

Path analysis for selection of feijoa with greater pulp weight

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ABSTRACT: *The objective of this paper was to identify the direct and indirect effects of feijoa fruits (*Acca sellowiana*) traitson pulp weight, in order to use these traits in indirect genotypes selection. Fruits of five feijoa plants were collected in Rio Grande do Sul, in the years of 2009, 2010 and 2011. Six traits were evaluated: diameter, length, total weight, pulp weight, peel thickness and number of seeds per fruit. In the path analysis, with or without ridge regression, pulp weight was considered as the basic variable, and the other traits were considered as explanatory variables. Total weight and fruit diameter had high direct effect, and are the main traits associated with pulp weight. These traits may serve as criteria for indirect selection to increase feijoa pulp weight, since they are easy to be measured.*

Key words: *Acca sellowiana, multicollinearity, ridge path analysis, indirect selection.*

Análise de trilha para seleção de goiabeira-serrana com maior peso de polpa

RESUMO: *O objetivo deste trabalho foi identificar os efeitos diretos e indiretos de caracteres dos frutos de goiabeira-serrana (*Accasellowiana*) sobre o peso de polpa, visando à utilização desses caracteres para a seleção indireta de genótipos. Foram coletados frutos de cinco grupo de plantas de goiabeira-serrana no Rio Grande do Sul, nos anos de 2009, 2010 e 2011. Seis caracteres foram avaliados: diâmetro, comprimento, peso total, peso de polpa, espessura da casca e número de sementes por fruto. Na análise de trilha com e sem regressão em crista para controle da multicolinearidade, o peso de polpa foi considerado variável básica e os demais caracteres considerados explicativos. O peso total, seguido do diâmetro de fruto, tiveram efeito direto elevado e são os principais caracteres associados ao peso de polpa. Estes caracteres podem servir de critérios de seleção indireta para aumentar o peso de polpa de goiabeira-serrana, pois são de fácil mensuração.*

Palavras-chave: *Accasellowiana, multicolinearidade, regressão em crista, seleção indireta.*

INTRODUCTION

Feijoa [*Acca sellowiana* (Berg) Burret, family Myrtaceae - synonym *Feijoa sellowiana*] is native to the Brazilian Southern plateau and Uruguay (MATTOS, 1990). Feijoa fruit present translucent jelly-like pulp, with sweet acidulated flavor, and unique aroma (MATTOS, 1990). Due to its chemical composition, several studies have demonstrated the pharmacological properties of the species (WESTON, 2010), such as antibacterial, anti-inflammatory and antioxidant activities (KELES et al., 2012; MONFORTE et al., 2014); and to their antifungal activity against *Candida glabrata*, which is resistant to fluconazole (MACHADO et al., 2016).

The species is at the initial domestication stage in its area of natural occurrence in Brazil, including the selection of genotypes with superior traits (SANTOS et al., 2005), and the development of four feijoa cultivars adapted to the conditions of the state of Santa Catarina (DUCROQUET et al., 2007, 2008). Owing to the fruits with unique characteristics, feijoa has potential for commercialization; however, it is still little commercially explored in Brazil (DUCROQUET et al., 2000; SANTOS et al., 2011). The species is cultivated in a domestic or extractive way, and few commercial orchards, since no improved cultivars adapted to all different regions of its natural occurrence have been developed yet.

Colombia and New Zealand are among the largest feijoa producers, and these countries have made significant advances in the breeding of the species (THORP & BIELESKI, 2002). In several other parts of the world, the plant has also undergone breeding process, such as in France, Israel, Italy, the former Soviet Union, and in the United States (THORP, 2006), Turkey (BEYHAN et al., 2011; BEYHAN & EYDURAN, 2011). It has also been introduced in China, where adaptation studies are underway (TANG et al., 2016).

Indirect selection may facilitate and accelerate the selection of promising genotypes in feijoa genetic breeding, which requires the study of correlations between traits. Simple correlation coefficients are estimates of linear association between two traits; however, they do not consider the influence of other traits in the association. Conversely, the path analysis, proposed by WRIGHT (1921), demonstrates the unfolding of the correlation coefficient into direct and indirect effects of a group of traits (explanatory variables) on the expression of a more important trait for selection (basic variable), generating more accurate estimates of cause and effect (CRUZ & CARNEIRO, 2006). For this reason, path analysis is a very useful tool in genetic breeding, especially of long selection cycle species, such as feijoa. Only one work has been reported in the literature regarding path analysis on fruit species of the family Myrtaceae in Brazil, which detected fruit traits for indirect selection of pulp percentage and anthocyanin content of jabuticaba peel (SALLA et al., 2015).

