Genetic variability in pecan genotypes in Brazil

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ABSTRACT

Pecan crops has been expanding in recent years, mainly in southern Brazil. Genotypes that compose Brazilian orchards come from the USA and from selections of plants made by Brazilian producers. This study aimed to characterize with microsatellite markers pecan cultivars registered for cultivation in Brazil, including some selections made in the country. It is important to know the genetic variability of the pecan tree, as it facilitates the identification of possible phytotechnical deficiencies due to the genetic similarity between the plants, in addition to helping in the conservation of the species, among other. Thirty-four, out of forty collected accessions, were genotyped with 11 selected SSR (Simple Sequence Repeats) loci. Twenty-four polymorphic alleles were identified. The genetic similarity matrix, based on the Jaccard coefficient, ranged from 0.125 to 1.0; general mean of similarity was 0.46. The cluster analysis, which was carried out by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), classified pecan accessions into four main groups. Results showed that there is high genetic variability in germplasm evaluated, although some accessions may be duplicates.

Keywords: Carya illinoinensis; molecular markers; microsatellites.

INTRODUCTION

Pecan trees are native to the United States and Mexico, even though they have been commercially grown in several countries. They bear a dry fruit called pecan nut which has arisen producers' and consumers' interest due to its price, product diversification in rural areas and health benefits that result from its frequent consumption (Wells, 2017a; Fronza *et al*., 2018).

The crop was introduced into southeastern Brazil by North-american immigrants in 1870 (Ortiz & Camargo, 2005; Bilharva *et al*., 2018; Poletto *et al*., 2020). Rio Grande do Sul (RS) state is the largest pecan producer, especially in the cities Anta Gorda (28° 58'13''S e 52° 00'17''W) and Cachoeira do Sul (30°02'21''S e 52°53'38''W) are the municipalities with the largest production areas. It is estimated that in the southern region there are 6.91 thousand hectares implanted with the crop (Bilharva *et al*., 2018; Gatto *et al*., 2008; Fronza *et al*., 2018).

These differences between pecan tree plants can be seen in the characteristics, such as fruit shape and size, quality, plant architecture and reproduction. In Brazil, 43 cultivars have been registered at the Ministry of Agriculture, Livestock and Food Supply (Mapa, 2020). In addition, there is genetic material of unknown origin which resulted from selections carried out by farmers and nurserypersons, taking into account morphological, productive and genetic aspects (Hamann *et al*., 2018; Poletto *et al*., 2020).

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Pecan is a perennial plant, deciduous and monoecious fruit tree (having pistillate and staminate flowers on the same plant). In addition, the pecan has a natural mechanism called dichogamy, where the period of pollen release and stigma receptivity are different, and this period may be complete or partial. However, there is a possibility that a small percentage of self-pollination will occur, normally pollination is anemophilous (Thompson & Romberg, 1985; Sparks, 1992; Wells, 2017b).

To know the magnitude of the genetic variability available in the country is an important step to ensure the development of pecan crop in the future. An alternative to help to identify and characterize genetic diversity of a species is the use of molecular markers, which enable differences among plants to be revealed by DNA analysis. Several studies have described different techniques, such as the Polymerase Chain Reaction (PCR) (Ferreira & Grattapaglia, 1996; Ferreira & Grattapaglia, 1998; Grauke *et al*., 2003).

Regarding the different types of markers based on the PCR, microsatellite markers or Simple Sequence Repeats (SSR), are the most successful ones in studies of genetic variation, mapping, genotype identification and others (Caixeta *et al*., 2009; Wang *et al*., 2010; Librelon *et al*., 2013).

This study aimed to characterize with microsatellite markers pecan cultivars registered for cultivation in Brazil, including some selections made in national territory, to know magnitude of the genetic variability cultivated in the country.

MATERIAL AND METHODS

Forty pecan genotypes were evaluated. Twenty-one are cultivars registered at the Ministry of Agriculture, Livestock and Food Supply (Mapa, 2020), while 19 are selections found in orchards in southern Brazil (Table 1). Considering all cultivars under analysis, farmers believe that about 38% of them are the most grown in the region.

Plant material was collected from young leaves (disease-free folioles were thoroughly extended) and taken to the Laboratory of Molecular Biology and stored in an ultrafreezer at -80 ºC, up to DNA extraction.

