

Contribution of production and seed variables to the genetic divergence in passion fruit under different nutrient availabilities

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Abstract – The objective of this work was to evaluate the relative contribution of variables related to fruit production and to seed morphophysiological characteristics to the genetic divergence in passion fruit (*Passiflora edulis*) progenies, aiming at selecting progenies potentially responsive to fertilization. Ten progenies were evaluated under conditions of low (50% of the recommended dose of fertilizers) and high soil fertility (dose 50% higher than the recommended one), regarding variable sets related to production and to the morphological and physiological characteristics of seeds. The association between these sets was determined by the canonical correlation analysis and by the nearest-neighbor clustering method. The nutritional environments interfered in the relative contribution of the variables to the genetic divergence of the progenies. The accelerated aging test of seeds – from the set of seed physiological quality – did not contribute significantly to the selection of genotypes responsive to soil fertilization. The most responsive progenies to the increased availability of nutrients were grouped according to the production variables that were evaluated under high soil fertility. Irrespectively of the evaluated environment, the set of production variables is the one that contributes more expressively to the identification of the genetic divergence of passion fruit progenies.

Index terms: *Passiflora edulis*, canonical analysis, cluster analysis, fruit quality, responsiveness to fertilization, seed physiological quality.

Contribuição de variáveis de produção e de semente para a divergência genética em maracujazeiro-azedo sob diferentes disponibilidades de nutrientes

Resumo – O objetivo deste trabalho foi avaliar a contribuição relativa de variáveis relacionadas à produção de frutos e a características morfofisiológicas das sementes para a divergência genética em progênes de maracujazeiro-azedo (*Passiflora edulis*), com vistas à seleção de progênes potencialmente responsivas à fertilização. Dez progênes foram avaliadas em condições de baixa (50% da dose recomendada de fertilizantes) e alta fertilidade do solo (dose 50% superior à recomendada), quanto a conjuntos de variáveis de produção e de características morfológicas e fisiológicas das sementes. A associação entre esses conjuntos foi determinada pela análise de correlação canônica e pelo método de agrupamento do vizinho mais próximo. Os ambientes nutricionais interferiram na contribuição relativa das variáveis para a divergência genética das progênes. O teste de envelhecimento acelerado das sementes – do conjunto de variáveis de qualidade fisiológica – não contribuiu significativamente para a seleção de genótipos responsivos à adubação. As progênes mais responsivas ao aumento da disponibilidade de nutrientes foram agrupadas de acordo com as variáveis de produção avaliadas em condições de alta fertilidade do solo. Independentemente do ambiente avaliado, o conjunto de variáveis de produção é o que mais expressivamente contribui para a identificação da divergência genética de progênes de maracujazeiro-azedo.

Termos para indexação: *Passiflora edulis*, análise canônica, análise de agrupamento, qualidade do fruto, responsividade à adubação, qualidade fisiológica das sementes.

Introduction

Passion fruit (*Passiflora edulis* Sims) has achieved an outstanding position in the national fruit market, due

to the increased demand from the fresh and industrial markets, and to the interest of producers in a crop with a cycle shorter than that of other fruit trees. Brazil produces more than 800 thousand tons of passion fruit

per year; however, its average productivity is only of 14 Mg ha⁻¹ (IBGE, 2014).

Genetic diversity is fundamental for breeding programs. In the case of passion fruit, the development of cultivars with better agronomic traits is closely related to the genetic variability in segregating generations (Bruckner et al., 1995; Viana et al., 2007). The multivariate analysis is an efficient method to calculate the similarity between genotypes representative of the population. It uses specific variables of interest and allows the formation of groups with minimal variation between individuals and maximum variation between groups (Crossa & Franco, 2004).

