

Agronomic evaluation of a Colombian passion fruit (*Passiflora edulis* Sims) germplasm collection

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Abstract. Passion fruit (*Passiflora edulis* Sims) is one of the most promissory crops in Colombia with approximately 7,400 ha cultivated, an average production of 89,000 t and an average yield of 15 t ha⁻¹. However, low yields as well as harvest problems including fruit quality, generate important losses, mainly due to lack of improved cultivars with particular characteristics for each market (fresh consumption, agroindustry) and specific adaptations to biotic and abiotic stress factors in producer areas. Therefore, as a pre-breeding phase, this study aimed at characterizing preliminary the production and physiochemical characteristics of 60 passion fruit germplasm accessions maintained in Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). We cultivated these accessions in the field for 22 months between 2014 and 2015, measuring 17 variables associated to both yield production and fruit quality. We analysed data using phenetic and multivariate methods to establish levels of genetic diversity. Additionally, we constructed a selection index (SI) with the most informative variables to select individuals according to fruit weight, shape and colour, as well as juice percentage. Results of genetic grouping and principal components showed a broad genetic diversity across the working collection, without any population substructure. Fruit volume, pulp and seed weight were the variables that explained 80% of the phenotypic variation. Finally, we identified 30 promissory accessions as parental for the first cycle of recurrent selection using the SI. We conclude that this core collection represents the diversity included in the working collection with promissory accessions to initiate an intra-population recurrent selection program.

Key words: passion fruit, plant breeding, selection index, genetic diversity, germplasm collection.

INTRODUCTION

Passion fruit (*Passiflora edulis* Sims) belongs to the genus *Passiflora* L. that is one of the most important genera in economic terms of the Passifloraceae botanical family. This gender comprises a wide diversity with ca. 530 species, all with an American origin (Yockteng et al., 2011). Colombia is the country with the most extensive *Passiflora* diversity worldwide with 162 species, followed by Brazil with 127 species (Ocampo et

al., 2010). Nevertheless, the materials with the highest economic importance for Colombia are the two botanical forms of *P. edulis* Sims, f. *edulis* Sims (gulupa) and f. *flavicarpa* Degener (passion fruit or maracuyá). Some authors prefer to simplify the taxonomy of both forms, using only the name *P. edulis* Sims (Bernacci et al., 2008). However, in this study, we recognize passion fruit and gulupa as different botanical forms as stated by Ocampo & Coppens (2017).

Passion fruit is a diploid ($n = 9$) self-incompatible species with extensive cross-pollination mediated mainly by insects (Bruckner et al., 1995). The genetic diversity within *P. edulis* worldwide has been evaluated using morphological descriptors and agronomic characteristics (Meletti et al., 2005a; Abreu et al., 2009). Moreover, several microsatellite molecular markers have been used (Oliveira et al., 2005; dos Santos et al., 2011; Cerqueira-Silva et al., 2014b; Araya et al., 2017). In Colombia, the genetic diversity of *P. edulis* has partially been studied using molecular markers as random amplified microsatellites (RAM) and amplified fragment length polymorphisms (AFLP) in gulupa (*P. edulis* f. *edulis*) (Fonseca-Trujillo et al., 2009; Ortiz et al., 2012). Furthermore, in yellow passion fruit (*P. edulis* f. *flavicarpa*), a preliminary diversity analysis was carried out in materials collected in producer regions assessing physicochemical fruit characteristics (Ocampo et al., 2013) and using single sequence repeat (SSR) molecular markers (Ocampo et al., 2017). Additionally, pre-breeding activities have been carried out to evaluate compatibility between interspecific crossings among commercial and wild cultivars (Ocampo et al., 2016).

