DOI: 10.5433/1679-0359.2022v43n5p2123

Characterisation and pre-selection of Acca sellowiana genotypes by multivariate analysis

Caracterização e pré-seleção de genótipos de Acca sellowia por análise multivariada

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Highlights _

Individuals with superior characteristics were selected using principal component analysis. More rounded fruit had a greater percentage of pulp. Fruit with a greater total mass showed excessive peel thickness.

Abstract _

Feijoa (*Acca sellowiana*) is a native Brazilian fruit with a peculiar flavour, a considerable amount of bioactive compounds and antioxidant activity. Even though this fruit tree is currently cultivated in several countries around the world, in Brazil, the process of domestication is underway, and the selection and breeding of new genotypes that are more productive and with better fruit quality is necessary. The objective of this work was to evaluate phenotypic diversity among and within progeny and to study the correlations among the quality variables of Feijoa fruit, seeking to select individuals with superior characteristics using principal component analysis. The parents who formed the progeny (families) were selected from a participatory breeding programme. We observed that individuals 47 and 93 had a combination of desirable fruit characteristics for selection, and individuals 15, 910, 98 and 410 should be selected for future crossings, as they had a high total fruit mass and soluble solid content or the highest percentage of pulp and rounded fruit shape. Larger fruit, in general, had a lower percentage of pulp. Principal component analysis is a viable tool in the preselection of new genotypes and potential progenitors for Feijoa breeding programmes. **Key words:** Brazilian native fruit. Feijoa. Fruit quality. Pineapple-quava.

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Resumo .

Feijoa (*Acca sellowiana*) é uma fruteira nativa do Brasil com sabor peculiar, com considerável quantidade de compostos bioativos e atividade antioxidante. Mesmo sendo cultivada em diversos países, no Brasil, seu processo de domesticação está em curso e a seleção e o melhoramento para obtenção de novos genótipos, com maior produtividade e melhor qualidade de frutas, é necessário. O objetivo deste trabalho foi avaliar a diversidade fenotípica entre e dentro de progênies e estudar as correlações entre as variáveis de qualidade de frutos de Feijoa, buscando selecionar indivíduos com características superiores, usando análise de componentes principais. Os progenitores que formaram as populações (famílias) analisadas foram selecionados a partir de um programa de melhoramento participativo. Foi observado que os indivíduos 15, 910, 98 e 410 podem ser selecionados para futuros cruzamentos, pois possuem maior massa total de frutos e sólidos solúveis, ou maior porcentagem de rendimento de polpa e formato arredondado. Maior tamanho de frutos, em geral, tiveram uma menor percentagem de polpa. A análise de componentes principais é uma ferramenta viável para pré-seleção de genótipos e potencias progenitores em programas de melhoramento de Feijoa.

Palavras-chave: Feijoa. Fruteiras nativas do Brasil. Goiabeira-serrana. Qualidade de frutos.

Introduction _

Acca sellowiana (O. Berg) Burret. (Myrtaceae), popularly known as Feijoa, pineapple-guava, goiaba da serra or goiabaserrana, has as its centre of origin in the southern Brazilian highlands (Ducroquet et al., 2000), with subsequent dispersion having occurred in north-eastern Uruguay (Nodari et al., 1997; Weston, 2010). In Brazil, despite it being the centre of origin, with great diversity, and the release of some cultivars, such as Alcântara, Helena, Mattos and Nonante (Ducroquet et al., 2007, 2008), little cultivation of the species occurs, and it is in the process of domestication (Donazzolo et al., 2020). However, this fruit tree has high economic, commercial and food potential, especially in countries, such as France, Arzebaijan, Israel, the United States of America, Chile, Colombia, Iran and New Zealand (Thorp & Bieleski, 2002; Santos et al., 2011; Karami et al., 2013; Pasquariello et al., 2015; Rasekh et al., 2021), and its cultivation success is attributed to the peculiar flavour of the fruit (Nodari & Guerra, 2017), nutraceutical components, and antimicrobial activity (Basile et al., 1997; Vuotto et al., 2000; Pasquariello et al., 2015; Phan et al., 2019). The fruit is rich in proteins, fibre, vitamin C, essential oils and minerals, such as potassium, phosphorus and iron (Zhu, 2018; Phan et al., 2019; Amarante et al., 2019). Feijoa fruit contains high amounts of bioflavonoids and polyphenols, such as catechin, leucoanthocyanins, proanthocyanidins and naphthoguinones, which confer many health benefits to the fruit (Weston, 2010; Karami et al., 2013; Zhu, 2018; lelpo et al., 2000).

