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Genetic divergence among *Psidium* accessions based on single nucleotide polymorphisms developed for *Eucalyptus*

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ABSTRACT. The goal of this study was to analyze the genetic divergence among *Psidium* species accessions based on SNPs developed for *Eucalyptus*. Fifty-three *Psidium* accessions, including 47 *P. guajava*, were genotyped with EUCHIP60K. The dendrogram similarity ranged from 0.58 to 1.00, with a cophenetic value of 0.97. Five groups were identified at dendrogram cut point of 0.7: the first with 44 guava accessions, the second with 1 guava accession, the third with 3 *P. guineense* accessions, the forth with 2 guava accessions, and the fifth with 3 *P. cattleianum* accessions. The Bayesian analyses suggested seven subpopulations, with formation of two additional groups with guava accessions. Primers designed with *Eucalyptus* SNP sequences resulted in reliable *Psidium* amplicons on 6% polyacrylamide gels. In general, the SNP dendrogram agreed with biological genus structure, since different species were not grouped, indicating that transferability among Myrtaceae genus was possible and reliable.

Key words: SNP; Transferability; Dendrogram

INTRODUCTION

The Myrtaceae family consists of approximately 130 genera and 3000 species of trees and shrubs distributed mainly throughout the tropics and subtropics. Brazil is a great representative of this diversity, where a total of 60 species can be found, including 47 endemic species (Sobral et al., 2016). Guava species is one of the highest economic values in *Psidium*. It is an important fruit in Brazil and in the world, and can be consumed both *in natura* and in its industrialized form. The fruit has good content of vitamin C (ascorbic acid), citrus juices being good sources (Pommer et al., 2013).

The root-knot nematode *Meloidogyne enterolobii* is currently responsible for the declining production of guava in Brazil. For presenting important sources of alleles, wild species of *Psidium* have been introduced in guava improvement program as a source of resistance to the nematode *M. enterolobii*, as reported by Costa et al. (2012), with the development of *P. guajava* x *P. guineense* hybrid. Several studies regarding characterization of guava and / or wild *Psidium* were carried out by using molecular markers.

Hernández-Delgado et al. (2007) analyzed 52 *Psidium* accessions from a Mexican collection based on AFLP markers. These authors observed the formation of two main groups, the first consisting of *P. cattleianum* and *P. friedrichsthalianum* accessions, and the second by *P. guajava* accessions. Rueda et al. (2006) characterized 27 guava accessions of *Psidium* ssp from a Colombian Research Center collection based on RAPD markers, reporting the formation of 16 groups, with a 0.78 similarity index. Pessanha et al. (2011) evaluated the genetic similarity of 20 *Psidium* ssp accessions using RAPD markers and the dendrogram generated two groups: the first containing guava species and the second containing *P. guineense* accessions. Valdés-Infante et al. (2007) reported the characterization of guava accessions of Cuban origin with microsatellites. da Costa and Santos (2013), in a study of four *Psidium* species, comprising 61 accessions, reported similarity between *P. guajava* and *P. guineense* of 82.4% through SSR markers. Kidaha et al. (2014) characterized 58 guava cultivars in Kenya through SSR and ISSR markers. To our knowledge there are no genetic divergence studies based on single nucleotide polymorphisms (SNPs) in *Psidium* and no reported attempts to transfer SNPs from one species to another, in general.

The goal of this study was to analyze the genetic divergence among *P. guajava* and *Psidium* sp accessions based on SNPs developed for *Eucalyptus* species in order to orientate guava genetic resources and breeding programs.

MATERIAL AND METHODS

Plant materials

Fifty-three *Psidium* accessions were analyzed, including 47 accessions of *P. guajava*, 3 *P. guineense* accessions, and 3 *P. cattleianum* accessions belonging to the germplasm bank of guava and wild *Psidium* of Embrapa Semiarid, located in the experimental field of Bebedouro, Petrolina, PE (Table 1).