In Colombia during 2015, approximately 7,400 ha were cultivated with passion fruit obtaining a production of 89 thousand tons, being the departments of Huila, Meta and Valle del Cauca the main producers with an average yield of 15 t ha⁻¹ (MADR, 2017). In contrast, Brazil shows a cultivated area that is more than six times larger (51,187 ha) than in Colombia with a production that is ten times higher, i.e. 921,275 t (IBGE, 2016), and a potential yield for new cultivars of 50 t ha⁻¹ (Meletti et al., 2005b). In Colombia, one of the reasons for the low productivity is the lack of improved cultivars, either explicitly adapted to each producer zone with better response to biotic and abiotic stress factors or with specific characteristics for each market (i.e. fresh consumption or agroindustry) (Quintero et al., 2012). Moreover, the first and unique cultivars registered in the 1970s in Colombia were selections called Hawaii, Brasil, and Venezuela. Since then, no research in plant breeding in this species has been carried out (Jaramillo et al., 2009). Currently, AGROSAVIA is interested in starting a breeding program in passion fruit using its working collection. However, this collection is not well conserved nor characterized. Therefore, the aim of this study was to characterize for the first time the passion fruit working collection maintained in AGROSAVIA and provide insights about the most promissory materials identified for future breeding programs.

MATERIALS AND METHODS

Study site and variables measured

During 2014, 1,313 passion fruit plants were obtained from seeds belonging to 60 accessions maintained in the working collection of AGROSAVIA (Corpoica). This material was previously not conserved or characterized properly, and therefore, we performed this first assessment with the material available, i.e. without repetitions or any experimental design in the field. The field trial was established in Centro de Investigación

Palmira of Corpoica (AGROSAVIA), located in the department of Valle del Cauca, between January 2014 and October 2015. The study site has an average annual temperature of 24 °C, a mean annual precipitation of 1,032 mm, an average relative humidity of 72%, an average sunshine of 5.8 hours day⁻¹, and a wind speed of 1.5 m s⁻¹; climatic variables were calculated using data collected from a meteorological station located on site.

Once plants were obtained, they were established in a deep Vertisol soil, with a clay-loam texture, good fertility conditions and rich in organic matter. Moreover, plants were cultivated using a trellis system establishing a distance of 2.5 m between plants, and 1.5 m between rows (Jaramillo et al. (2009)). Two variables related to yield components, including total plant production and number of fruits per plant, were registered in the entire population. Finally, a random subgroup of 295 plants was selected for fruit quality evaluation including 15 variables. Among these, fruit colour (i.e. scale from one to ten, where one is white and ten is yellow), fruit shape (diameter in mm/height in mm ratio), fruit volume (mL), fruit density (g mL⁻¹), peel thickness (mm), and peel weight (g). Moreover, pulp weight (g), seed weight (g), juice weight (g), juice (%), Brix degrees (°Brix), acidity (%), vitamin C (%), juice pH and average fruit weight (g).

Statistical analysis

A principal component analysis (PCA) and grouping was carried out with the software NTSYS-pc® version 2.02 g (Rohlf, 1990), standardizing the data with the function *STAND*. Principal components were identified with the *EIGEN* and *PCA* functions. Moreover, Pearson's correlation and variance-covariance matrices were constructed using the *CORR* and *VAVCOV* functions, respectively. For grouping analysis, each genotype was considered as an operational taxonomic unit (OTU), and a dissimilarity matrix was constructed with an average taxonomic distance using the function *DIST*. Furthermore, we grouped and constructed a dendrogram with the *UPGMA* function. For the principal coordinate analysis, we used a similarity matrix with the algorithms *DCENTER* and *EIGEN*. Finally, we developed a selection index based on yield and market requirements using the Eq. 1:

$$SI = P(0.4) + J(0.2) + S(0.2) + C(0.2) \quad (1)$$

where *P* is fruit weight per plant, *J* is juice percentage, *S* is fruit length and width relation, and *C* is fruit colour.