The size, firmness, titratable acidity value, soluble sugar content, taste, aroma and texture are fundamental quality attributes for many fruit species (Crossa-Raynaud & Audergon, 1991; Bassi & Bartolozzi, 1993). Furthermore, antioxidant activity and a considerable amount of bioactive compounds, especially phenols, flavonoids and ascorbic acid, were detected in Feijoa but are variable among cultivars (Pasquariello et al., 2015). These quality parameters may not be independent of each other. Therefore, findina genotypes from segregating populations that bring together the greatest number of desirable quality parameters can improve fruit quality. This improvement could benefit breeding programmes and orchard management because knowledge of the relationship between fruit quality parameters, possibly using multivariate analyses, would make it possible to reduce the number of pomological traits available for study (Gurrieri et al., 2001; Pasquariello et al., 2015) and to identify the best genitors for future crosses.

During the process of domestication and breeding, species are impacted due to the occurrence of evolutionary factors, as well as the selection and recombination of specific genotypes (Donazzolo et al., 2020). This process can be negative, when the frequency of deleterious genes increases (Wettberg et al., 2018), or positive, due to the screening of mother-trees with traits of interest and the rejection of plants not desired or seedlings considered to be unsuitable (Korshikov et al., 2004). In some cases, the managed plantations of a tree species may preserve high indices of genetic diversity, having the potential for conservation of the species' genetic resources (Stefenon et al., 2008; Levis et al., 2017). In southern Brazil, a participatory research proposal with Feijoa has been implemented as a strategy for genetic improvement and adaptation to different environments, as well as for conservation using this genetic resource, with a strong emphasis on the traditional knowledge of farmers (Santos et al., 2018; Donazzolo et al., 2020). From this germplasm kept on farms or in situ, <u>plants have been</u> selected and crossed with each other, forming segregating populations. These populations are distributed in different environments (locations). The Caçador area population was used in this study.

Started in 2008, the UFSC/UTFPR participatory breeding programme for Feijoa adopts the following desirable selection criteria: fruit weight > 60 g, soluble solid content > 12 °Brix, pulp yield > 35% and rounded fruit shape (Volpato et al., 2011), meaning that plants that produce fruit within these criteria have superior characteristics and are more attractive for consumption.

Multivariate analysis has been used efficiently in the selection of the best progeny in hybrid populations of fruit trees, for example, in the selection of cacao progeny (Maharaj et al., 2011), peach (Cantín et al., 2010), papaya (Ramos et al., 2012), and other non-fruit species, such as for the selection of droughttolerant bean progeny (Sousa et al., 2015).

For Feijoa, there is limited information on the global assessment of fruit quality in breeding progeny and their correlations. Thus, the objective of this work was to evaluate phenotypic diversity among and within progeny and to study the correlations between the quality variables of Feijoa fruit, seeking to select individuals with superior characteristics using principal component analysis.

Materials and Methods ____

The progeny were planted in 2012 in the middle of a fragment of the natural forest of the EMBRAPA/EPAGRI Forest Reserve, located in Caçador-SC, Brazil, coordinates

26°51'41.81" S, 50°56'15.32" W, elevation of 1065 m. The local climate is classified as Cfb according to the Köeppen classification, with an average temperature of 16.6°C and an average annual precipitation of 1613 mm. The terrain of the site was subdivided into three blocks containing 99 trees. The individuals were randomly arranged and identified by family according to the following crosses: 1001 x 'Helena' (family 1), 1067 x 1003 (family 2), 1013 x 1051 (family 3), 1004 x 1035 (family 4), 1079 x 'Branca' (family 5), 1051 x 1035 (family 6), 1006 x 'Helena' (family 7), 1006 x Pomar (family 8) and 1013 x Pomar (family 9). The orchard was maintained under minimum management conditions without fertilisation, pruning, thinning or phytosanitary treatments.

