

Original article

## Canonical correlation between vegetative and fruit characters in guava families (*Psidium guajava* L.)

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### ABSTRACT

Guava is a crop of a great economic importance in tropical and subtropical regions of the world. The knowledge of the associations among agronomic importance characters has a great utility in breeding and cultivar selection programs. In the present study, canonical correlation analysis was used to estimate the relationship between vegetative and fruit traits, evaluated in three full-sib families of guava. The families were obtained from intraspecific controlled crosses, made at the Tropical Fruits Research Institute. Positives and significant genetic correlations were observed among the most of fruit traits. Few associations were detected among vegetative and fruit traits. A significant canonical correlation coefficient was only detected in the first pair of canonical varieties. The analysis indicated that, among vegetative traits, leaf width was the trait with the most contribution to the canonical varieties explanation in the guava full-sib families. Among fruit traits, number of seeds, fruit width and internal flesh thickness were the traits that most contribute to the canonical varieties'

estimates. With the use of canonical correlation analysis, it was determined that there is a low association between the groups of vegetative and fruit traits. For that reason, in these guava families, some of these traits group must not be used to predict the behavior of the other.

**Key words:** plant breeding, selection, genetic correlation, multivariate analysis

## INTRODUCTION

Guava (*Psidium guajava* L.), is one of the best known fruit trees, which is widely cultivated in more than 60 countries, in the tropical and subtropical regions of the world <sup>(1)</sup>. It is included in the genus *Psidium* that groups around 150 species of trees and shrubs, of which only 20 produce edible fruits <sup>(2)</sup>.

The demand and expansion of the guava crop has been developed by the need to obtain cultivars, with good quality and long-lasting fruits, resistant to diseases and with high nutrient content <sup>(3)</sup>. The selection of promising genotypes and the proposal of new cultivars requires the evaluation of characters of agricultural interest, which have quantitative inheritance and have a negative environmental effect. Many of these characters are associated with others, so the selection of one character can affect others positively or negatively <sup>(4)</sup>. For this reason, it is important to determine the correlations between characters of interest for improvement, which can be caused by gene linkage or pleiotropy <sup>(5)</sup>.

When the characters are correlated, the selection criterion that considers a single variable may not generate a satisfactory genetic gain for all the evaluated characters <sup>(5)</sup>. Correlation analyzes are feasible to determine the mutual relationships between several characters and suggest the advantage of a selection scheme for more than one character at a time <sup>(1)</sup>. However, the magnitude and value of the correlation coefficients is not sufficient to clarify the relationships between the characters, because there is no cause-effect relationship between them <sup>(6)</sup>.

Canonical correlation is a multivariate analysis method used to examine the relationship between two groups of characters <sup>(7)</sup>. This analysis allows grouping of characters of interest, in such a way that the determination of associations between the groups makes it possible to indirectly select characters <sup>(4)</sup>, thus providing valuable information for the selection of ideotypes in breeding programs <sup>(8)</sup>. In this technique, correlation estimates are made between linear combinations of the two groups of characters, called canonical variables. The

correlation between the corresponding pairs of canonical variables is defined as canonical correlation <sup>(9)</sup>.

In agronomy, canonical analysis has been used to estimate the association between two different groups of characters; for example, between morphological and biochemical characters; vegetative characters and performance components; agronomic and seed quality characters; physiological and morphological characters, among others. These studies have been carried out in various crops such as red pepper (*Capsicum annuum* L.) <sup>(10)</sup>, wheat (*Triticum aestivum* L.) <sup>(8,9,11)</sup>, corn (*Zea mays* L.) <sup>(12,13)</sup>, soy (*Glycine max* L.) <sup>(14)</sup>, but they have been little used in fruit trees.

In all the previously mentioned studies, the canonical analysis was performed with the phenotypic values of the characters. Some authors have suggested that it is more effective to perform it with the predicted genetic values <sup>(6)</sup>. Genetic values can be predicted through the methodology of mixed models (REML–BLUP, procedures of maximum restricted likelihood–best linear unbiased), which provide more precise and exact inferences <sup>(6,15)</sup>. These authors used the canonical correlation analysis to determine the association between vegetative characters and yield components in families of complete guava siblings, based on the predicted genetic values.