RESULTS AND DISCUSSION

The primary goal of this study was to characterize for the first time the production and physiochemical characteristics of 60 passion fruit germplasm accessions maintained in AGROSAVIA. We found a considerable variation associated with the number of plants within each accession (Fig. 1). We obtained on average 21 plants per accession. In some cases, only one plant per accession was obtained (e.g. 12.2Cenicafe, 40.5Cenicafe, 5.1Cenicafe, 7.1Cenicafe and La Union S1P3). In comparison, accessions that came from collections with masal selection as La Union 2/2 and La Union 1/2 (L. Arango, 2017 personal communication) showed the highest number of individuals, i.e. 167 and 120 plants, respectively. We found that seed viability and adaptation during their initial crop growth phases are probably the main factors associated with the

considerable variation observed in the phenotypic characteristics in our data. The diversity in germination patterns observed across different passion fruit populations is due to the effect of the exterior seed layer that limits water absorption, and therefore, inhibits germination (Santos et al., 2015). Consequently, to achieve uniform germination in passion fruit seeds, it is necessary to use several treatments with warm water, sulphuric acid and sucrose (Ghosh et al., 2017).

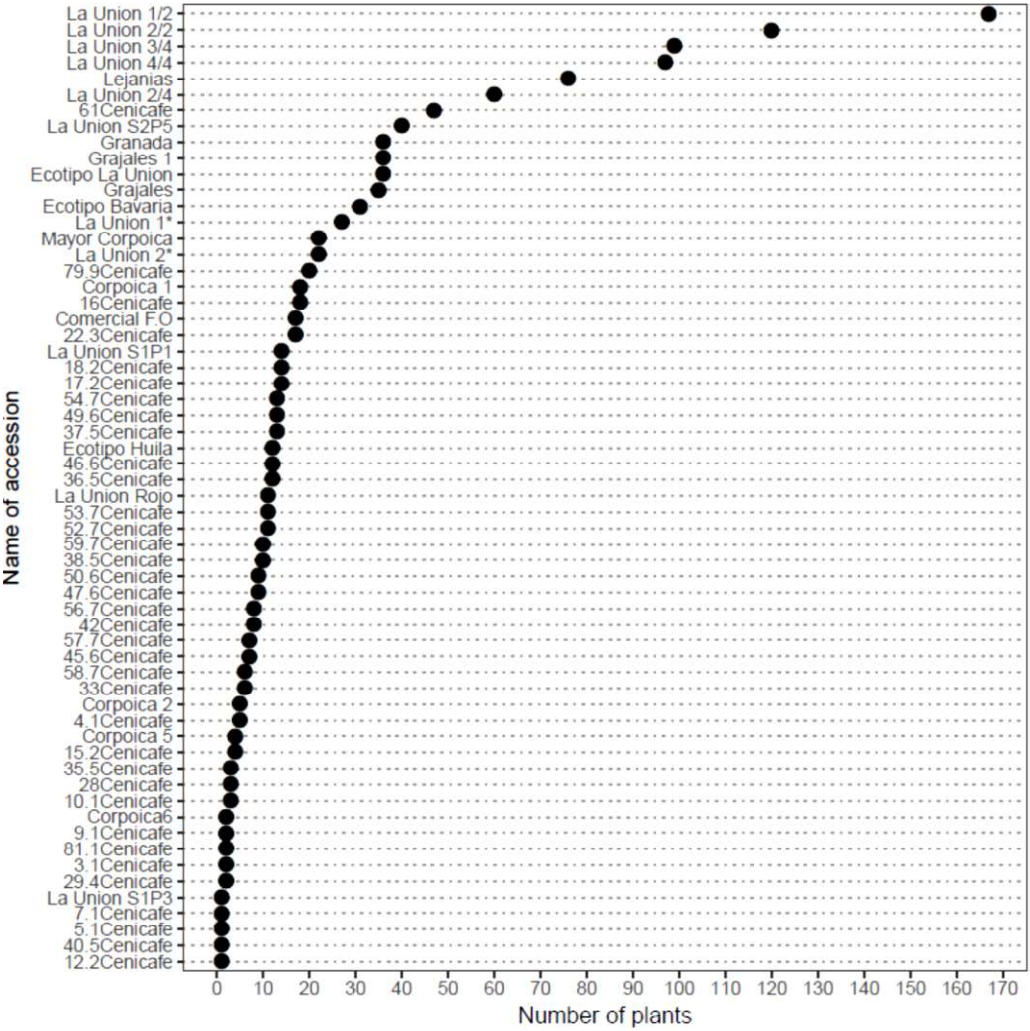


Figure 1. Total number of plants per accession ($n = 60$) from the *Passiflora edulis* working collection of AGROSAVIA assessed in this study.

Regarding the two yield components considered (i.e., plant weight and fruit number) as well as the 15 variables associated with fruit quality, we found a wide variation in the data (Fig. 2). This variation is mainly due to their forced cross-pollination condition. Therefore, passion fruit populations are heterogeneous and heterozygous. In the case of plant weight, the average found in our collection was 5.9 kg with variation

ranging from 0.2 kg to 19 kg. These results show materials that can potentially reach up to 50 t ha⁻¹, similarly to the maximum yield obtained by improved hybrid materials from Brazil (Meletti et al., 2005b). However, detailed studies with controlled pollination must be carried out to establish heritability in a broad and narrow sense, and in this way, establish recurrent selection schemes that will be used to achieve a higher genetic gain (Gonçalves et al., 2007). Similarly, the broad variation of fruit quality traits, such as Brix degrees, acidity and percentage of juice within this working population is very important to develop new cultivars for the agroindustry and the fresh market. Therefore, further studies evaluating the environmental stability of these traits as well as other nutrition properties such as antioxidant potential have to be developed to increase nutritional value as a functional food (dos Reis et al., 2018).