Cruz et al. (2012) point out the importance of using canonical correlations to evaluate the maximum correlation between two sets of variables. This analysis has been used in fruit trees to assess: the relationship between soil and plant, in peach trees (Terra et al., 2014); agronomic traits of passion fruit (Neves et al., 2013); physical and chemical properties of passion fruit and juice (Espitia-Camacho et al., 2008); soil biological and chemical attributes in the apple crop (Maluche-Baretta et al., 2006); and fruit quality and production traits of passion fruit (Viana et al., 2003).

The hierarchical clustering method can be used to unify genotypes by similarity measures, until a dendrogram is established (Cruz et al., 2012). Several studies have been developed to quantify and characterize *Passiflora* genetic diversity, mainly seeking to identify superior genotypes (Viana et al., 2006; Gonçalves et al., 2007; Araújo et al., 2008; Silva et al., 2009; Krause et al., 2012; Paiva et al., 2014). Plant morphological and production variables are commonly used for this purpose; however, the variables related to seed morphological and physiological quality (germination and vigor) are not commonly employed (Brum et al., 2011).

In addition, little is known about the impacts of soil fertility or nutrient availability on the evaluation of passion fruit genetic divergence. This may be a problem, since progenies usually present different responses to fertilization management, which affects fruit productivity, as well as seed size and weight (Borges et al., 2006; Nascimento et al., 2011; Carvalho & Nakagawa, 2012).

The objective of this work was to evaluate the relative contribution of variables related to fruit production and to seed morphophysiological characteristics to the

genetic divergence in passion fruit progenies, aiming to select progenies potentially responsive to fertilization.

Materials and Methods

Ten progenies of passion fruit – 57x15, 144x130, 112x42, 117x19, 68x135, 81x117, 132x15, 144x42, 68x15, and 46x14 –, from the third cycle of the intrapopulation recurrent selection program of Universidade Estadual do Norte Fluminense Darcy Ribeiro (Uenf), were subjected to two levels of fertilization, according to the results of the soil analysis. The dose used for fertilization 1 was 50% lower than the recommended average for the crop (Carvalho et al., 2000), with 135 g urea per plant per year and 270 KCl per plant per year. In fertilization 2, the dose used was 50% higher than the recommended average for the crop, with 405 g urea per plant per year and 810 g KCl per plant per year. Fertilizations were split and applied monthly.

The experiment was implemented at the experimental unit of Uenf, in the municipality of Itaocara, located in the northwest of the state of Rio de Janeiro, Brazil, from September 2012 to May 2014. A completely randomized block design was used, in a split plot with ten progenies in the main plot and two levels of fertilization in the subplots, with two replicates of three plants each.

The production traits evaluated were: productivity (Mg ha⁻¹); average fruit mass (g), for which ten fruits per treatment were weighed in a scale with two decimal places; pulp mass (g), as the average of ten fruits, also in a scale with two decimal places; and number of fruits per plant.

The seeds of these fruits were obtained by friction in a steel mesh sieve in running water until the aril was removed. Then, they were dried at room temperature, for 48 hours, to assess the following variables: length/width ratio of 15 seeds, measured with a digital caliper; average thickness of the 15 seeds (mm), also using a digital caliper; 1,000 seed weight (g), obtained by the average of eight replicates, in a scale with three decimal places (Brasil, 2009); germination test, in which the means of the treatments were obtained 28 days after the start of the test, from four replicates with 50 seeds, which were arranged between three germination paper sheets moistened with distilled water, at a ratio of twice the substrate mass, at alternating temperatures of 20–30°C, in a

germination chamber (Brasil, 2009); first germination count, evaluated together with the germination test, for which normal seedlings were recorded at 14 days; accelerated aging, in which the seeds were placed on an aluminum screen, in a transparent plastic box, with 40 mL of water, where they remained for 48 hours, at 40°C, in a germination chamber (Larré et al., 2007), before being subjected to the same methodology of the germination test, for which normal seedlings were recorded at 28 days (Brasil, 2009); first count of the accelerated aging test, for which the normal seedlings were counted at 14 days; seedling size, by using ten seedlings located at the top row of the paper roll at the end of the germination test (Negreiros et al., 2008); rootlet size, obtained from the measurement of the rootlets of ten seedlings located at the top row of the roll at the end of the germination test (Negreiros et al., 2008); and germination rate index, for which the number of normal seedlings was recorded every four days (Maguire, 1962).