Based on the elements described above, this paper aims to evaluate the association between vegetative and fruit characters in families of complete siblings of guava, by calculating canonical correlations.

## MATERIALS AND METHODS

### Plant material used in the experiments

The study was developed at the Basic Scientific-Technological Unit (UCTB) of Alquizar, Artemisa province, belonging to the Tropical Fruit Research Institute (IIFT) of Cuba, which is located at 22° 47 'north latitude and 82° 31 'west longitude, 11 m above sea level, on an eutrophic Ferralsol soil, with a flat topography of zero slope <sup>(16)</sup>.

Three families of complete siblings of guava (*Psidium guajava* L.), obtained from intraspecific crosses, were carried out by means of controlled pollination in 2001. Three plants of the cultivar 'Cuban Red Dwarf' ('EEA) were used as female progenitors. 18-40', which is used on a commercial scale in the country. As male parents the cultivars were used:

'N6', 'Suprema Roja' and 'Belic L-207'. These cultivars were selected as progenitors, because they presented great phenotypic and genotypic variability, detected when evaluating the germplasm bank of the culture by means of morphological and molecular markers <sup>(17)</sup>.

The seeds obtained from each cross were sown in seedbeds and subsequently transplanted into individual 26 x 46 cm bags containing Ferralsol soil and organic matter (cachaza) at the 3:1 ratio. When the plants were between 50 and 60 cm high, they were planted in areas of the UCTB, following a 6 x 5 m planting frame. Each of the plants obtained can be considered a different genotype, because they were obtained from seeds, so there is only one replica per genotype. The three families were planted adjacent to each other, forming a compact block in the same lot, next to the parents.

The plants were maintained with localized irrigation, using the drip irrigation technique with RAM brand emitters of 2.3 L h<sup>-1</sup>, spaced at 0.65 m within a 20 mm diameter side. Irrigation was applied with fixed doses and fixed intervals (alternate days) and was suspended during heavy rain events. Cultural work, fertilization and phytosanitary control were carried out according to the Technical Instructions of the crop <sup>(18)</sup>.

### **Quantitative Character Evaluation**

In the progenies resulting from the three crosses and their parents, ten quantitative characters of those proposed as guava descriptors were evaluated <sup>(19,20)</sup>. The measurements were made taking into account the recommendations of the crop descriptor, published by UPOV <sup>(19)</sup>. The characters evaluated were: leaf length (mm); blade width (mm); plant height (m); fruit mass (g); fruit length (mm); fruit width (mm); external thickness of the pulp (mm); internal thickness of the pulp (mm); number of seeds per fruit and total mass of seeds per fruit (g).

The plants began to be evaluated at five years of age, starting in 2006 and measurements were made for three consecutive years (2006–2008), in each of the genotypes of the three families. The vegetative characters were evaluated in the March-April period and the fruit characters in the August-September period, which is the peak of summer harvest. The fruits were harvested at their physiological maturity and were evaluated in full ripening, two or three days after being harvested. For the measurement of these characters, technical scales, millimeter rulers and king's foot were used.

## **Evaluation of the association between vegetative and fruit characters, with the use of canonical correlation analysis**

In order to identify and quantify the linear association between the group of vegetative characters and the group of characters of the fruit, a canonical correlation analysis was developed, which focuses on the correlation between linear combinations belonging to the character groups. This analysis was performed with the use of the predicted genetic values, based on the best unbiased linear predictors (*BLUP*) that were obtained by the maximum restricted likelihood method (*REML*) with the use of the *MIXED* procedure. The equation of the mixed model used was as follows:

$$Y = Xr + Zg + Wa + e$$

where:

and is the vector of observations.

r is the vector of measurements that includes the general mean and is considered of fixed effects.

g is the vector of individual genotypic effects considered random.

a is the vector of environmental effects (years of evaluation) considered of fixed effects.

e is the vector of errors or residuals (random).

X, Z and W are the known incidence matrices, associated with the vectors r, g and a, respectively.