The pulp is the edible part of the fruit, and also the most economically important (MATTOS, 1990). Therefore, it is one of the main traits to be improved in the obtainment of new genotypes. Proportion of pulp in relation to the total weight of the fruit is still low for the species, being around 30% in cultivars already released in Brazil (DUCROQUET et al., 2007, 2008); although, some genotypes may present proportion that can reach close to 50% (MATTOS, 1990; DEGENHARDT et al., 2003). In addition, the measurement of pulp weight is laborious, since it is firmly adhered to the peel, and it can only be removed manually, using spoon-like devices, which hinders the obtainment of information. Thus, the objective of this research was to identify feijoa fruits traits for indirect selection of pulp weight.

MATERIALS AND METHODS

Five to ten mature fruits were collected per feijoa plants originated from five groups of plants

from Rio Grande do Sul. Groups were denominated: 1) "Farmers": plants cultivated by farmers in the municipalities of Ipê, Monte Alegre dos Campos, and Antônio Prado. Fruits were collected from 17, 42, and 37 plants in the years of 2009, 2010, and 2011, respectively; 2) "Backyards": plants grown in urban yards in the municipality of Vacaria. Fruits were collected from 10, 43, and 27 plants in the years of 2009, 2010, and 2011, respectively; 3) "Natural populations": plants originated from forest fragments in regeneration, in the municipalities of Ipê and Antônio Prado. Fruits were collected from 24 and 27 plants in the years of 2010 and 2011, respectively; 4) "SelCAV": plants grown in agroforestry systems, originated from mass selection of matrices, carried out in 2002. Fruits were collected from 20 plants in 2011; 5) "Chileans": plants grown in agroforestry systems in the municipality of Ipê and Antônio Prado, originated from germplasm imported from Chile. Fruits were collected from 16 plants in 2011.

In the laboratory, the following parameters were evaluated: diameter (measured in the equatorial part of the fruit), length (measured between opposite fruit sides), total weight, pulp weight, peel thickness, and number of seeds per fruit. Fruit diameter, fruit length and peel thickness were measured with a digital caliper. Weights were obtained using a precision scale, weighing the whole fruit and then the peel (after pulp removal).

Data of all the populations and years were grouped in the statistical analyses of each trait. Descriptive statistics were calculated to verify the variability of traits data. Assumptions of normality and homogeneity were verified by the Lilliefors and Bartlett tests. Afterwards, the Pearson's correlation coefficients were estimated between traits, whose significance was determined by the Student's *t* test ($P \leq 0.01$).

Subsequently, the multicollinearity diagnosis was performed by the condition number analysis (CN), which represents the ratio between the largest and the smallest eigenvalue of the correlation matrix. If $CN < 100$, collinearity is weak; If $100 \leq CN \leq 1,000$, collinearity is moderate to strong; And if $CN > 1,000$, collinearity is severe (MONTGOMERY & PECK, 1981). Path analysis was performed considering pulp weight as the basic variable (main dependent), and the other five traits were considered as independent or explanatory. With the multicollinearity, indicated by the condition number of 209.7 (moderate to strong) among the six traits, the ridge path analysis method, also called path analysis under multicollinearity, was applied (CARVALHO, 1994). In this strategy, a constant *k*

is used, whose value must be the smallest as possible, in order to stabilize the path coefficients, and at the same time keep the variance inflation factor (VIF) lower than 10, in all explanatory variables. This technique solves the problem of the multicollinearity effect, which can cause bias in the estimates of the direct and indirect effects between the variables. This technique avoids the exclusion of explanatory variables to maintain multicollinearity at acceptable levels. (CRUZ & CARNEIRO, 2006; RIOS et al., 2012). For comparison purposes, a second single chain path analysis method (with no multicollinearity control) was performed, since the CN was close to the lowest limit to be considered weak multicollinearity (CN<100). All statistical analyses were carried out using the Genes software (CRUZ, 2013). Interpretation of the effects of the path coefficients was performed using the Pearson's correlation coefficients, according to LÚCIO et al. (2013).

RESULTS AND DISCUSSION

High variability was observed among feijoa fruit for the six traits, especially for total weight, pulp weight, and number of seeds per fruit (Table 1). This is very important for the selection of genotypes, since the existing variability allows selecting multiple traits, aiming to increase pulp yield, which is the *in natura* edible part, or that used in processed food or drinks (frozen pulp, juice, liquor, candies, etc). All the evaluated traits fulfilled the assumptions of normality and homogeneity by the Lilliefors and Bartlett tests, respectively.

