Original Article

GENETIC DIVERSITY AMONG SOURSOP GENOTYPES BASED ON FRUIT PRODUCTION

DIVERSIDADE GENÉTICA ENTRE GENÓTIPOS DE GRAVIOLA BASEADA NA PRODUTIVIDADE DE FRUTOS

Carlos Felipe Barrera SÁNCHEZ[1]; Bruno Ermelindo LOPES[2]; Paulo Eduardo TEODORO[2]*; Andrea Del Pilar GARCIA[1]; Leonardo De Azevedo PEIXOTO[2]; Lidiane Aparecida SILVA[2]; Leonardo Lopes BHERING[2]

1. Universidad Nacional de Colombia, Medellin, Colombia; 2. Universidade Federal de Viçosa, Viçosa, MG, Brasil.
*eduteodoro@hotmail.com

ABSTRACT: This study aimed to estimate the genetic diversity among soursop genotypes in terms of fruit yield evaluated in different crop years. Sixteen measurements for fruit yield in 71 soursop genotypes were carried out from 2000 to 2016. Based on ANOVA it was verified the existence of genetic variability among genotypes in the different measurements (harvests). The genotypes were clustered according to their respective fruit yield averages in the 16 years as well as from their means obtained in each measurement year by the Scott-Knott test (p<0.05). To study genetic diversity among genotypes, three methodologies were compared: Tocher hierarchical optimization, UPGMA method and principal components. Five groups were formed for all clustering methods used. It was identified that crossings between genotypes 124 and 145 with genotypes 59 and 170 are potential to generate population with large genetic variability and high fruit yield average. New researches should be developed aiming to exploit the genetic variability among soursop genotypes based on the results found in this study.

KEYWORDS: *Annona muricata*. Clustering. Plant breeding. Biometry.

INTRODUCTION

The soursop (*Annona muricata* L.), belongs the *Annonaceae* family, and is one of the approximately 119 species that compose this genus *Annona*, which is widely distributed in different parts of the world. São josé et al. (2014) reported that the center of diversity of the species in this genus is widespread among Tropical America and Central Africa. Nevertheless, there is a controversy about the exact region of origin of the soursop, which according to Campos et al. (2008) is more probably that the origin was in the Central America, on the other hand FALCAO et al. (1982) considered that this species originated in the Antilles and spread in tropical America.

High diversity can be observed among species into the genus *Annona*, however only nine species produce edible fruits, and only five are cultivated with economic purpose (SÃO JOSÉ et al., 2014). Soursop stands out among the other species cultivated due to it presents fruits of considerable size, approximately 3 kg, which can be used for several purposes. Soursop pulp is commonly consumed fresh or can be frozen, processed and used industrially for the production of juices, ice cream, jellies, and another foods. However, the soursop production chain is largely composed of small producers and is often unsustainable because the fruits are highly perishable and lack adequate production and post-harvest techniques (FALCAO et al., 1982; SÃO JOSÉ et al., 2014).

Several parts of the plants can also be used in medicine for the production of herbal products standing out in the current market (MOGHADAMTOUSI et al., 2014). Increasingly, interest in studies in the field of medicine reinforces the need for greater knowledge and importance of soursop cultivation. Jaramillo et al. (2000) verified the action of their extracts against promastigotes of *Leishmania braziliensis* and *L. panamensis*, BORIES et al. (1991) tested its antiparasitic activity against *Entamoeba. histolytica*, *Nippostrongylus. brasiliensis*, and *Artemia. salina*, and several other authors reported the importance of this species in the treatment and prevention of important diseases, such as cancer (GEORGE et al., 2012; MOGHADAMTOUSI et al., 2014).

In this context, it is necessary to study soursop cultivation, aiming for increasing fruit yield, emphasizing the increase of nutritional quality, application of adequate techniques for seedling production, improvement of existing methodologies and efficient exploitation of genetic resources (CAMPOS et al., 2008; OLIVEIRA, 2001), to make the crop economically more attractive to farmers.