Extraction and quantification of DNA

New and healthy leaves of 53 *Psidium* accessions were collected in paper bags, duly

identified, and conditioned in a freezer at -80°C until DNA extraction. DNA was extracted according to the method proposed by Doyle and Doyle (1990) with modifications as described by da Costa and Santos (2013). The DNA was quantified on 0.8% agarose gel stained with ethidium bromide by visually comparing the intensity of the DNA bands extracted with those of bands of Lambda phage DNA. The samples were diluted to 10 ng/μL and stocked at -20°C.

Table 1. Origin of guava and wild *Psidium* accessions from the Embrapa Semiarid germplasm collection.

Acession	Origin	State	Accession	Origin	State
GUA87AM	Iranduba	AM	GUA68RO	Buritis	RO
GUA88AM	Iranduba	AM	GUA72RO	Monte Negro	RO
GUA90AM	Iranduba	AM	GUA73RO	Ariquemes	RO
GUA92AM	Manacapuru	AM	GUA81RO	Porto Velho	RO
GUA97AM	Autazes	AM	GUA82RO	Porto Velho	RO
GUA98AM	Autazes	AM	GUA133RR	Iracema	RR
GUA62BA	Antonio Gonçalves	BA	GUA 135RR	Iracema	RR
GUA146BA	Valença	BA	GUA136RR	Rorainópolis	RR
GUA147BA	Valença	BA	GUA137RR	Caracaraí	RR
GUA150BA	Nilo Peçanha	BA	GUA138RR	Boa Vista	RR
ARA153BA	Ituberá	BA	ARA138RR	Boa Vista	RR
GUA117GO	Morrinhos	GO	ARA140RR	Boa Vista	RR
GUA120GO	Goiás Velho	GO	ARA55RS	Pelotas	RS
GUA121GO	Goiás Velho	GO	ARA58RS	Pelotas	RS
GUA124GO	Santa Isabel	GO	ARA105RS	Pelotas	RS
GUA127GO	Mimoso de Goiás	GO	GUA104RS	Pelotas	RS
GUA02MA	Caxias	MA	GUA106RS	Pelotas	RS
GUA03MA	Coelho Neto	MA	GUA109RS	Pelotas	RS
GUA05MA	Buriti	MA	GUA110RS	Pelotas	RS
GUA07MA	Mata Roma	MA	GUA51SE	Capela	SE
GUA26MA	Paraibano	MA	GUA52SE	Capela	SE
GUA33PE	Ibimirim	PE	GUA53SE	Japoratuba	SE
GUA34PE	Ibimirim	PE	GUA55SE	Pirambu	SE
GUA36PE	Pesqueira	PE	GUA59SE	Umbamba	SE
GUA38PE	Pesqueira	PE	GUA61SE	Riachão dos Dantas	SE
GUA39PE	Belo Jardim	PE	PEDRO SATO	Commercial cultivar	PE
GUA67RO	Jaru	RO			

GUA = *Psidium guajava* (guava); ARA = *Psidium* spp (wild *Psidium*).

Annotation and analysis of SNP data

DNA samples of accessions were genotyped with 60,904 SNPs of the EUChip60K chip, developed by Silva-Junior et al. (2015). The genotyping services were performed by GeneSeek (Lincoln, NE, USA). SNPs were also amplified in an F2 population of 189 individuals from a cross between *P. guajava* x *P. guineense* in order to select those with amplifications in both *Psidium* sets for divergence analysis.

The data generated by SNPs were transformed into binary codes for presence (1) or absence (0) of alleles, to build a matrix of Jaccard similarity index. The dendrogram with distances of accessions was made by the UPGMA grouping method (grouping method not based on weighted average arithmetic). The evaluation of the dendrogram adjustment was performed by cophenetic correlation, or the correlation between the true distances and the graphically represented ones. For these analyses, the NTSYSpc computer application was used (Rophlf, 2000).