Prior to the calculation of the canonical correlation, the adjustment of the values of each of the characters to the normal distribution was evaluated, to verify compliance with the premise of multivariate normality that the analysis has <sup>(21,22)</sup>, with the use of the goodness of fit tests of Kolmogorov-Smirnov, Shapiro Wilk, Cramer-von Mises and Anderson-Darling, which provides the *UNIVARIATE* procedure <sup>(23)</sup>.

The *CANCORR* procedure was used to perform the canonical correlation analysis, which first determined the genetic correlations between all the evaluated characters, based on the genetic values estimated with the *BLUP* procedure <sup>(15)</sup>. Subsequently, the association was identified between the group of vegetative characters (plant height, length and width of the fruit) and the group of fruit characters (mass, length and width of the fruit, external and internal thickness of the pulp, number of seeds and total mass of seeds per fruit), with the calculation of the canonical correlation. Three functions or canonical variables were determined. The

procedure uses the F statistic to test a series of null hypotheses that state that each canonical correlation is equal to zero in the population <sup>(21)</sup>. Also, for each pair of canonical variables ( $U_i$ ,  $V_i$ ), the values of the canonical correlations, the average variance explained, the redundancy values and the canonical coefficients were determined.

All statistical analyzes were developed using the SAS program, version 9.3 <sup>(24)</sup>.

## RESULTS AND DISCUSSION

With the completion of the *MIXED* procedures, the BLUPs were obtained, which constitute the predictors of the genetic values (Breeding value). These genetic values were used to determine the association between vegetative characters (height of the plant, length and width of the leaf) and of the fruit (external and internal thickness of the pulp, number and total mass of the seeds by fruit, mass, length and fruit width), based on the canonical correlation analysis. This analysis is a special case of a general linear model that is based on the correlation matrix of all the evaluated characters <sup>(25)</sup>. Its objective is to look for the relationships that may exist between two groups of characters and their validity <sup>(6)</sup>.

It was observed that, taking into account all values obtained in the three families for each character, the adjustment to the normal distribution for each of them was achieved.

Table 1 shows the values of the correlation coefficients among the ten characters evaluated, with their statistical significance. Most of the fruit's characters showed significant and positive correlations between them, with the exception of the length of the fruit, with the internal thickness of the pulp and the number of seeds and the external thickness of the pulp with the number of seeds, in which negative coefficients were obtained. Among the vegetative characters, only a positive and significant correlation was observed between the length and width of the leaf. This last character is positively correlated with all of the fruit, with the exception of the length. Plant height was not associated with any of the characters evaluated in the three guava families.

**Table 1.** Values of the correlation coefficients obtained for the characters evaluated in three families of complete siblings of guava

Characters	AH	AP	MF	LF	AF	EEP	EIP	NS	MTS
LH	0,66***	-0,03 <sup>ns</sup>	0,04 <sup>ns</sup>	0,06 <sup>ns</sup>	0,05 <sup>ns</sup>	0,16*	-0,08 <sup>ns</sup>	0,03 <sup>ns</sup>	0,002 <sup>ns</sup>
AH	-	0,05 <sup>ns</sup>	0,14*	-0,04 <sup>ns</sup>	0,23***	0,15*	0,16*	0,24***	0,17*
ALT		-	0,005 <sup>ns</sup>	-0,07 <sup>ns</sup>	0,02 <sup>ns</sup>	-0,06 <sup>ns</sup>	0,04 <sup>ns</sup>	0,02 <sup>ns</sup>	-0,03 <sup>ns</sup>
MF			-	0,66***	0,89***	0,74***	0,46***	0,16*	0,35***
LF				-	0,32***	0,60***	-0,16*	-0,17*	0,01 <sup>ns</sup>
AF					-	0,63***	0,69***	0,30***	0,44***
EEP						-	-0,03 <sup>ns</sup>	-0,18**	0,05 <sup>ns</sup>
EIP							-	0,60***	0,62***
NS								-	0,70***

LH: leaf length, AH: leaf width, AP: plant height, MF: fruit mass, LF: fruit length, AF: fruit width, EEP: external pulp thickness, EIP: thickness internal pulp, NS: number of seeds per fruit, MTS: total mass of seeds per fruit. \*, \*\*, \*\*\* - Indicate the statistical significance for the significance levels: 0.05; 0.01 and 0.001, respectively

The greatest correlations were observed between the mass and width of the fruit (0.8866), the mass of the fruit and the external thickness of the pulp (0.7445) and the number and total mass of the seeds per fruit (0, 7049) (Table 1). The high values of the correlation coefficients can be the result of the pleiotropism effect, in which the same gene affects the expression of more than one character <sup>(26)</sup>. This information is useful for plant breeding, because it favors the simultaneous selection of two or more characters, by selecting only one of them.

