1.55	0.24	0.43	USA	Grauke et al. (2010)
cpSSR	2.80	0.54	0.83	Brazil	Nagel et al. (2022)
Codominant markers					
nSSR	3.77	0.52	1.20	USA	Grauke et al. (2010)
nSSR	1.42	0.41	--	China + USA	Jia et al. (2011)
nSSR	1.38	0.24	--	USA	Jia et al. (2011)

For instance, trees 29 and 30, which present the largest nuts, clustered together based on the combined genetic dataset, with moderate bootstrap support (BS = 62%; Figure 3b). When the AFLP and S-SAP datasets are evaluated separately, genotypes 29 and 30 cluster together in the S-SAP dendrogram, but not in the AFLP dendrogram (Figure S2), suggesting that some S-SAP genetic markers may be linked to genomic regions of interest for selection and breeding. Accordingly, retrotransposons are recurrently detected in the regions flanking plant genes (White et al. 1994).

In addition to holding the largest nuts among all sampled plants, genotype 29 also demonstrated resistance to anthracnosis (our unpublished data), an important trait considering the expansion of plantation areas around the world, that may cause more frequent epidemics caused by fungal pathogens (Poletto et al. 2021). Thus, the establishment of core collections with high genetic diversity will enable the elaboration of more detailed studies related to nuts characteristics, resistance to diseases, phenology, and other stable agronomic characteristics, at least partially adapted to the edaphoclimatic conditions of southern Brazil. This can assist in obtaining new commercial cultivars that may attend the Brazilian market and other countries with similar environmental characteristics. Farmers have already subjected these genotypes to an empirical selection process when they were preserved in the orchards. Although it was not a classic breeding program, most genotypes were selected because they present some characteristic of specific interest that deserves preservation. These results also support the need for further sampling expeditions looking for more ancient pecan plants. Considering the advance of climate changes, such established genotypes are likely more adapted to the edaphic-climatic

conditions of the region and hold important alleles and genotypes to broaden the genetic diversity of pecan germplasm collections.

Perspectives for studies with pecan are advancing, although specialized laboratories and studies are relatively scarce in comparison to other crop species. However, the establishment of core collections for breeding programs should be grounded in phenotypically and genetically well-characterized genotypes. Further studies are necessary, especially in regions where the growth of production is expected. Genetic studies may contribute to selecting superior cultivars and establishing germplasm collections interrelated with productivity. Additionally, establishing multidisciplinary research groups with collaboration among local pecan producers through a participatory breeding program may improve the conservation of local genetic diversity and traits neglected by formal and centralized breeding programs (Poletto et al. 2021).

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SUPPLEMENTARY MATERIAL

Figures S1, S2, S3.

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LOO, DDS, and TP performed the laboratory and data analyses; TP and IP identified and sampled the accessions; DCB, and JNOZ contribute to data analysis; VMS conceived the study, contributed to materials and reagents, coordinated the study, and wrote the manuscript; MFBM contributed to materials and reagents. All authors read and approved the manuscript for publication.