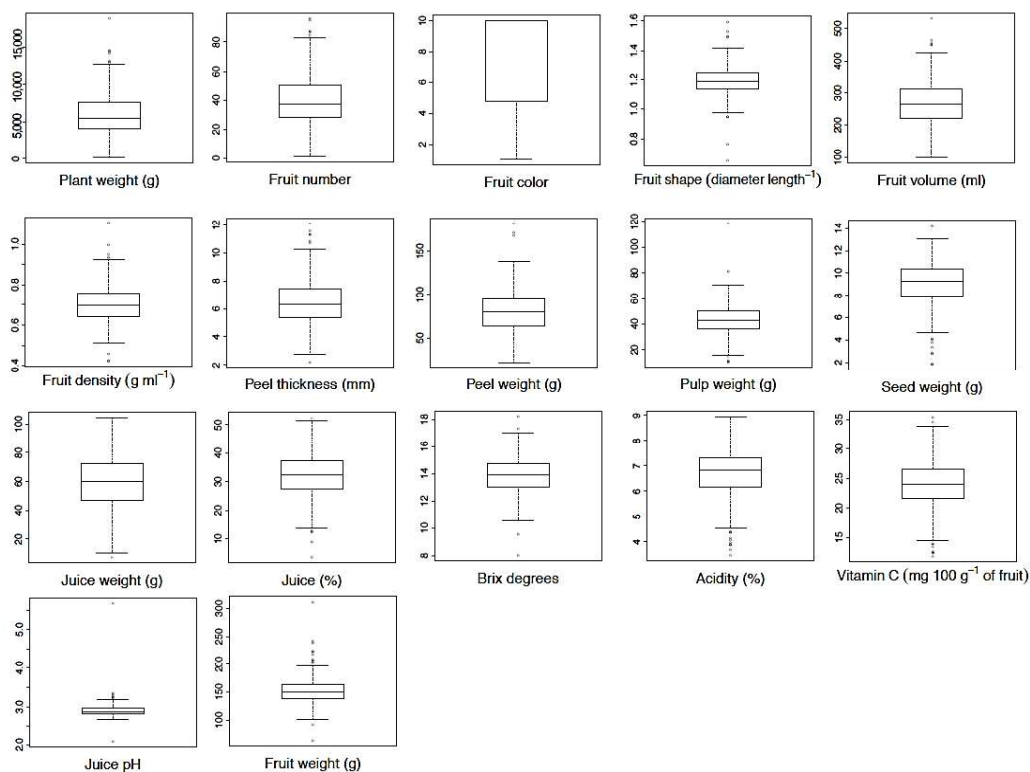


Figure 2. Box plots for each of the 17 variables measured in the *Passiflora edulis* working collection of Agrosavia.

Principal component analysis for fruit quality characteristics (Table 1) shows that variables like fruit volume, peel weight, pulp weight, seed weight, juice weight and peel thickness explain best the first component (PC1). For the second component (PC2), the variables juice weight, juice percentage, and total weight are the ones that have more relevance. We found a positive correlation between the seven variables that explain much better the principal component analysis with high ranges varying from 0.1 to 0.75. However, juice (%) is negatively correlated with four variables (i.e. fruit volume, peel thickness, peel weight and pulp weight with a range from -0.07 to -0.45) (Fig. 3, A).

The analysis of 25 morpho-agronomic descriptors to identify improved cultivars in Brazil showed similar results (Fonseca, 2017). In this study, seed weight and peel thickness were highlighted as the most important variables that explained the phenotypic variation. Additionally, in the first genetic diversity screening of a Colombian passion fruit collection carried out through the evaluation of 40 accessions, peel thickness and seed weight were also the variables that explained most of the variability (Ocampo et al., 2013). Further studies in a diversity panel as well as with a segregating population will establish possible heterotic groups in passion fruit and the subsequent use of hybrid vigour in new cultivars (Silva et al., 2014).

Table 1. Principal component analysis for 17 yield and fruit quality variables measured in passion fruit. Bold values indicate the variables that explain each component

Variable	PC1	PC2	PC3
Fruit colour	0.095	0.135	0.161
Fruit shape	-0.323	0.101	0.174