Similar results were obtained by estimating the genetic correlations in the same guava families, but by the progeny-parent covariance method <sup>(27)</sup>, in which the progenie-progenitor covariance of each character and the covariance between two characters must be calculated. In another study, the genetic correlations between characteristics associated with the yield in maize lines (*Zea mays* L.) <sup>(28)</sup> were estimated, using a manual method that uses the formula proposed by other authors <sup>(26)</sup> and the maximum likelihood restricted, through a *MIXED* procedure, in which the *GCORR* option was specified for genetic correlations to be calculated. These authors also found equivalent results by the two methods, when comparing the genetic correlations obtained by both methods, with the use of a Student's t-test of paired samples and finding no significant differences. This result allowed them to conclude that genetic correlations can be estimated through the *PROC MIXED* that is simpler and quicker to do <sup>(28)</sup>.

In an evaluation of fruit characters and yield components in families of complete guava siblings, low values of the genetic correlation coefficients were obtained <sup>(5)</sup>. These results are similar to those obtained in the present work in the coefficients calculated between vegetative and fruit characters. The authors stated, based on the results, that the selection of these characters will be carried out without having a correlated response.

It is possible to make a profit with correlated answers in the characters that show positive and significant associations. The existence of a positive genetic association between two characters implies that changes in one of them can cause alterations in the other <sup>(4)</sup>. Since all correlations were positive with the mass of the fruit, the width of the fruit and the total mass of the seeds, simultaneous selection can promote gain in the mass of the fruits and the mass of the seeds, more efficiently <sup>(29)</sup>, as stated, when the characters that contribute positively with a character of interest are selected, a more effective use of the correlation is made <sup>(26)</sup>.

The correlation studies between different characters of the plant and the fruit, in guava genotypes, can provide an idea of which characters could be used for the selection of desirable parameters in future crop improvement programs. The positive and significant correlations between characters of interest are favorable for the improver, because they can help in the simultaneous improvement of both characters. On the other hand, the negative correlation could overlap the synchronized expression of both characters <sup>(30)</sup>.

The knowledge of the genetic correlations can be very useful in the improvement, for the selection of characters with low heritability and difficulties in their measurement, because under these conditions, the indirect selection of them can be carried out; That is why genetic correlations must be estimated in improvement programs <sup>(4)</sup>.

As low values of the genetic correlation coefficients between vegetative and fruit characters were obtained, they are not important indicators of each other, that is, they cannot be used to make the selection of more than one character. In addition, correlations between pairs of characters are more difficult to explain simultaneously <sup>(10,21)</sup>. For this reason, three canonical correlation coefficients were estimated to explain the interrelationship between the two groups of characters, since the number of canonical correlations that need to be interpreted is equal to the lower number of characters in the groups <sup>(21,25)</sup>, that in this study corresponds to the group of vegetative variables.

Table 2 shows the results obtained when performing the canonical analysis between the two groups of characters. Only the first pair of canonical variables or functions ( $U_1$ ,  $V_1$ ) showed



a significant canonical correlation ( $p=0.001$ ) between the vegetative and fruit characters, in relation to the likelihood ratio test. In this first canonical function, which is the most important because it explains the highest percentage of the variance of the set of variables and indicates the maximum correlation between the two groups of characters, a correlation coefficient value of 0.3282 was obtained. This value represents the greatest possible correlation between any linear combination of the vegetative characters and any linear combination of the fruit characters <sup>(21)</sup> and can be interpreted as the simple correlation between the weighted summation or linear combination of the values in each character group, with the weighting belonging to the first canonical function <sup>(25)</sup>.