Leaf genomic DNA was extracted with the protocol of DNA isolation based on cetyltrimethylammonium bromide (CTAB), in agreement with the methodology proposed by Ferreira & Grattapaglia (1996).

Sample quantification was carried out by a NanoVue Plus™ spectrophotometer which enabled to estimate resulting DNA concentration and quality, based on the A260/280 nm ratio. Only samples whose A260/280 was between 1.8 and 2.1 were used. In order to check DNA integrity, 1% agarose gel electrophoresis, with GelRed (coloring) and Hind III (marker), was also carried out. Afterwards, samples were diluted in ultrapure water and adjusted to 20 ng μL^{-1}.

PCR reactions were carried out in agreement with the protocol described by Grauke *et al*. (2003). Final volume of reaction was 10 µL, which consisted of: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.0 mM $MgCl_2$, 0.16 mM dNTPs, 30 ng of every primer (*Invitrogen*™), 30 ng DNA and 0.5 µL Taq DNA polymerase (*Invitrogen*™). SSR primers were PM- CA 07, PM- CA 10, PM- CA 12, PM- CTA 24, PM- GA 19, PM- GA 23, PM- GA 28, PM- GA 31, PM- GA 38, PM-GA 39, PM-GA 41 and PM- GA 44 (Grauke *et al*., 2003). Table 2 shows the primers, sequences, sizes and annealing temperatures under study.

Amplifications were carried out by the Gene Amp® PCR System 9700 thermocycler (Applied Biosystems). Amplification cycles were a denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 seconds; in the annealing phase, temperatures ranged to be adequate for every primer for 30 seconds, at 72 °C for 1 min and ended with an extension cycle at 72 °C for 3 min and a cycle at 4 °C up to sample removal from the thermocycler (Grauke *et al*., 2003).

Amplification products were separated by 2% agarose gel electrophoresis with the use of the molecular marker 1 Kb plus DNA Ladder (Invitrogen™). Amplified fragments were visualized by the Gel Logic 2200 Imaging System (Kodak).

Amplification results of every locus under analysis were registered qualitatively, i. e., presence (1) or absence (0). Although SSR markers are codominant markers, due to the amplification of some locis in more than one region, the markers were treated as dominant.

The genetic distance matrix was generated using the complement of the Jaccard similarity coefficient. Based on the resulting genetic distance matrix, the cluster analysis was performed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), with the help of the NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, version 2.02 (Rohlf, 1998).

Table 1: Pecan genotypes under evaluation and origins of every accession

Number	Genotype	Genealogy Paternal	Genealogy Maternal	Classification	Place of origin	Year	Collection area
5	Apache	Schley	Burkett	Cultivate	Texas	1940	Cachoeira do Sul
25	Barton 01	Success	Moore	Cultivate	Texas	1937	Pelotas
38	Barton 02	Success	Moore	Cultivate	Texas	1937	Pelotas
39	Barton 03	Success	Moore	Cultivate	Texas	1937	Pelotas
40	Barton 04	Success	Moore	Cultivate	Texas	1937	Pelotas
13	Caddo	Alley	Brooks	Cultivate	Georgia	1922	Cachoeira do Sul
15	Cape Fear	-	Schley	Cultivate	Carolina do Norte	-	Cachoeira do Sul
2	Cherokee	Evers	Schley	Cultivate	Texas	1948	Cachoeira do Sul
21	Cheyenne	Odom	Clark	Cultivate	Texas	1942	Cachoeira do Sul
14	Chickasaw	Evers	Brooks	Cultivate	Texas	1944	Cachoeira do Sul
24	Choctaw	Mahan	Success	Cultivate	Texas	1946	Cachoeira do Sul
1	Cowley	-	-	Cultivate	Oklahoma	-	Cachoeira do Sul
22	Desirable	Jewett	Success	Cultivate	Mississipi	1900	Cachoeira do Sul
23	Elliot	-	-	Cultivate	Florida	-	Cachoeira do Sul
31	Farley	-	-	Cultivate	Florida	1918	Pelotas
11	Gratex	Success	Ideal	Cultivate	Texas	1945	Cachoeira do Sul
28	Imperial	-	-	Cultivate	Texas	1958	Pelotas
30	Importada	-	-	Cultivate	-	-	Pelotas
33	Jackson	Success	Schley	Cultivate	Mississipi	-	Canguçu
10	Mahan	-	-	Cultivate	Mississipi	1910 (planted)	Cachoeira do Sul
27	Melhorada	-	-	Cultivate	-	-	Pelotas
26	Mohawk	Mahan	Success	Cultivate	Texas	1946	Cachoeira do Sul
19	Shawnee	Barton	Schley	Cultivate	Texas	1949	Cachoeira do Sul
20	Shoshoni	Evers	Odom	Cultivate	Texas	1944	Cachoeira do Sul
12	Sioux	Camichael	Schley	Cultivate	Texas	1943	Cachoeira do Sul
3	Stuart	-	-	Cultivate	Mississipi	1874 (planted)	Cachoeira do Sul
29	Success	-	-	Cultivate	Mississipi	-	Pelotas
18	Sumner	-	-	Cultivate	Georgia	-	Cachoeira do Sul
9	Western	-	-	Cultivate	Texas	-	Cachoeira do Sul
17	Tejas	Risien	Mahan	Cultivate	Texas	-	Cachoeira do Sul
16	Wichita	Mahan	Halbert	Cultivate	Texas	1940	Cachoeira do Sul
4	17CF	-	-	Selection	-	-	Cachoeira do Sul
6	Crioula	-	-	Selection	-	-	Cachoeira do Sul
7	17PF	-	-	Selection	-	-	Cachoeira do Sul
8	17PFB	-	-	Selection	-	-	Cachoeira do Sul