Fruit volume	0.858	-0.060	0.099
Fruit density	-0.038	0.086	0.248
Peel thickness	0.501	-0.385	-0.239
Peel weight	0.777	-0.445	-0.160
Pulp weight	0.781	0.099	0.093
Seed weight	0.785	0.126	0.123
Juice weight	0.623	0.509	0.465
Juice percentage	-0.091	0.693	0.557
Brix degrees	0.181	0.239	0.147
Acidity %	0.174	0.000	-0.253
Vitamin C	0.188	-0.093	-0.371
Juice pH	-0.025	-0.145	0.099
Total weight	0.184	0.674	-0.644
Number of fruits	0.081	0.687	-0.685
Fruit weight	0.323	-0.077	0.203

Despite the genetic variability that was seen using fruit quality features, the PCA analysis did not show a defined structure within the population (Fig. 3, B). Similar results using molecular markers show the low genetic structure in passion fruit mostly due to self-incompatibility and cross-pollination (Ocampo et al., 2017). However, this information is useful to select genetically contrasting materials that can be used as parental to increase segregation in following generations. Additionally, because of the selection pressure that these materials have suffered to select the best plants, much of the phenotypic variability is due to environmental effects with a narrow genetic base. Therefore, any pre-breeding and breeding program in passion fruit must start with the collection, conservation, and characterization of new materials in both *ex situ* and *in situ* germplasm banks. After this process has been carried out and the information has been analysed, it is possible to identify a core collection which represents the minimum number of materials with the highest allelic variability (Cerqueira-Silva et al., 2014a). Recent studies have used SSR markers with high polymorphism and with a broad genome coverage as a complementary tool for characterization of the genotypic diversity within the *Passiflora* genus (Araya et al., 2017).