To evaluate the quality of the fruit, 10 pieces of fruit were harvested from the plants of the orchard in February and March of 2017 and 2018, respectively. The maturation stage considered for harvesting was when the fruit came off the tree with a light touch of the hand or slightly shaking the plant. After harvesting, fruit was placed in plastic bags and stored in a refrigerator for up to three days for analysis in the laboratory.

For the evaluations, the following quantitative and qualitative parameters were considered: diameter/length ratio (D/L), obtained by the ratio between the transversal and longitudinal lengths, measured in centimetres; average fruit mass, obtained by dividing the total mass by the number of fruit, expressed in g; pulp yield, average obtained by the percentage of the pulp weight in relation to the total weight of each fruit, expressed as a percentage; soluble solids, obtained by extracting the juice from the pulp of each fruit and measured using a manual refractometer, expressed in °Brix.

The data were analysed using a multivariate approach, in which the principal component methodology was used, which consists of transforming a set of 'p' variables belonging to 'n' individuals or populations into a new set of variables of equivalent dimension, called principal components. The values for total mass, SS content, diameter/ length ratio and percentage of pulp were utilised. The analyses were performed using the FactoMineR package (Lê et al., 2008) of the R language.

Results and Discussion ____

For principal component analysis, components 1 and 2 were selected for the graphical representations due to their greater representation of the data variance, with 37.96% for the first component and 27.25% for the second component. Thus, it is possible to represent 65.21% of the diversity of the genotypes based on the analysed characteristics. The variables total mass and pulp yield (%) had a high correlation with CP1, with values of 0.64 and -0.85, respectively, and the variable °Brix had a high correlation with CP2, with a value of -0.90 (Table 1). The diameter/length ratio, however, had an average correlation with both components, with correlation values of -0.56 for CP1 and 0.50 for CP2.

Table 1

Factor weight matrix of the quality variables of the Feijoa (Acca sellowiana) for the two main components selected

Variable	CP1	CP2
Total mass	0.64	-0.11
% of pulp	-0.85	-0.15
Soluble solids - SS (°Brix)	-0.24	-0.90
Diameter and Length ratio (D/L Ratio)	-0.56	0.50
Eigenvalue	1.52	1.09
% of variance explained	37.96	27.25
% of variance accumulated	37.96	62.21

The genotypes of the right quadrants of Figure 1 had higher indexes of total mass and SS when they negatively departed from the origin of CP2, while the genotypes of the lower left quadrant had higher pulp yield (%) and SS indexes when they negatively distanced themselves from the CP2 origin. In contrast, genotypes in the upper quadrants close to CP2 had lower levels of SS content, pulp yield (%) and total mass. The genotypes present in the upper left quadrant had a high D/L ratio, which characterises fruit with a more rounded shape.

Individuals 47, 410 and 46 were characterised by higher pulp yield, lower total mass and average SS (°Brix). Individuals 82, 93, 99 and 98 had a higher SS content in their fruit, especially 82 and 98, the latter of which had the highest total fruit mass. Individuals 34, 15, 94 and 12 had a greater fruit mass, although 34 had a low SS content, while the others had average SS values.

For the families, the values of CP1 and CP2 explained 73.07% of the variation in the data. Families 4 and 9 were the most contrasting regarding the variability of the measurements studied, while family 5 was close to the origin of the components, which indicates average values for all variables (Figure 2). Individuals in Family 1 had large fruit but did not stand out for any other variable, including a low percentage of pulp. Family 9, on the other hand, had fruit with better total mass and an SS content above the general average; however, similar to family 1, they had a low pulp percentage. Family 4, in addition to the average SS content, stood out for producing fruit with a better percentage of pulp, while Family 8 showed higher values for SS content and average pulp yield. Families 7 and 6 presented low levels of SS and average values for pulp percentage and total fruit mass.