Continue

Continuation

Number	Genotype	Genealogy Paternal	Genealogy Maternal	Classification	Place of origin	Year	Collection area
34	Selection M	-	-	Selection	-	-	Canguçu
32	Mioto	-	-	Selection	-	-	Pelotas
35	Selection PS	-	-	Selection	-	-	Canguçu
36	Selection MI	-	-	Selection	-	-	Uraí
37	Selection MII	-	-	Selection	-	-	Uraí

*Adapted from: Hamann et al., 2018; Martins et al., 2018; Grauke LJ & Thompson TE –USDA- Pecan Breeding Program- Naticional Collection of Genetic Resources for Pecans and Hickories-https://cgru.usda.gov/carya/pecans/cvintro.htm.

Table 2: Sequence, size and annealing temperatures of SSR primers used for genetically characterizing pecan accessions

Primer Identification	Sequences forward 5´-3´ e reverse 5´-3´	Size (bp)	Annealing temperature (˚C)
PM-CA07	CAATCAACCCTACGACATACAGTG CGCGCACTCA CACATACTCATAC	199	56
PM-CA10	AAATCAACCCTGTAGCATACAATG GCTCAGACATGCAAACGTACC	113	48
PM-CA12	AGATCGAAAAGCGTGGAGCAAC ACACCGAATTCTCAATGAGCCAAAC	173	51
PM-CTA24	AAATGGTGAGGATGAGGAAGTGAA GATATGAA GCCCCTTATACAGTTCTACCTCTCTC	123	53
PM-GA19	CAAAAGGTTCAGGAAAGGTCTAGAGA GGTAAAAAAGTTATTAGCCCGCACT	80	54
PM-GA23	CAGACCATCATCAACCCATCTCT AGTGTAATGGTTTTGAGGGAGTGA	131	57
PM-GA28	AATGAGATGACTACATACACAAGTC GG GGGCTCGCATACCTTCATGA	111	48
PM-GA31	TGAACTCCAAAAGCCTCCTCTC GTATTTGTATTTTTTCCTTGAGCTTTCTC	83	48
PM-GA38	AAAAGTTTTAGGGTTGTTTGCTCTCT GTAAAGCCTACAACCTACAACAGTCTATG	89	48
PM-GA39	TGTAAATGCGTGCTATTGCTGAT GAATAGACAAAGAAACGAAACTCATTGA	89	48
PM-GA41	TCTTCAGAAAAAACCCTTACCTCTCT GAAAAATATAAACTCCCATAGTACCCACAT	89	48
PM-GA44	AATTACAGAGTCTCGGTAACACAGAGAG CAGCTTCTCCGGTATCTCCTATCT	107	48

*Sequence of SSR primer pairs and annealing temperature calculated in agreement with fragment size. However, some primers underwent some adaptation. Source: Grauke et al., 2003.

RESULTS AND DISCUSSION

Eleven, out of 12 SSR primer pairs under study, were selected for the analysis. Primer PM-CA 12 was removed because it exhibited low-quality amplification patterns, besides a high percentage of failures. It didn't amplified in 30.77% of genotypes.