Grouping based on model implemented in the Structure software was also used (Pritchard et al., 2000). The Bayesian algorithm in this software identifies genetically distinct subpopulations based on allele frequencies. The analysis to set the number of K or existing

subpopulations consisted of four independent replicates, with cutoff values of 100,000 permutations (burning) and 100,000 simulations of Monte Carlo chains.

PCRs and resolution on polyacrylamide gels of *Eucalyptus* SNPs in *Psidium* samples

Primer pairs of selected *Eucalyptus* SNPs were designed in the public software Primer3 public software (http://bioinfo.ut.ee/primer3/) to amplify *Psidium* genome regions, including eight samples of *P. guajava*, *P. guineense*, *P. cattleyanum*, and *P. friedrichsthalianum* and eight F2 samples of a *P. guajava* x *P. guineense* cross.

The PCR amplification was carried out for a final volume of 10 µL, containing 30 ng DNA, 0.2 µL of each primer, 1X Taq DNA polymerase buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, and 0.75 U of the enzyme Taq DNA polymerase. The amplification program consisted of denaturation of the initial cycle at 94°C for 4 min; 37 cycles at 94°C for 45 s, 54°C for 60 s, and 72°C for 60 s; and one stage of final extension at 72°C for 5 min. Amplified PCR products were separated on 6% polyacrylamide gels as described by da Costa and Santos (2013).

RESULTS AND DISCUSSION

In the experiment, 3523 SNPs of *Eucalyptus* for *Psidium* were generated, and 888 SNPs of these were selected for the present diversity analysis, because they also amplified in the F2 population of an interspecific hybrid. The chip EUChip60K is composed of 60,904 SNPs, of which 51,204 were polymorphic in 14 species of *Eucalyptus* (Silva-Junior et al., 2015). Thus, the amplification of *Eucaliptus* SNPs to *Psidium* was nearly 7%, and the effective transferability, by validation criteria in another *Psidium* sample, almost 2%.

Primers designed based on the EuBR03s4249801, EuBR08s41303533, EuBR03s13633658, EuBR11s17302993, EuBR02s23677860, EuBR03s47452480, EuBR06s21493635, and EuBR10s9300572 *Eucalyptus* SNPs presented reliable amplifications in *Psidium* genome (Figure 1), attesting the effective SNP transferability and the presence of conserved genome regions among Myrtaceae species.

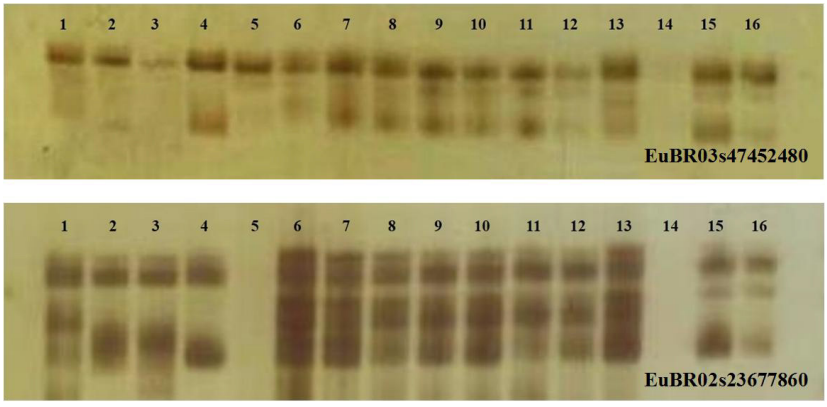

Figure 1. Profile of the PCR products of eight *Psidium* accessions: *Psidium guajava* allele (*lanes 14*, *15*, *16*); *P. guineense* allele (*lanes 1* and *5*); *P. cattleianum* allele (*lanes 2* and *3*); *P. friedrichsthalianum* allele (*lane 4*), and eight F2 plants (*lanes 6-13*) of a *Psidium* hybrid allele. Amplicons were amplified by *Eucalyptus* primers and resolved on silver- stained polyacrylamide gel.