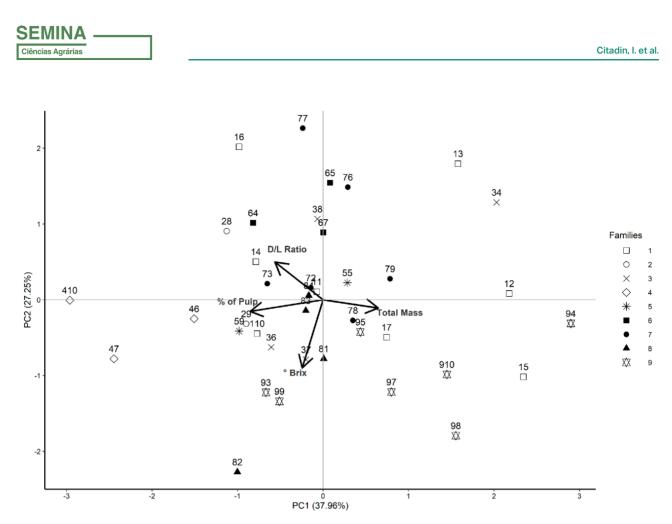


Figure 1. Principal component analysis (PCA) of each individual for four characteristics (percentage of pulp, soluble solids content, total mass and the diameter/length ratio) of fruit from nine Feijoa (Acca sellowiana) families grown in Caçador-SC, Brazil.

The fruit with a greater total mass showed excessive peel thickness, which affected pulp yield. Degenhardt et al. (2003) demonstrated negative correlations between total fruit weight and pulp yield. This was observed for most of the individuals evaluated in this study, in which larger fruit did not necessarily have a higher percentage of pulp. For the consumer, this characteristic is undesirable, as the fruit is bought by the kilo, and thus, most of it is discarded if they do not know how to reuse the peel of the fruit. Despite the low pulp yield, fruit with a greater peel thickness tend to have greater resistance to handling and post-harvest transport (Amarante et al., 2008; Silveira et al., 2015). The edible fruit peel possesses significantly (p < 0.05) higher amounts of antioxidant flavonoids and vitamin C than the fruit pulp. This is most likely the reason for the observed strong antimicrobial activity of peel extracts against a wide range of food spoilage microorganisms (Phan et al., 2019). For this reason, the potential use of Feijoa peel as a natural food perseverative needs to be investigated in follow-up studies.

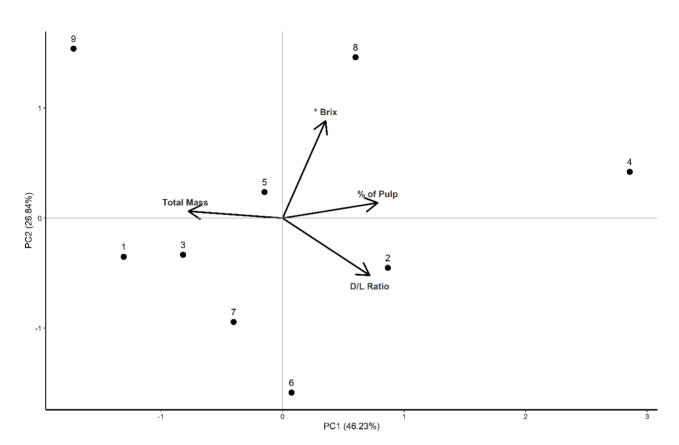


Figure 2. Principal component analysis (PCA) of four characteristics (percentage of pulp, soluble solids content, total mass and the diameter/length ratio) of fruit from nine Feijoa (*Acca sellowiana*) families grown in Caçador-SC, Brazil. 1001 x 'Helena' (family 1), 1067 x 1003 (family 2), 1013 x 1051 (family 3), 1004 x 1035 (family 4), 1079 x 'Branca' (family 5), 1051 x 1035 (family 6), 1006 x 'Helena' (family 7), 1006 x Pomar (family 8) and 1013 x Pomar (family 9).