In addition, all Pearson's correlation coefficients among the six traits were significant by the t test ($P \leq 0.01$), except for the number of seeds with diameter and pulp weight (Table 2). Coefficients were considered moderate (0.50) to strong (0.96) among the traits, except for the number of seeds,

which had the lowest correlations with the other traits, demonstrating that the number of seeds in the fruits has low effect on the total weight and pulp weight, despite the wide variability reported for this traits in the evaluated fruits.

Comparing the two strategies of path analysis, without multicollinearity control (single chain path analysis) and with multicollinearity control (ridge path analysis), similar analysis performance was observed, since the determination coefficients were high ($R^2=0.94$ and 0.95), and the effect of the residual variable was low (0.25 and 0.23) (Table 3).

This suggested that the two methods were accurate in estimating the direct and indirect effects (CRUZ & CARNEIRO, 2006; RIOS et al., 2012). This similarity between the performance of the two methods of path analysis was due to the low degree of multicollinearity (CN = conditions number = 209.7), being close to the limit considered as weak, CN<100 (MONTGOMERY & PECK, 1981). Thus, the effect of bias correction by the method of multicollinearity control (ridge path analysis) was not as expressive as that of the method without multicollinearity control.

In contrast, for Jaboticabeira fruit traits set (SALLA et al., 2015), in which multicollinearity was severe (CN = 5.15×10^9), the use of ridge path analysis was necessary in order to correct the bias caused to the coefficient, even with more accurate estimates than the exclusion of the trait that caused multicollinearity (peel percentage).

The high determination coefficient and the effect of the low residual variable indicated the goodness of fit of the path analysis model, as well as the effective explanation of the effects of the five explanatory variables regarding the basic feijoa variable (pulp weight) (CRUZ & CARNEIRO, 2006).

The total fruit weight had the highest direct effect on the basic trait (pulp weight). Fruit diameter

Table 1 - Descriptive statistics of six traits of feijoa fruit (*Acca sellowiana*).

Variable	N	Minimum	Mean	CI (95%)	Median	Maximum	SD	CV(%)
Diameter (cm)	2414	1.7	4.2	4.16-4.24	4.2	7.1	0.9	21.4
Length (cm)	2414	2.4	5.1	5.05-5.14	5.0	9.6	1.1	21.6
Total weight (g)	2414	6.0	54.9	53.65-56.15	48.0	209.0	31.4	57.2
Pulp weight (g)	2399	2.0	19.2	18.73-19.67	16.1	94.0	11.8	61.5
Peel thickness (cm)	2381	0.2	0.5	0.49-0.51	0.5	1.6	0.2	40.0
N. of seeds	239	21.0	90.6	85.97-95.23	85.0	229.0	36.5	40.3

CI: confidence interval of the mean, at 95% probability. SD: standard deviation of the mean. CV: coefficient of variation.

Table 2 - Pearson's correlation coefficients in the upper diagonal, and associated probability, by the t ($P \leq 0.01$) test, on the lower diagonal, among feijoa (*Acca sellowiana*) traits.

Variables	Diameter	Length	Total Weight	Pulp Weight	Peel Thickness	N. of seeds
Diameter (cm)	-	0.833*	0.958*	0.890*	0.772*	-0.196 ^{NS}
Length (cm)	<0.0001	-	0.884*	0.814*	0.659*	-0.253*
Total weight (g)	<0.0001	<0.0001	-	0.911*	0.771*	-0.235*
Pulp weight (g)	<0.0001	<0.0001	<0.0001	-	0.500*	-0.136 ^{NS}
Peel thickness (cm)	<0.0001	<0.0001	<0.0001	<0.0001	-	-0.257*
N. of seeds	0.0119	0.0010	0.0024	0.0824	0.0009	-

had significant explanatory effect on the definition of pulp weight. Both traits presented high and positive Pearson's correlation coefficients (0.91 and 0.89) with pulp weight. However, in the path analysis, which demonstrated the cause and effect relationships, the effect of fruit weight on pulp weight is greater than that of the diameter.

The high and significant effect (0.81) of the length on pulp weight, detected by the simple correlation, was due to the indirect effect, mainly of the total weight, since the direct effect between fruit length and weight was low, proving not to be suitable for indirect selection of feijoa fruits with greater pulp weight. Thus, the evaluation of the Pearson's correlation coefficients between these two traits would lead to the error of using it in the selection. This indicated the limitation of the coefficient of linear correlation and the advantage of the path analysis in the indication of the actual cause and effect relationships, since this analysis decomposes the coefficient of linear correlation, and demonstrated the direct effect of the explanatory trait on the basic trait, removing the indirect effects, and quantifying them (WRIGHT, 1921; CRUZ & CARNEIRO, 2006). However, path analysis requires the evaluation of more than two response variables, which can be related, in order to identify the interrelationships between the traits, regardless of the others.