A total of 24 polymorphic bands were identified in the 11 selected primer pairs. Due to their low DNA quality, six genotypes – 'Caddo', 'Chickasaw', 'Mohawk', Success 02, 'Importada' and Selection Mi01 – also had to be removed from the analysis. Therefore, 34 out of 40 collected accessions were evaluated.

Mean similarity of genotypes under study was 46%. It shows that the percentage of genetic difference among accessions is higher than the similarity among them. The similarity matrix (Figure 1) shows that some pecan genotypes exhibited more than 80% genetic divergence, i. e., less than 20% similarity. Success exhibited the highest genetic divergence with cultivars Cowley, Choctaw and Selection PF 17 b, i. e., 80%, 82% and 87%, respectively. However, some pecan genotypes, such as 'Choctaw' and 'Checokee' (17%), Selection M1 and 'Farley' (25%) and 'Farley' and Selection PS (18%), exhibited less than 30% genetic difference.

Based on the cluster analysis, with a cophenetic correlation coefficient was 73.10% with the genetic similarity matrix, four large groups were identified (Figure 2). The cut off point corresponSded to the mean genetic similarity of about 46%.

Group I comprised exclusively by 'Success'. This cultivar was genetically very different from the other genotypes under study. It was also found to be distant from genotypes with which it has some parental in common, such as cultivars 'Barton', 'Gratex', 'Desirable', 'Choctaw' and 'Jackson'. The hypothesis that can explain the distance from 'Success' is that the markers used may be in distinct, random regions, and perhaps not linked to the region where the common ancestor is located.

Group II comprised only two cultivars: one of unknown origin ('Sumner') and 'Shoshoni', which resulted from a cross between 'Evers' and 'Odom'. Both cultivars Summer and Shoshoni exhibited 67%. It was higher than the mean, which was 0.46, revealing the proximity of both materials. Group III was composed of cultivars Stuart, Barton (2, 3 and 4), Elliot and Cheyenne.

Conner & Wood (2001) used RAPD markers to study pecan cultivars and found similarity between 'Elliot' and 'Barton' (0.46). The comparison of their results and the ones found by this study shows that values were similar, since genetic similarity between Elliot and Barton 3 and 4 was 0.47. Both studies confirm that there is similarity between these cultivars, based on the analysis of the DNA level.

Subgroup IV-A comprised most genotypes that exhibited proximity in their origins. For instance, cultivars Desirable, Choctaw and Gratex had a progenitor in common, i. e., Success. Thus, they were included in the same group. Besides, cultivars Choctaw, Wichita and Tejas, whose origin had the participation of the cultivar Mahan, and were grouped close to each other, mainly Wichita and Tejas, which had high degree of genetic similarity. In addition, they were quite close to Mahan since the degree of similarity was 0.833.

Grauke *et al*. (2003) used SSR and studied some pecan accessions, such as cultivars Wichita and Mahan, genetically. Both exhibited genetic similarity (0.63). The results found in the present study does not corroborate those observed by Grauke *et al*. (2003), since similarity was higher (0.833). High similarity between both cultivars was expected because Mahan is in the origin of Wichita.

Jia *et al*. (2011) also observed the close grouping relationship of the cultivars Mahan and Wichita, which presented a genetic similarity of 0.86. Confirming this close genetic relationship between these two cultivars, as can also be observed in the present study.

The cultivar Cowley and the Selection Crioula showed the same molecular profile and formed a group whose genetic similarity was 0.667 in relation to Desirable. The Selection Crioula is a genotype that was selected by farmers and probably is a duplicate of 'Cowley', a further study, with the aid morphological descriptors can help to elucidate this.

Both cultivars, Apache and Shawnee, were classified into the same group. Both cultivars have an ancestral in common, the cultivar Schley. 'Shawnee', results from a cross between 'Schley' and 'Barton', is a cultivar that exhibits moderate yield, precocity and low tolerance to scabies. The cultivar Apache, which results from a cross between 'Burkett' and 'Schley', is more tolerant to this disease (Walker *et al*., 2016).