The genus *Psidium* and *Eucalyptus* belong to the same Myrtacea family and transferability of markers between species of the same family has been applied in many studies, including among Myrtaceae species (Rai et al., 2013). In their study, with SSRs markers, these authors reported transferability rate of almost 80%. SNP markers have not been developed to *Psidium* yet and have been widely used in genetic analysis, due to the limitations of microsatellites and AFLP markers.

Similarity genetic based on dendrogram of Jaccard coefficient

Based on the numerical matrix, it was possible to obtain the distance matrix between individuals with use of the Jaccad Index. The correlation between the matrix of cophenetic values and the similarity distance matrix was 0.97, indicating that the dendrogram (Figure 2) presented a great adjustment in groups of *Psidium* accessions.

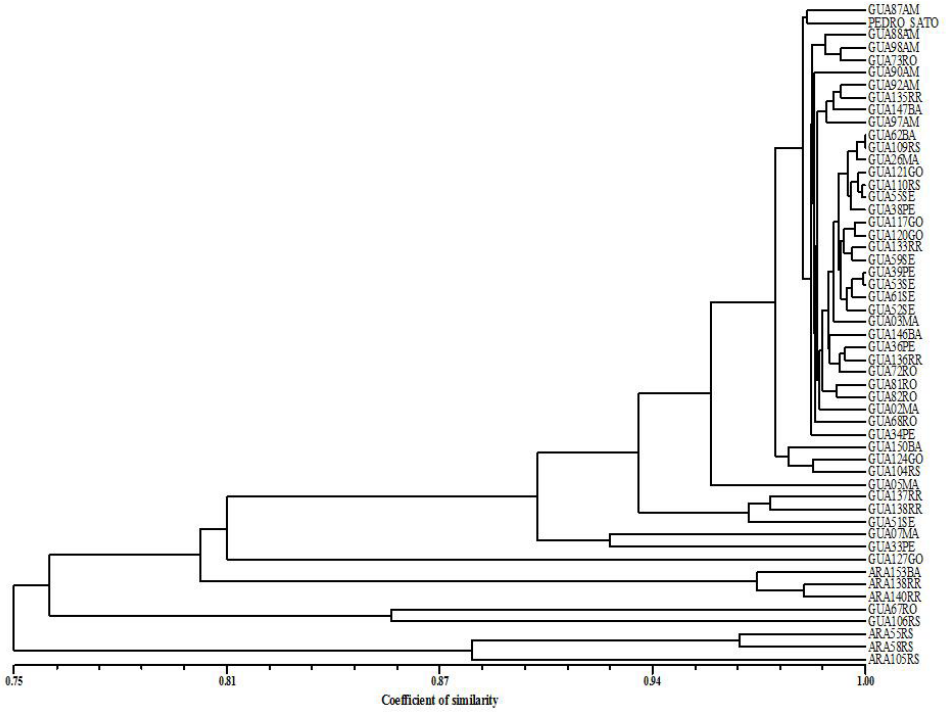

Figure 2. UPGMA dendrogram of Jaccard coefficient among 53 *Psidium* accessions of the Embrapa Semiarid germplasm collection based on 888 SNPs of *Eucalyptus*. Cophenetic correlation coefficient = 0.97.

The similarity among accessions ranged from 0.58 to 1.00 reflecting the existence of high genetic variability among studied accessions. High genetic variability was also reported by da Costa and Santos (2013) among the 61 *Psidium* accessions of that same collection evaluated by microsatellite marker. Corrêa et al. (2011) also reported high genetic variability among 88 *Psidium* accessions, with similarities ranging from 0.28 to 0.98 based on AFLP markers. These results indicate high discriminative power of SNP markers, even among

genetically related materials when compared to results obtained with AFLP and SSR markers for the same collection of accessions.