Another characteristic observed through the analysis is that more rounded fruit had a greater percentage of pulp compared with more elongated fruit, thus highlighting the importance of shape evaluation, represented by the diameter/length ratio. The average of 0.74 shows a tendency towards fruit with rounded shapes. The genotypes showed low variability in relation to fruit shape, which reduced the separation of the genotypes by the principal components through this variable.

The SS content varied from 7.09 to 14.06 °Brix, with an average of 10.70 °Brix (Table 2). This result was similar to that obtained by Borsuk et al. (2017), who evaluated 18 Feijoa populations in the South Region. These levels are in line with those described by Ducroquet et al. (2007, 2008) for the cultivars Helena, Nonante and Mattos.

Table 2

Characterisation of fruit from Feijoa (Acca sellowiana) plants introduced to Caçador, SC. The highlighted cells represent those that met at least one established selection criteria

Plant code	Total mass (g)	Pulp yield (%)	SS (ºBrix)	D/L
11	50.56	26.56	10.81	0.75
12	108.14	21.59	9.87	0.68
13	76.17	19.93	7.90	0.75
14	60.89	36.98	9.61	0.75
15	157.11	21.72	12.30	0.76
16	68.53	33.29	8.65	0.89
17	64.01	31.34	9.90	0.6
28	43.32	37.66	9.00	0.75
29	62.16	36.38	11.06	0.75
34	68.34	17.60	8.20	0.68
36	42.99	33.11	11.21	0.69
37	79.21	32.64	11.80	0.74
38	66.42	34.07	8.45	0.72
46	44.10	34.74	11.72	0.8
47	66.34	44.73	12.50	0.84
55	44.19	28.47	9.62	0.67
59	75.31	38.56	11.22	0.77
64	52.25	25.48	10.87	0.9
65	79.49	27.63	9.07	0.84
67	35.14	26.95	9.17	0.72
72	62.13	27.64	10.92	0.78
73	43.28	31.30	10.45	0.75
76	65.70	24.07	9.18	0.82
77	56.20	26.31	8.22	0.86
78	64.43	26.31	11.10	0.73
79	54.97	19.23	10.80	0.76
81	42.48	23.37	12.33	0.74
82	41.23	33.01	14.06	0.69
83	48.55	31.78	10.33	0.69
84	58.67	30.41	10.50	0.74
93	73.16	36.10	12.30	0.73
94	137.06	22.45	9.90	0.65
95	44.69	22.81	11.37	0.7
97	49.38	26.91	11.30	0.59
98	91.78	21.08	13.10	0.67

continue...

99	44.32	31.93	12.32	0.68
110	50.46	30.15	11.93	0.79
410	50.90	42.77	12.17	0.91
910	107.75	23.80	12.00	0.72
Mean	64.92	29.25	10.70	0.74
SD	25.55	6.43	1.47	0.07
CV	0.39	0.22	0.14	0.09

continuation...

Selection criteria used: fruit weight > 60g; sugar content > 12 °Brix; and pulp yield > 35% (Volpato et al., 2011). D/L = Diameter and Length Ratio.

The average obtained for the total mass (64.9 g) was low when compared with that observed for the cultivars Alcântara, Mattos, Nonante and Helena, all with average weights above 90 g. However, the pulp yield was within the standards determined for the cultivars (25-33%) (Ducroguet et al., 2007, 2008; Santos et al., 2018), which means that there is less peel thickness and a greater quantity of pulp, on average, for the studied progeny compared with the cultivars, with pulp yields of individuals ranging from 35-42%. Thus, it is important to evaluate several variables at the time of selection to prevent any easy-tomeasure variable, such as fruit weight, from overlapping the variable of greatest relevance to the quality of fruit for consumption, such as the percentage of pulp and SS content. Anzanello et al. (2013), while evaluating commercial kiwi cultivars, demonstrated that there is a direct relationship between the number of seeds and the final size of the fruit, emphasising the importance of efficient pollination for obtaining large fruit. Thus, measuring the weight and quantity of seeds per fruit is recommended in future studies to more accurately determine the influence of these parameters on the final weight of the fruit. Feijoa has hermaphrodite flowers; however, it is a predominantly allogamous species that presents barriers to selffertilisation, such as dichogamy by protogyny (Stewart & Craig, 1989). In addition, lateaction self-incompatibility occurs through the rejection/abscission of self-pollinated flowers precisely after the formation of the singamia and zygote (Finatto et al., 2011). This mode of reproduction influences the number of seeds, size and fruit set.