For the development and fixation of this crop in the market it is essential the availability of superior cultivars, with desirable traits such as yield (SANCHÉZ et al., 2017). Borém (2005) reported that 50% of the gain in production is related to cultural factors, present in the crop, while the other 50% are covered to the genetic attributes. However, there are few reports of research that investigated the stages of soursop genetic breeding (RONNING et al., 1995; SÁNCHEZ et al., 2017; SURATMAN et al., 2015). Among these stages, the initial phase characterized by the formation of the base population in which the selection will be practiced, pre-breeding studies are necessary to help breeders to identify superior genotypes to be used in the crossing blocks. Therefore, the identification of genotypes with desirable characteristics and with high diversity can contribute to generate segregating populations with high genetic variability and productive potential.

Therefore, the objective of this work was to estimate the genetic diversity among soursop genotypes in terms of fruit yield evaluated in different years, in order to obtain information to support future studies of soursop genetic breeding programs.

MATERIAL AND METHODS

Experimental design

The experiment was laid out at campus Halcones Farm, in Cerritos - Risaralda, Colombia (4°48'48.00"N, 75°42'58.63"W, at approximately 1,345 m asl). Region climate is AF according to Köppen's classification, with rainfall scattered throughout the year, with average temperatures of 23 ºC and minimum of 18 ºC, with annual rainfall of 2,100 mm and relative humidity around 75%.

The experiment was carried out using 8x8 m spacing. Plants were conducted without irrigation and the other cultural practices were carried out according to the recommended management practices for soursop (OLIVEIRA, 2001). Cultural practices were similar throughout the period of conduction of the experiment.

Fruit yield was evaluated in sixteen years from 2000 to 2016 in the 71 soursop genotypes.

Data analysis

Data were submitted to ANOVA, where the statistical model adopted considering two variation factors was: $Y_{ij} = \mu + g_i + a_j + \varepsilon_{ij}$ where Y_{ijk} is the observation regarding the ith soursop genotype, in the jth measurement; μ is the overall mean; g_i is the fixed effect of the ith genotype, under influence of the permanent environment; a_j is the fixed effect of the temporary environment in the jth measurement, where $\sum_{j=1}^{n} a_j = 0$; and ε_{ijk} is the experimental error established by the temporary effects of the environment in the jth measurement of the ith genotype.

The genotypes were clustered by the Scott-Knott test at a 5% probability according to their respective fruit yield means in the 16 years of evaluation, as well as from their means obtained in each measurement year.

Aiming to study the genetic diversity among genotypes, Tocher's optimization and UPGMA method, based on the standardized Euclidean mean distance, and principal components were applied. The criterion of Singh (1981) was used to evaluate the contribution of each measurement to the observed genetic diversity. Statistical data analyzes were performed using software Genes (CRUZ, 2013) and Microsoft Excel®.

RESULTS AND DISCUSSION

Table 1 shows the analysis of variance for fruit yield evaluated in 71 soursop genotypes in the 16 measurements from 2000 to 2016. The significant effect for genotype revealed that there was a large genetic variability in the population and consequently it is possible to select soursop genotypes with high fruit yield. The F test showed the significant effect for measurements that indicated that the genotypes mean changed among the years.

Table 1. Analysis of variance for fruit yield evaluated in 71 soursop genotypes in 16 measurements. The experiment was laid out from 2000 to 2016**.**

SV	Degree of freedom	Fruit yield
Measurements	15	93,981.61*
Genotypes	70	42,282.05*
Error	1,050	4,388.99

*: Significative for 1% of the probability by F Test. SV – source of variation.

The Skott-Knott clustering test grouped the 71 soursop genotypes in 5 homogeneous groups based on fruit yield (Table 2). Group I was formed by three genotypes (59, 124, and 45) that represented the highest average during the measurements. Group II was formed by genotypes 1, 10, 15, 24, 47, 79, 81, and 170 that also presented a high average for fruit yield during the measurements. Other groups were formed by genotypes with low average for fruit yield.