	Cowley	Cherokee	Stuart	17C_PF	Apache	Crioula	PF17	PF17_B	Western_S	Mahan	Gratex	Sioux	Cape_Fear	Wichita	Tejas	Sumner	Shawnee	Shoshoni	Cheyenne	Desirable	Elliot	Choctaw	Barton01	Melhorada	Imperial	Success	Farley	Jackson	SelM	SelPS	SelM-4	Barton2	Barton3	Barton4
Cowley	1.000																																
Cherokee	0.538	1.000																															
Stuart	0.429	0.467	1.000																														
17C_PF	0.538	0.467	0.467	1.000																													
Apache	0.636	0.538	0.429	1.000	1.000																												
Crioula	1.000	0.571	0.375	0.571	0.667	1.000																											
PF17	0.538	0.571	0.375	0.571	0.538	0.571	1.000																										
PF17_Barros	0.538	0.667	0.538	0.429	0.500	0.538	0.333	1.000																									
Western_Sahley	0.538	0.692	0.294	0.467	0.538	0.571	0.571	0.429	1.000																								
Mahan	0.429	0.571	0.571	0.571	0.538	0.467	0.692	0.333	0.571	1.000																							
Gratex	0.500	0.429	0.176	0.538	0.636	0.538	0.538	0.286	0.667	0.333	1.000																						
Sioux	0.583	0.400	0.235	0.400	0.462	0.615	0.400	0.357	0.500	0.313	0.462	1.000																					
Cape_Fear	0.462	0.313	0.167	0.500	0.583	0.500	0.400	0.357	0.400	0.235	0.727	0.538	1.000																				
Wichita	0.538	0.692	0.467	0.692	0.667	0.571	0.833	0.429	0.692	0.833	0.429	0.400	0.313	1.000																			
Tejas	0.538	0.692	0.467	0.692	0.667	0.571	0.833	0.429	0.692	0.833	0.429	0.400	0.313	1.000	1.000																		
Sumner	0.385	0.333	0.333	0.538	0.500	0.333	0.538	0.200	0.333	0.429	0.385	0.313	0.462	0.538	0.538	1.000																	
Shawnee	0.636	0.429	0.538	0.667	0.636	0.667	0.818	0.385	0.429	0.667	0.500	0.462	0.462	0.667	0.667	0.500	1.000																
Shoshoni	0.429	0.294	0.294	0.250	0.400	0.353	0.353	0.590	0.278	0.353	0.167	0.467	0.294	0.438	0.438	0.500	0.400	0.438	1.000														
Cheyenne	0.400	0.438	0.643	0.438	0.400	0.353	0.353	0.500	0.278	0.353	0.167	0.467	0.294	0.438	0.438	0.500	0.400	0.438	1.000														
Desirable	0.636	0.538	0.333	0.538	0.636	0.667	0.429	0.636	0.538	0.429	0.385	0.583	0.400	0.538	0.538	0.200	0.500	0.176	0.400	1.000													
Elliot	0.538	0.571	0.571	0.571	0.667	0.467	0.667	0.375	0.429	0.294	0.438	0.583	0.400	0.375	0.375	0.429	0.333	0.222	0.533	0.429	1.000												
Choctaw	0.667	0.833	0.375	0.375	0.429	0.692	0.571	0.538	0.571	0.467	0.429	0.400	0.313	0.571	0.571	0.333	0.429	0.375	0.353	0.533	0.467	1.000											
Barton01	0.385	0.429	0.333	0.538	0.500	0.429	0.538	0.286	0.429	0.538	0.250	0.583	0.357	0.667	0.667	0.500	0.429	0.158	0.533	0.538	0.571	0.375	1.000										
Melhorada	0.500	0.333	0.250	0.333	0.385	0.538	0.429	0.286	0.429	0.333	0.500	0.900	0.583	0.333	0.333	0.286	0.538	0.333	0.313	0.385	0.250	0.333	0.385	1.000									
Imperial	0.538	0.375	0.222	0.375	0.429	0.571	0.467	0.538	0.375	0.294	0.429	0.615	0.750	0.375	0.375	0.333	0.538	0.294	0.353	0.538	0.294	0.375	0.429	0.667	1.000								
Success	0.200	0.250	0.333	0.538	0.500	0.250	0.333	0.125	0.250	0.538	0.250	0.357	0.357	0.429	0.429	0.500	0.385	0.250	0.400	0.385	0.250	0.176	0.667	0.286	0.250	1.000							
Farley	0.636	0.667	0.250	0.429	0.500	0.667	0.667	0.385	0.667	0.538	0.500	0.615	0.583	0.667	0.667	0.538	0.500	0.538	0.400	0.500	0.333	0.667	0.636	0.636	0.667	0.385	1.000						
Jackson	0.636	0.429	0.250	0.429	0.500	0.667	0.429	0.385	0.538	0.333	0.500	0.909	0.583	0.429	0.429	0.333	0.500	0.429	0.400	0.500	0.333	0.429	0.500	1.000	0.667	0.667	1.000						
SelçãoM	0.462	0.313	0.235	0.313	0.357	0.500	0.400	0.267	0.400	0.313	0.462	1.000	0.538	0.313	0.313	0.267	0.500	0.313	0.375	0.462	0.235	0.313	0.462	0.969	0.615	0.357	0.583	1.000					
SelçãoPS	0.500	0.538	0.176	0.333	0.385	0.538	0.538	0.286	0.538	0.429	0.385	0.615	0.462	0.538	0.538	0.429	0.385	0.429	0.400	0.636	0.250	0.538	0.800	0.500	0.538	0.500	0.818	0.538	1.000				
SelçãoM-4	0.462	0.500	0.167	0.313	0.357	0.500	0.500	0.462	0.615	0.400	0.462	0.467	0.667	0.500	0.500	0.400	0.357	0.500	0.294	0.462	0.235	0.500	0.462	0.462	0.750	0.267	0.750	0.500	0.429	1.000			
Barton2	0.333	0.467	0.467	0.692	0.667	0.375	0.375	0.429	0.294	0.375	0.333	0.313	0.313	0.467	0.467	0.333	0.429	0.158	0.533	0.538	0.571	0.375	0.538	0.176	0.222	0.538	0.250	0.250	0.235	0.333	1.000		
Barton3	0.429	0.571	0.571	0.571	0.538	0.467	0.467	0.538	0.375	0.467	0.250	0.400	0.235	0.571	0.571	0.250	0.538	0.222	0.643	0.667	0.467	0.467	0.667	0.250	0.294	0.429	0.333	0.333	0.313	0.429	0.833	1.000	
Barton4	0.429	0.571	0.571	0.571	0.538	0.467	0.467	0.538	0.375	0.467	0.250	0.400	0.235	0.571	0.571	0.250	0.538	0.222	0.643	0.667	0.467	0.467	0.667	0.250	0.294	0.429	0.333	0.333	0.313	0.429	0.833	1.000	1.000