Number of seeds per fruit and peel thickness were also non-explanatory traits for the basic variable (pulp weight). In addition, both traits are difficult to be measured, and are traits of lesser relevance in the breeding of a fruit species. Therefore, these traits are not suitable for indirect selection of great pulp weight feijoa fruit.

Mainly the total fruit weight and fruit diameter can be measured and used for indirect selection of greater pulp weight of feijoa fruit. Since this association is directly proportional (positive sign

of the correlation coefficient and of the direct effect), the selection of fruits with greater weight and greater diameter will be effective in the selection of fruits with greater pulp weight. This fact is important, since these two traits are easy to be measured by using a scale and a caliper, and also for they are not destructive, and the whole fruit can be used for other purposes after the measurements.

Other authors have determined the importance of path analysis to accelerate and facilitate the selection of genotypes with superior traits in breeding program of fruit species based on fruit morphological traits. In assai palm (*Euterpe oleracea*), fruit weight per bunch, number of bunches, and number of rachilles per bunch were determinant factors in the variation of fruit production of half-sib progenies, and can be used in the indirect selection of genotypes (TEIXEIRA et al., 2012). The mean pulp weight and number of fruits had a greater effect on the total weight of passion fruit (*Passiflora edulis*), and can be used to determine fruit yield in commercial cultivars and progenies of controlled hybridization originated from the breeding of the species (LÚCIO et al., 2013). Similar to the present results, pulp weight presented greater direct effect with fruit weight and equatorial diameter in a study carried out with 42 half-sib progenies of *Passiflora edulis* (NEGREIROS et al., 2007). In a study carried out with a species native to the south of Brazil, *Plinia cauliflora*, the peel percentage is the most determinant variable of pulp percentage, and the indirect selection for a smaller peel percentage may increase pulp percentage in the species (SALLA et al., 2015). In the present study, pulp percentage presented wide variation (between 7.3 and 64.9%), with a mean of 35.4%, which is slightly greater than the mean value of pulp yield (27 and 33%) of the cultivars available in the market (DUCROQUET et al., 2007, 2008).

Table 3 - Direct and indirect effects of feijoa fruit traits on pulp weight, estimated by Ridge path analysis (with multicollinearity) and single chain path analysis (without multicollinearity).

Means of Association	Ridge path analysis	Single chain path analysis
-----Diameter-----		
Direct effect on pulp weight	0.451	0.421
Indirect effect via length	0.041	0.014
Indirect effect via total weight	0.789	0.867
Indirect effect via peel thickness	-0.389	-0.407
Indirect effect via n. of seeds	-0.006	-0.006
Total effect (Pearson's correlation)		-0.89**
-----Length-----		
Direct effect on pulp weight	0.049	0.017
Indirect effect via diameter	0.376	0.351
Indirect effect via total weight	0.728	0.800
Indirect effect via peel thickness	-0.332	-0.348
Indirect effect via n. of seeds	-0.007	-0.007
Total effect (Pearson's correlation)		-0.81**
-----Total weight-----		
Direct effect on pulp weight	0.824	0.906
Indirect effect via diameter	0.432	0.403
Indirect effect via length	0.043	0.015
Indirect effect via peel thickness	-0.388	-0.406
Indirect effect via n. of seeds	-0.007	-0.007
Total effect (Pearson's correlation)		-0.91**
-----Peel thickness-----		
Direct effect on pulp weight	-0.503	-0.527
Indirect effect via diameter	0.348	0.325
Indirect effect via length	0.032	0.011
Indirect effect via total weight	0.635	0.698
Indirect effect via n. of seeds	-0.007	-0.007
Total effect (Pearson's correlation)		-0.50**
-----N. of seeds-----		
Direct effect on pulp weight	0.029	0.029
Indirect effect via diameter	-0.088	-0.082
Indirect effect via length	-0.012	-0.004
Indirect effect via total weight	-0.194	-0.213
Indirect effect via peel thickness	0.129	0.135
Total effect (Pearson's correlation)		-0.14 ^{NS}
-----Analysis performance-----		
Determination coefficient (R ²)	0.936	0.946
Effect of residual variable	0.254	0.232
CN – Multicollinearity diagnosis		209.7
K value used in the analysis	0.0087	-
Maximum VIF	7.3	-

NC=Condition Number; Maximum VIF: the highest value of the variance inflation factor verified after applying the ridge path analysis.
 **Pearson's correlation coefficient significant by the t test ($P \leq 0.01$).

CONCLUSION

Fruit weight and diameter are the main determining factors of Feijoa weight. For being easily measured, these two traits can serve as criteria for indirect selection to increase pulp weight in feijoa in breeding programs.

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