Considering the criteria adopted by the Feijoa participatory breeding programmes, plants 93 and 47 were the only plants with fruit that had suitable values for all characteristics considered desirable by the participatory breeding criteria (Table 2). The matrices of the analysed crosses were selected using the same criteria. However, several plants possessed potential characteristics for preselection, principally when assessing the distribution in the PCA analysis. Plants with an SS content above 10 °Brix and a pulp vield above 30% could be considered, as these are the characteristics of cultivars bred by Epagri (Ducroquet et al., 2008). Thus, other plants would meet the selection criteria, namely: 29, 36, 37, 46, 59, 73, 82, 83, 84, 99, 110 and 410,

with emphasis on genotype 410 due to its high pulp yield (42.77%).

In addition, genotypes 15, 910 and 98 stand out for their total mass and high levels of SS and should be used in future crosses. Genotypes 12 and 94 had similar characteristics in relation to mass, with an emphasis on 94 but with intermediate SS, but eventually they could also be used for crossings aimed at improving the total mass. For the pulp percentage, genotype 410 had the potential for selection as a parent, which, despite its low weight, presented excellent SS content and pulp percentage and a rounded fruit shape.

The evaluated characteristics of the individuals showed quite variable values in relation to the commercial cultivars described, showing the magnitude of diversity that can be used for selection and breeding, especially considering those that showed superior performance in a particular variable. This result is due to the segregating characteristics of the population evaluated, combined with the use of parents with a high degree of heterozygosity. However, it is important to consider climatic conditions and the possibility of new assessments to determine greater stability for the highlighted characteristics. In this aspect, Cacador-SC has characteristics very similar to those of lpê-RS, the place of origin of part of the matrices of the evaluated crosses. Beyhan and Eyduran (2011), while evaluating Feijoa progeny in Turkey, found that a drop in temperature and an increase in rainy periods influenced the shape and weight of the fruit, showing better potential when climatic characteristics were similar to the centre of origin. These factors should be

evaluated individually and over the long term so that their influence on the variability of the characteristics and the importance of these sites for the adaptation and domestication of Feijoa can be determined.

The selection of parents within families 1, 4, 8 and 9 is interesting for the mentioned characteristics. In two of the highlighted families, the Pomar selection was present as the parent. Within these families, plants can also be selected for future hybridisations, considering the contrasting characteristics.

Selection between families allowed us to identify the best parents for future crosses. For the evaluated characteristics, the Pomar selection presents itself as a good option for increasing the concentration of SS content, as well as fruit size, since all individuals of the progeny that had 'Pomar' as a parent presented these characteristics. As for the percentage of pulp, selections 1004 and 1006 were the best parents.

Conclusion _

individuals Feiioa 47 and 93 presented a combination of desirable fruit characteristics for selection. Individuals 15, 910 and 98 should be selected for future crossings, as they had both a high total mass and soluble solid content. Individual 410 had the highest percentage of pulp, with a rounded fruit shape. Larger fruit generally had a lower percentage of pulp. The best parents to perform crosses were selections Pomar, 1004 and 1006. Principal component analysis proved to be a viable tool in the pre-selection of new genotypes of Feijoa.

Acknowledgments _____

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for financial support and for incentivising scientific research and Maria Izabel Radonski *(in memoriam)* for being responsible for the implementation of the experiment.

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