Table 2. Scott-Knott clustering for fruit yield (kg/plant) evaluated in 71 soursop genotypes among 16 measurements**.** The experiment was laid out from 2000 to 2016**.**

Group	Genotype	Mean ± Standard deviation
I	59, 124 and 145	269.03 ± 12.56
II	1, 10, 15, 24, 47, 79, 81 and 170	209.20 ±10.07
III	18, 46, 51, 54, 77, 89, 125 and 126	173.39 ± 10.44
IV	2, 12, 16, 22, 23, 36, 40, 41, 42, 48, 53, 82, 90, 107, 116, 117, 118, 119, 120, 121, 122, 123, 129, 130, 131, 132, 144, 166, 167, 168 and 169	129.57 ± 13.79
V	3, 11, 19, 20, 21, 35, 38, 44, 49, 50, 52, 55, 57, 58, 78, 83, 115, 133, 135, 136 and 164	80.44 ± 13.39

Based on Scott-Knott test it is possible to verify that crossings between genotypes from group 1 and 2 may be a suitable strategy to improve fruit yield in soursop since breeders should cross genotypes with high average and divergent (BORÉM; MIRANDA, 2013).

Aiming to study the genetic diversity among soursop genotypes, fruit yield for each measurement was considered as a trait, and primarily Tocher's optimization procedure was performed (VASCONCELOS et al., 2007). The genotypes were classified in five groups by Tocher's optimization (Table 3). Group 1 was the largest group composed by almost all genotypes with low fruit yield average among the measurements. Genotypes with highest fruit yield average among the measurements were allocated into groups 4 and 5.

Table 3. Tocher's optimization procedure for 71 soursop genotypes based on 16 measurements for fruit yield. The experiment was laid out from 2000 to 2016**.**

| Groups	Genotypes
1	21, 58, 35, 57, 38, 55, 135, 115, 11, 133, 78, 20, 44, 132, 83, 136, 19, 40, 107, 167, 48, 130, 22, 90, 2, 49, 52, 82, 3, 41, 50, 123, 53, 12, 166, 116, 120, 169, 164, 23, 36, 117, 118, 51, 122, 131, 42, 144, 121, 119, 129, 168, 54
2	1, 15, 81, 79, 77, 126, 24, 18, 10, 89
3	16, 46, 47, 131
4	124, 145
5	59, 170

Interestingly, genotypes 124 and 145 were the genotypes with highest fruit yield average in the early measurements, while the genotypes 59 and 170 were the genotypes with the highest fruit yield average after the seventh measurement. This may happens because genes that affected fruit yield in the early stage are not the same genes that will affect this trait in the later stages (RESENDE, 2015). Therefore, crossings between genotypes from group 4 and genotypes from group 5 can be a useful strategy to generate population with large genetic variability and high fruit yield average in all growth stages.

Hierarquical clustering was also used to verify the diversity among soursop genotypes. UPGMA clustering based on the standard Euclidean distance allowed to visualize in a graphic how the genotypes were grouped (Figure 1). Two groups were formed, which the genotypes 124 and 145 belonged to group 1 and the remaining genotypes

belonged to group 2. Therefore, it was possible to prove that the crossing between genotypes 124 and 145 with the genotypes 59 and 170 may be potential to improve the fruit yield in soursop because they belonged to different group by Tocher's optimization and UPGMA method, and they also