**Table 2.** Summary of the results obtained in the canonical correlation analysis for the characters evaluated in three families of complete siblings of guava

Canonical Variables	Canonical correlation	Canonical square correlation	Self-worth	Cumulative Proportion	Likelihood ratio	F value	p
U <sub>1</sub> V <sub>1</sub>	0,39	0,15	0,18	0,67	0,78	2,60	0,000
U <sub>2</sub> V <sub>2</sub>	0,25	0,06	0,07	0,93	0,92	1,54	0,11
U <sub>3</sub> V <sub>3</sub>	0,14	0,02	0,02	1,00	0,98	0,83	0,53

Legend: F - Value of statistic F. p - probability value associated with statistic F

Other studies have also obtained only a significant canonical correlation value, in the first canonical function. For example, when relating morphological and productive characters in families of half-brothers of *Jatropha curcas* L. <sup>(31)</sup>, between agronomic and physiological quality characters of the seed in segregating soy populations <sup>(14)</sup> and when associating morphological and germination variables in wheat cultivar seeds <sup>(32)</sup>.

The second (U<sub>2</sub>V<sub>2</sub>) and the third (U<sub>3</sub>V<sub>3</sub>) canonical functions will explain the highest percentage of the variance left by the first canonical function, that is, the residual or remaining variance of the first functions. In addition, canonical correlations decrease as additional functions are calculated; that is, the first function reflects the greater correlation between the two groups, the following the second correlation and so on <sup>(21)</sup>.

The canonical functions significance test uses an approximation of the F test to determine the significance of the Wilks Lambda, which is equal to the likelihood ratio test <sup>(21)</sup>. This test is used to assess whether the variables of one group are correlated with those of the other group. This is done, sequentially, if all the canonical functions are significant or not <sup>(25)</sup>. First, the

three canonical functions are observed together and it is evaluated whether the three canonical correlations are zero; If the null hypothesis is rejected ( $p < 0.05$ ), the second and third correlations are evaluated <sup>(21)</sup>. In order to determine the statistical significance of the other two functions, the first function, which is the most significant one, is eliminated and if the second test is also significant, it is possible to proceed only with the third one, to determine if the remaining function is also significant <sup>(25)</sup>. If only the first canonical function is statistically significant, as in the present work, it is the only one that must be interpreted. Although the canonical variables are artificial, they can be identified in terms of the original variables. For this, the standardized canonical coefficients for each group of characters can be interpreted in the three functions or canonical variables ( $U_1V_1$ ,  $U_2V_2$  and  $U_3V_3$ ), as well as, the correlations between the canonical variables and the original variables, which are shown in the Tables 3 and 4, respectively. The canonical coefficients are standardized to allow their interpretation, because the original variables do not have to have homogeneous variances, nor be measured in the same unit <sup>(21)</sup>. The magnitudes of the canonical coefficients indicate the relative contributions of each variable in the canonical linear combination <sup>(25)</sup>; that is, the coefficients indicate the effects of vegetative characters on those of the fruit in guava genotypes.

When observing the values obtained for the standardized coefficients (Table 3), in the first function that is the only one significant, it can be said that, if the values of leaf width and plant height increase, the width will increase of the fruit, the external thickness of the pulp and the number of seeds and will decrease the internal thickness of the pulp, the total mass of the seeds, the mass and the length of the fruit. The characters that contributed most to the first canonical variable ( $V_1$ ) among those of the fruit were: the width of the fruit (1.0750) and the number of seeds (0.7803), while the width of the leaf (1.2832) was the one with the greatest contribution to the first canonical variable of the vegetative ( $U_1$ ). These three characters showed positive and significant genetic correlations between them and with the majority of the remaining fruit characters (Table 1). The remaining vegetative and fruit characters had a lower contribution to the first pair of canonical variables  $U_1$ ,  $V_1$ .