Figure 1: Genetic similarity matrix of 34 pecan genotypes. Pelotas, RS, 2020.

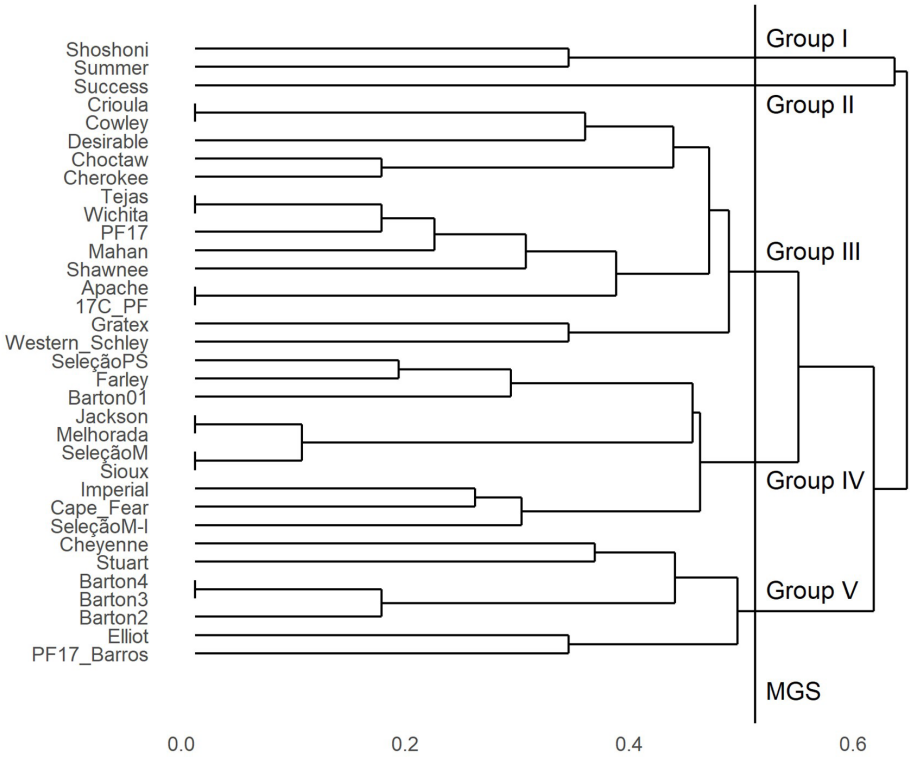

Figure 2: Dendrogram of genetic relations among pecan genotypes based on SSR markers, generate of by Jaccard coefficient. Mean genetic similarity (MGS). Pelotas, RS, 2020.