presented a high fruit yield average during the years. The genetic diversity should be explored aiming to choose genotypes with high general and specific combination ability that makes these genotypes useful to be crossed and generate hybrids with high heterosis.
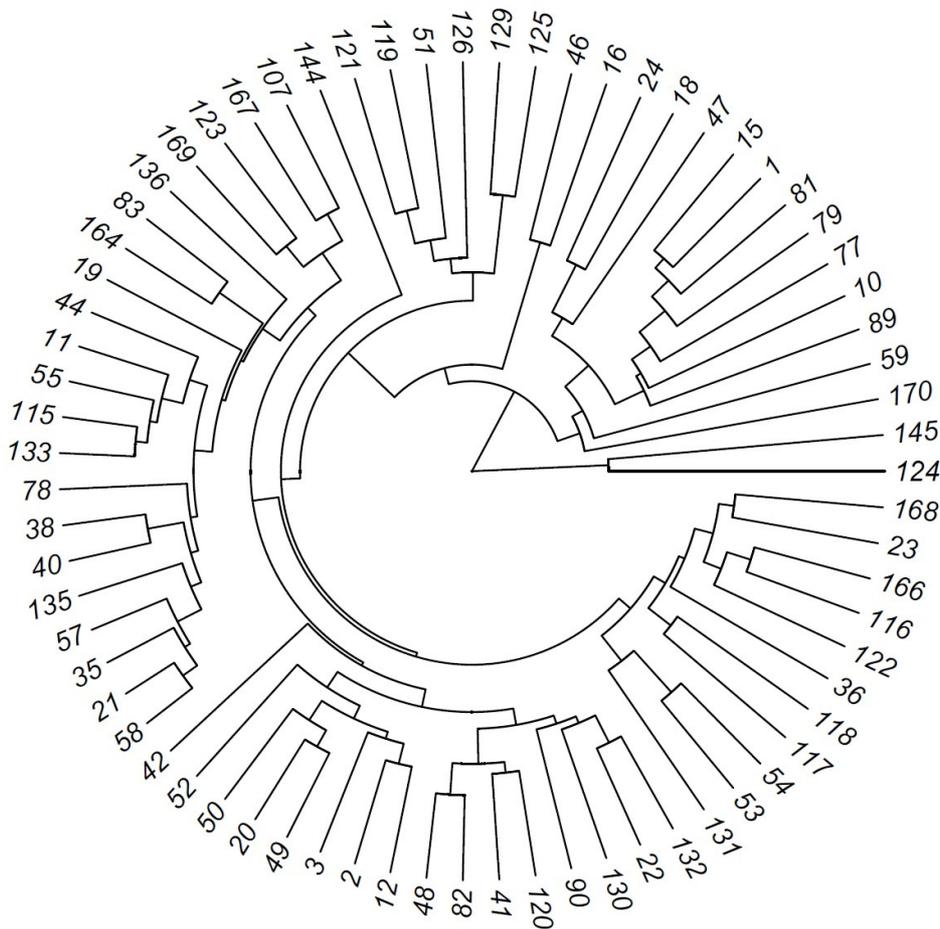
Figure 1. Clustering of the 71 soursop genotypes using UPGMA method based on standard Euclidean distance. The trait used to estimate standard Euclidean distance was fruit yield evaluated during 16 measurements. The experiment was laid out from 2000 to 2016.

Genetic diversity study has a big importance for the soursop breeding program success since this is one of the first step and it may help breeders to choose superior genotypes for crossings (CRUZ et al., 2012). Therefore genetic diversity study will help breeders to choose the best strategy based on their objectives (ALLARD, 1999).

Another methodology that was used in this study to calculate the genetic diversity among soursop genotypes was principal component analysis (Figure 2). This methodology is widely used in the genetic diversity study when there is no replication in the experiment, which means when there is just one plant represent each genotype.

To compare principal components analysis with Tocher's optimization, genotypes clustered in

the same group by Tocher's optimization were represented using the same symbols, and for each group was used a different symbol (Table 3 and Figure 2). It was possible to verify that the groups formed by Tocher's optimization was similar for the groups formed by principal components analysis. This result is important because it demonstrated the precision between methods.

The maintenance of the genetic diversity is important for guarantee the possibility to obtain selection gain in a long term since variability is one of the most important effect that affects selection gain (RESENDE, 2002; RESENDE, 2015). Based on this fact it ís necessary to cross soursop genotypes from different groups guaranteeing the variability on the soursop breeding program.
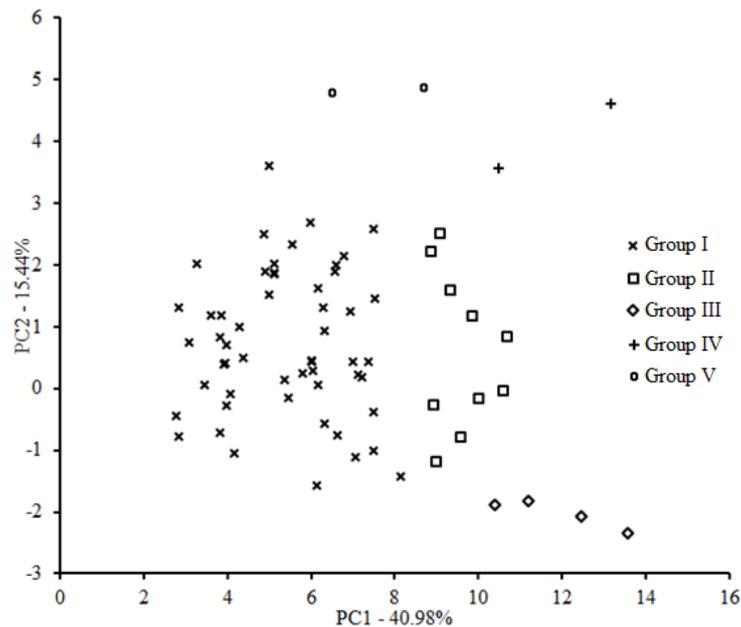
Figure 2. Clustering of the 71 soursop genotypes using the first two principal components (PCA 1 and PCA 2). Groups were established based on Tocher's optimization (each symbol represent one group based on Tocher's optimization – Table 3). The experiment was laid out from 2000 to 2016.