The 888 SNPs were efficient for separating the 53 *Psidium* accessions, indicating the formation of five groups at the cutoff point of 0.70 (Figure 2): group I was formed exclusively by guava accessions, from accession GUA87AM to GUA33PE; group II was formed by accession GUA127; group III was formed by the three accessions of *P. guineense*; group IV was formed by guava accessions GUA67 RO and GUA106 RS; and the group V was formed by the three accessions of *P. cattleianum*. The largest number of accessions were clustered in group I, comprising 44 of the 53 accessions, formed exclusively by guava accessions. The same grouping pattern was observed by da Costa and Santos (2013) based on microsatellite markers and Briceño et al. (2010) among Venezuelan accessions of guava and other species of *Psidium* such as *P. guineense*. The highest similarity (100%) was observed among the accessions GUA62BA and GUA109RS collected in different regions, Antonio Gonçalves, BA, and Pelotas, RS, respectively. This result was not observed in the study by da Costa and Santos (2013).

The accessions ARA55RS, ARA58RS, and ARA105RS present the least similarity in relation to the set of evaluated accesses. da Costa and Santos (2013) analyzed the genetic variability of these accessions by microsatellite markers and observed that these wild *Psidium* accessions also showed less similarity in relation to other accessions, confirming the results obtained with SNPs.

It was observed that the six wild *Psidium* accessions, ARA153BA, ARA138 RR, ARA140 RR (*P. guineense*), ARA55RS, ARA58RS, and ARA105RS (*P. cattleianum*), and two guava accessions, GUA67RO and GUA106RS, were positioned at the base of the dendrogram, suggesting greater similarity between accessions. These results are promising since the resistance of plants of the species *P. guineense* and *P. cattleianum* to the nematode was found (Almeida et al., 2009; Miranda et al., 2011; Castro et al., 2012) being indicated for interspecific crosses.

Sitther et al. (2014) reported the formation of six groups composed of eight accessions of *P. guajava*, including three species of wild *Psidium* accessions of *P. friedrichsthalianum*, *P. guineense*, and *P. sartorianum* in the United States germplasm. Oliveira et al. (2014) found greater proximity of *P. cattleianum* with guava accessions. However, these species possess proven differences concerning evolutionary distance in relation to guava, mainly because it is polyploid, being evidenced different ploidy levels, such as tetraploid, heptaploide, or octaploide (Costa et al., 2008; Ray, 2002; Singh and Sehgal, 1980; Hirano, 1967). Overall, the reported results and those obtained in this study indicate a clear separation of *Psidium* species, attesting the efficiency of *Eucalyptus* SNPs transfer to *Psidium.*

Genetic structure based on Bayesian analysis

The number of subpopulations suggested in the analyses of the Structure and Structure Harvester software was seven (K = 7) (Figures 3 and 4). The results based on Bayesian analysis were similar to those found by UPGMA (Figure 2), with the identification of the three main groups, separating the three *Psidium* species evaluated. There were minor discrepancies such as formation of two unique groups to GUA67R and GUA106RS in Bayesian analysis (Figure 3). UPGMA grouping number would be the same in the Bayesian analysis if the cutoff point in the dendrogram had been around 0.80.

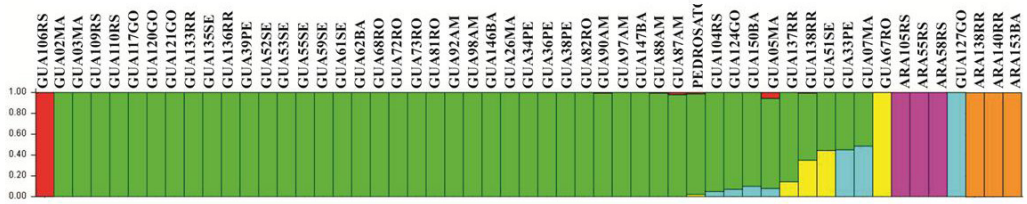

Figure 3. Genetic structure of 53 *Psidium* accessions generated based on Bayesian analysis, considering K = 7, obtained by the method ΔK. Each vertical line represents a *Psidium* accession. Each color represents the most probable lineage of the set from which the genotype or partial genotype was derived.