There are two possibilities to increase the genetic diversity bases of passion fruit in a pre-breeding program. The first strategy is to search for wild materials to increase the allelic variability of this botanical form. This strategy has been used in gulupa (Ortiz et al., 2012). Alternatively, a second strategy is to cross passion fruit with closest species. Fortunately, in Colombia, the following high compatible interspecific crosses have been identified as viable: *P. edulis* f. *flavicarpa* × *P. vitifolia*, *P. alata* and *P. cincinnata*; *P. maliformis* × *P. caerulea*; and *P. edulis* f. *edulis* × *P. edulis* f. (Ocampo et al., 2016).

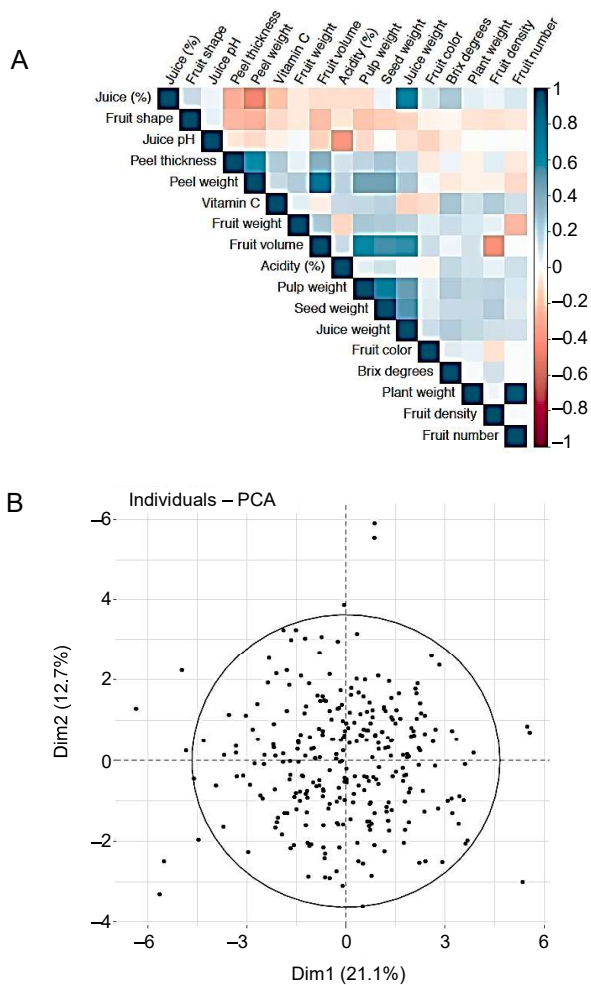


Figure 3. A – Correlation analysis among 17 variables analysed in the passion fruit collection; B – Variation of individuals in the two PCA dimensions that explains the data showing no evidence of subpopulation structure.

Finally, the SI analysis allowed us to identify 30 accessions from the working collection with most desirable characteristics to continue with the next recombination and/or selection cycle. Five of the materials with the highest SI were 38.5Cenicafe, 18.2Cenicafe, LaUnion 2/4, Lejanias, and Grajales with values of 2.09, 1.93, 1.44, 1.40 and 1.29, respectively (Fig. 4). Furthermore, with a selection pressure of 10%, thirty of the best-improved materials showed a SI superior to 0.65. The use of SI has been critical in plant breeding and particularly in passion fruit progeny evaluations. Furthermore, non-parametric SI have shown good gain predictions that are superior, in a balanced way, among the characteristics assessed (Silva & Viana, 2012). Moreover, SI give us the possibility to complement these results with future molecular analyses and response trials to specific biotic stresses. For example, Cerqueira-Silva et al. (2015) used 23 SSR markers together with plant response values (incidence and severity) for anthracnose, scab and passion fruit woodiness virus (PWV) to select the most promissory materials.