The criteria proposed by Singh (1981) was used to verify the contribution of each measurement for the genetic diversity among soursop genotypes, and it revealed that the 6th, 7th, and 8th measurements allowed to identify more accurately the genotypes more divergent (Table 4). Based on this results it may be inferred that the fruit yield in soursop has a cyclic behavior during the years. Therefore, it is needed that genotypes should have their fruit yield behavior established for studying the genetic diversity among soursop genotypes, because it is a quantitative trait and consequently its heritability is low.

Table 4. Contribution (S.j.) of the 16 fruit yield measurements for genetic diversity evaluated among 71 soursop genotypes based on Singh (1971) criteria. The experiment was laid out from 2000 to 2016.

Measurements	S.j.	S.j. (%)
1	283.91	7.61
2	240.72	6.45
3	172.63	4.63
4	119.65	3.21
5	219.48	5.88
6	324.71	8.70
7	316.20	8.47
8	290.03	7.77
9	261.32	7.00
10	176.09	4.72
11	228.04	6.11
12	237.57	6.37
13	244.75	6.56
14	146.37	3.92
15	279.90	7.50
16	190.80	5.11

Sánchez et al. (2017) verified that the optimum number of measurements to guarantee high accuracy to select superior genotypes in soursop is around eight and nine measurements. Therefore based on the genetic diversity analysis and the contribution of each measurement for the diversity on soursop the selection of superior genotypes should be done considering the measurements from 6 through 9 year more important since they represent a large diversity and are more accurate.

Based on the results found in this research other applications can be performed. For instance, in future research other agronomic traits might be evaluated and correlated with fruit yield only in the 6th, 7th, and 8th measurements. This information is very useful for the soursop breeding due to the financial resources and labor can be better distributed.

CONCLUSION

It was identified that crossings between genotypes 124 and 145 with genotypes 59 and 170 are potential to generate population with large genetic variability and high fruit yield average. New researches should be developed aiming to exploit the genetic variability among soursop genotypes based on the results found in this study.

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RESUMO: O objetivo deste estudo foi estimar a diversidade genética entre genótipos de graviola em termos de produção de frutos avaliados em diferentes safras. Foram realizadas 16 medições de rendimento de frutos em 71 genótipos de graviola no período de 2000 a 2016. Com base na ANOVA, verificou-se a existência de variabilidade genética entre genótipos nas diferentes medidas (colheitas). Os genótipos foram agrupados de acordo com suas respectivas médias de rendimento de frutos nos 16 anos, bem como suas médias obtidas em cada ano de medição pelo agrupamento de médias de Scott-Knott. Para estudar a diversidade genética entre genótipos, foram aplicados a otimização hierárquica Tocher, método UPGMA e componentes principais. Cinco grupos foram formados para todos os métodos de agrupamento utilizados. Foi identificado que os cruzamentos entre os genótipos 124 e 145 com os genótipos 59 e 170 são promissores para gerar população com grande variabilidade genética e alta média de produtividade de frutos. Novas pesquisas devem ser desenvolvidas com o objetivo de explorar a variabilidade genética entre os genótipos de graviola, com base nos resultados encontrados neste estudo.

PALAVRAS-CHAVE: *Annona muricata*. Agrupamento. Melhoramento de Plantas. Biometria.

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