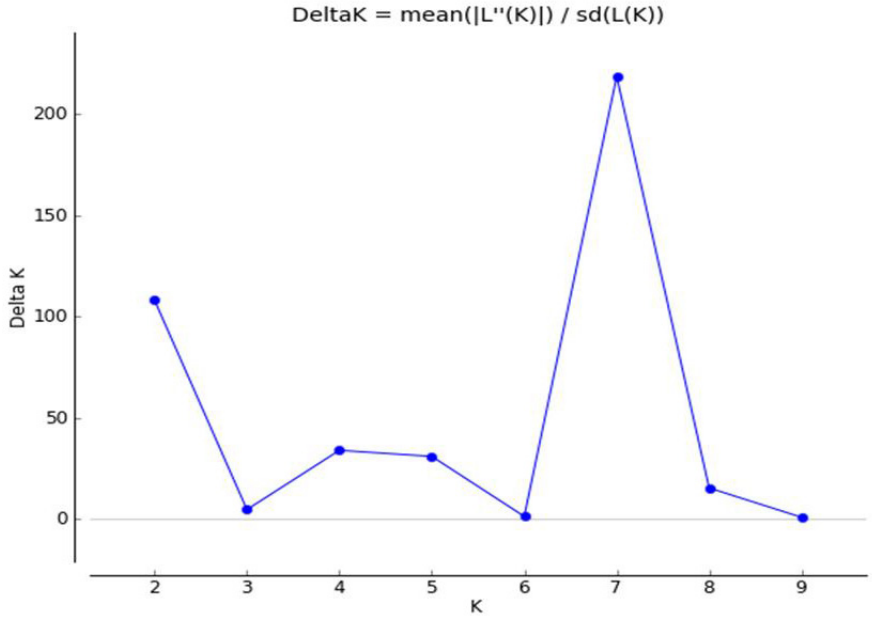

Figure 4. Delta K (filled circles, solid line), calculated with the average rate of second order of change of probability of K divided by the standard deviation probability of K m (| L00 (k) |) / s [L (R)].

When analyzing the information about UPGMA grouping along with Bayesian analysis, it was possible to observe that the guava accession GUA127GO is the one that proves to be more divergent, because it is found isolated from the other accessions. It was observed that the groups formed by wild *Psidium* accessions formed by Bayesian and UPGMA grouping remained in different groups, both species *P. cattleianum* and *P. guineense*.

da Costa and Santos (2013) while analyzing the genetic diversity of *Psidium* in that same collection based on microsatellite markers reported the formation of five groups, with a large group of guava accessions and three groups formed only by wild *Psidium* accessions, one formed by *P. guineense*, other with *P. cattleianum*, and another one containing only P. *friedrichsthalianum*. These results corroborate with the data presented in this study, demonstrating the efficiency of *Eucaliptus* SNPs applied in *Psidium.*

According to Arriel et al. (2006), studies involving more than one grouping method, due to differences in the hierarchy, optimization and ordering of groups, allow the classification to be complemented by the criterion that each technique uses, as well as prevent erroneous inferences from being adopted in the allocation of materials within a particular subgroup of genotypes.

This is the first study with the application of SNP markers to analyze the genetic diversity in *Psidium* accessions, adopting the strategy of transferability of SNPs developed for *Eucalyptus* species. The transferability of SNPs of different species is a pioneer and can assist in developing saturated linkage guava maps as well as mapping more accurately multiple-agronomical important traits to the crop. The SNP loci identified in this study can be used in studies about guava genetic diversity and can be added to SNPs that may be developed specifically for *Psidium* species, which will be the best scenario for this species.

CONCLUSION

The SNP transferability of *Eucalyptus* to *Psidium* species was very reliable since amplifications of *Eucalyptus* SNP sequences in *Psidium* genome occurred, agreeing with previous microsatellite genetic divergence studies and the SNP dendrogram with the biological *Psidium* genus structure, since different species were not grouped.

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