Subgroup IV-B exhibited high degree of genetic similarity (0.90) among genotypes. 'Sioux' and Selection M presented the same genetic profile, as well as 'Melhorada' and 'Jackson'. These local selections may be duplicates of these cultivars. Both cultivars, Sioux and Jackson, also showed high similarity (0.91), it can be explained by the common ancestral that they share, since their female progenitor is 'Schley'.

Silva *et al*. (2019) studied 22 plants selected from *Passiflora maliformis* L. and used RAPD and ISSR molecular markers to show variation among sweet calabash plants. Genetic similarity found by the authors ranged from 0.15 to 0.78. The study reported by this paper also found genetic differences among pecan accessions, whose similarity ranged from 0.125 to 1.0. Even though both crops are different, variations identified in the fruit trees are important. The difference found in a population, regardless of the species, is important, since it enables individuals to respond differently to environmental conditions as the result of their high genetic variation.

In order to compose the set of genotypes under evaluation, samples of the cultivar Barton were collected of distinct plant nurseries. High similarity was found among three of them; in Group III, it was 0.833. However, the sample identified as Barton 01 composed Subgroup IV-B, because it was very distant from the other three samples of this cultivar. The hypothesis that may explain the difference among the material – which should be closer – is that it was either switched for another sample or wrongly identified before commercialization or throughout propagation in the nursery. This result leads to the belief that the accession identified as Barton 01 does not belong to this cultivar.

Poletto *et al*. (2020) have recently studied morphometric, chemical and genetic aspects of pecan and found high genetic variation in the plants. Their study, which was based on AFLP markers, obtained 154 polymorphic fragments whose cophenetic coefficient was 0.84. Besides, the authors carried out morphometric evaluation of fruit, which also showed several differences in shape and size; as a result, fruit may aim at different markets.

Sharma & Sharma (2001) studied genetic divergence of *Juglans regia* L. plants and grouped them in 16 groups according to their characteristics. According to the authors, the difference among plants is often chosen as a requirement to conduct a good selection process. Ghanbari *et al*. (2019) also evaluated genetic difference among 31 European nut trees with the use of ISSR markers. In this case, they were classified into three large groups and 26 alleles; mean per locus was 7. Their results were different from the ones found by this study, but they showed differences among other species of fruit trees.

The most common cultivars in Brazilian orchards are 'Barton', 'Melhorada', 'Imperial', 'Importada', 'Jackson' and 'Shawnee' (Crosa *et al*., 2020). Among them, there are some genetically very similar. For instance, 'Melhorada' is closer to 'Jackson', 'Imperial' and 'Shawnee', with 1.000, 0.667 and 0.538, respectively (Figure 1). Nevertheless, 'Barton' 03 and 04 exhibited high genetic divergence in this group of cultivars, since its values of genetic similarity were 0.250 ('Melhorada'), 0.294 ('Imperial') and 0.333 ('Jackson').

Oliveira *et al*. (2021) highlight different studies and advances in the technology used in the characterization or genetic identification in the pecan culture, along with the increased need to seek improvements in production aspects among other characteristics that influence nut production. The authors also noted a progress in recent years in relation to studies directed to the area of molecular biology in relation to culture.

Since Pecan crop introduction in Brazil, several selections were made in the nurseries, resulting in an important source of germplasm (Bilharva *et al*., 2018; Poletto *et al*., 2020). Our study shows that, although some of the selections analyzed were very similar to some cultivars, others are genetically distinct, showing a unique molecular profile, highlighting the importance of this genetic variability for the development of pecan crop in Brazil.

CONCLUSIONS

Pecan genotypes under evaluation exhibit high genetic variation.

Some potential duplicates were identified in germoplasm evaluated.

The SSR loci analyzed in this study are good markers for genetic identification pecan germplasm cultivated in Brazil.

Based on the loci under evaluation and the number of identified alleles, genotypes could be classified into four main groups.

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