**Table 3.** Standardized canonical coefficients for canonical variables

Var.	Vegetative characters			Var.	Fruit Characters						
	Can.	LH	AH		AP	Can.	MF	LF	AF	EEP	EIP
U <sub>1</sub>	-0,59	1,28	0,04	V <sub>1</sub>	-0,34	-0,40	1,07	0,20	-0,38	0,78	-0,13
U <sub>2</sub>	1,07	-0,26	-0,36	V <sub>2</sub>	-1,79	0,67	0,63	1,21	-0,35	0,48	0,03
U <sub>3</sub>	-0,57	0,29	-0,94	V <sub>3</sub>	-1,03	0,82	-1,82	1,49	2,20	-0,37	0,58

Legend: LH: leaf length, AH: leaf width, AP: plant height, MF: fruit mass, LF: fruit length, AF: fruit width, EEP: external pulp thickness, EIP: internal pulp thickness, NS: number of seeds per fruit, MTS: total mass of seeds per fruit

The proportions of variance extracted in the two groups and the redundancy values are shown in Table 4. The values of proportion of variance extracted and redundancy indicate the magnitude of the total correlations between the two groups of variables, relative to the variance of the original variables. They are different from the square canonical correlation value, because the latter statistic expresses the proportion of the variance explained in the canonical variables <sup>(25)</sup>.

**Table 4.** Proportion of variance extracted, proportion of cumulative variance and redundancy of the two groups of characters in the three canonical functions

Canonical Variables	Caracteres vegetativos			Caracteres del fruto		
	Variance extracted	Accumulated variance	Redundancy	Variance extracted	Accumulated variance	Redundancy
U <sub>1</sub> V <sub>1</sub>	0,3877	0,3877	0,0446	0,2920	0,2920	0,0440
U <sub>2</sub> V <sub>2</sub>	0,2961	0,6837	0,0248	0,1279	0,4198	0,0082
U <sub>3</sub> V <sub>3</sub>	0,3163	1,0000	0,0061	0,0861	0,5059	0,0017

The first canonical variable extracted, on average, 38.77 % of the variance of the vegetative characters and 29.20 % of the fruit characters, values that can be considered low. The three canonical functions together extracted 100 % of the variance of the group of vegetative

characters and 50.59 % of the variance of the fruit characters. If the redundancy values are observed, with the fruit characters only 4.46 % of the variance in the vegetative characters can be explained, based on the first canonical function; while with the vegetative characters, 4.40 % of the variability in the fruit's characters is explained (Table 4).

Redundancy coefficients are used to measure the predictive capacity of one set of characters with respect to the other <sup>(33)</sup>. The results indicate that there is a weak association between the vegetative and fruit characters, so the behavior of the guava genotypes in the fruit characters cannot be predicted, based on the values of the vegetative characters and vice versa. This result confirms the one obtained previously, when estimating the genetic correlations between all the characters and obtaining low values of the correlation coefficients between vegetative characters and fruit characters.

It can be concluded then, that there is a weak correlation between the vegetative characters and the fruit characters evaluated in the three families of siblings full of guava, so the selection of certain vegetative characters will not imply that it carries that of the characters of the fruit and vice versa. Similar results were obtained in progenies of complete guava brothers in Brazil <sup>(6)</sup>. These authors, when using the canonical correlation analysis, determined that the correlation coefficient values for the canonical functions were low and that there was little genetic correlation between vegetative characters and yield components. The canonical coefficients are important to consider in the decisions that are made in the stages of selection of superior individuals, in the improvement programs, for characters of interest, when two different groups of characters are considered <sup>(6)</sup>. The knowledge of the degree of association, through correlation studies, can identify characters that can be used as indirect selection criteria for performance or as secondary characters, which improves the efficiency of the selection processes <sup>(34)</sup>. Several bivariate and multivariate correlation measures can be used to determine the relationships between variables; however, bivariate correlation coefficients may fail to determine complex relationships. Multivariate models may be appropriate to ensure relationships between a large number of variables. Canonical correlation analysis can be used to determine relationships between multiple dependent and independent variables; therefore, it is more successful to estimate complex relationships in the biological sciences <sup>(9)</sup>.

## CONCLUSIONS

- Among the fruit characters evaluated in the families of complete guava brothers, a high genetic correlation was observed.
- The canonical correlation analysis used detected a low association between the group of vegetative characters and the group of characters of the fruit, in the three families of complete siblings of guava.

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