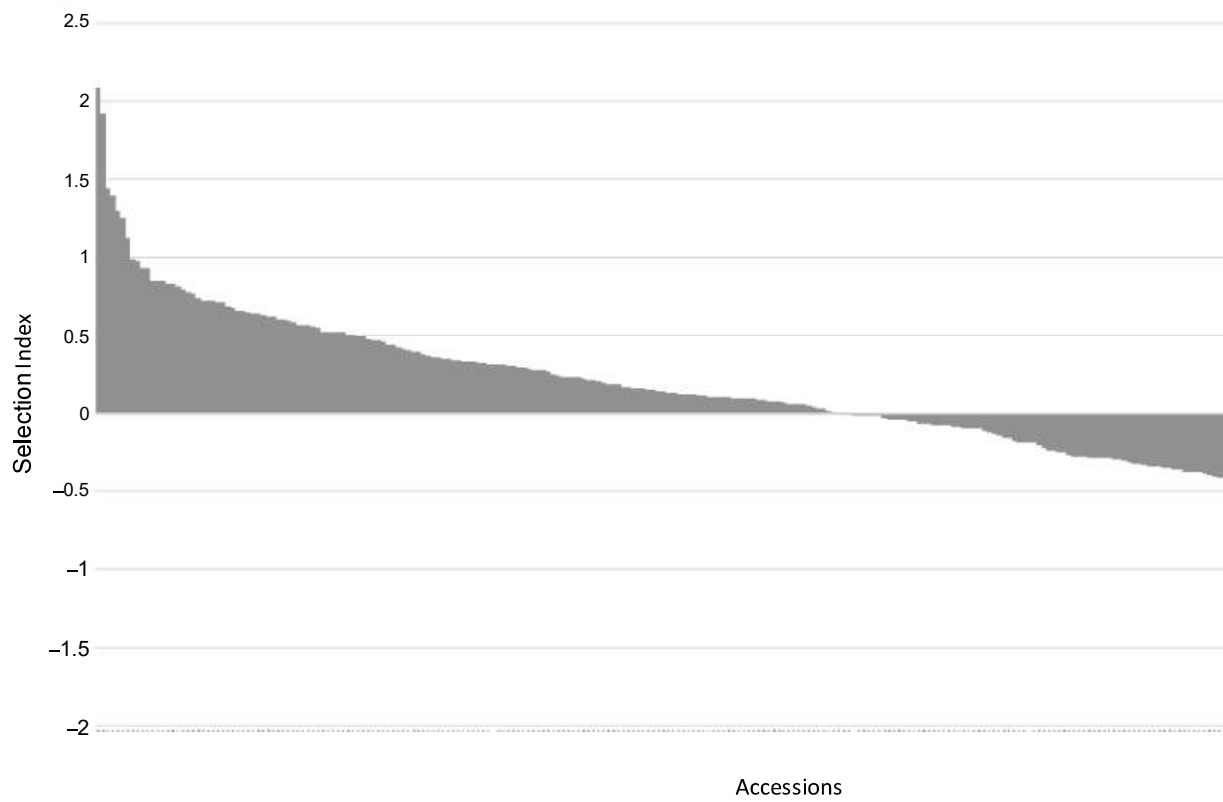


Figure 4. Selection index of 295 passion fruit plants that represent the diversity of 60 accessions from the working c

CONCLUSIONS

This study allowed us to establish for the first time the genetic diversity of the passion fruit germplasm collection of AGROSAVIA (Corpoica). We found a high diversity across 60 accessions including 17 variables associated to yield production and fruit quality. Despite this variability, we did not find evidence of population substructure within the collection. Therefore, we selected the 30 most promissory accessions to be used in the first recurrent selection cycle plan as part of a future breeding program for this species in Colombia. These accessions and their progenies are going to be genotyped across the genome and combined with current agronomic characterization to estimate their breeding values (GEBV), and reduce selection cycles in a future selection process (Viana et al., 2017).

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