The canonical correlation was used to estimate the maximum correlation of the linear combinations (Cruz et al., 2012) between production traits (set 1: productivity, pulp mass, fruit mass, and number of fruits per plant), seed morphological traits (set 2: seed length/width ratio, seed thickness, and 1,000 seed weight), and seed physiological traits (set 3: germination potential, first germination count, first count of the accelerated aging test, accelerated aging, seedling size, rootlet size, and germination rate index). The following models were used to obtain the linear combinations: $X_i = a_1X_1 + a_2X_2 + \dots + a_pX_p$; $Y_i = b_1Y_1 + b_2Y_2 + \dots + b_pY_p$, in which X_i and Y_i refer to one of the likely linear combinations between the traits from the sets 1, 2, and 3, in fertilizations 1 and 2.

The first canonical correlation maximizes the relationship between X_i and Y_i , and constitutes the first canonical pair, expressed by the equation:

$$r_1 = \text{Cov}(X_1, Y_1) / \sqrt{\widehat{V}(X_1) \widehat{V}(Y_1)} \text{ in which:}$$

$$\widehat{\text{Cov}}(X_1, Y_1) = a' S_{12} b; \widehat{V}(X_1) = a' S_{11} a; \widehat{V}(Y_1) = b' S_{22} b;$$

In this case, S_{11} is the $p \times p$ covariance matrix, between the traits of set 1; S_{22} is the $q \times q$ covariance matrix, between the traits of set 2; and S_{12} is the $p \times q$ covariance matrix, between the traits of sets 1 and 2.

The other canonical correlations and canonical pairs were estimated using the eigenvalues and eigenvectors of the described expressions, corresponding to the p - or q -th estimated correlation. The significance of the hypothesis that all possible canonical correlations were null was assessed by the chi-square (X^2) test:

$$X^2 = \log_e \left[\prod_{i=k+1}^s (1 - r_i^2) \right]$$

The analyses of canonical correlations were processed using the Genes software (Cruz, 2013). The evaluated variables were used to study the genetic divergence in different environments, based on Mahalanobis' generalized distance and on the unweighted pair-group method with arithmetic mean (UPGMA). The relative contribution of the traits to divergence was assessed by the Singh method, and the following parameters were evaluated: productivity, pulp mass, fruit mass, number of fruits per plant, seed length/width ratio, 1,000 seed weight, germination potential, first germination count, accelerated aging, seedling size, rootlet size, and germination rate index. The variables were calculated with non-standard means, and the grouping analyses among genotypes, at both fertilization levels, were performed by the Genes software (Cruz, 2013).

Results and Discussion

In the combination of variables set 1/set 2, the three canonical pairs were not significant for the fertilization levels 1 and 2 (Table 1). In the combination of variables set 1/set 3, the first and second canonical pairs were significant for fertilization 1, and only the first canonical pair was significant for fertilization 2. In the combination of variables set 2/set 3, the first and second canonical pairs were significant for the two levels of fertilization. The coefficients of the pairs were estimated in order to verify the relationship between the sets. When the tests detected significance, the first canonical pair was used for data interpretation, since it maximizes the relationship between X_1 and Y_1 (Cruz et al., 2012).

In the combination of seed morphological and physiological (germination and vigor) traits (set 2/set 3), the variable seed thickness showed greater effect on the following variables (Table 2): germination; first count of the accelerated aging test (FCAA); rootlet size

and the germination rate index (GRI) in fertilization 1, with lower nutrient availability. In fertilization 2, the variable 1,000 seed weight had effect on the variables germination, first germination count, and seedling size. Regardless of the level of fertilization, accelerated aging was the only unaffected variable in set 3.

Brum et al. (2011) verified significant canonical correlations between seed morphological and seedling variables of castorbean (*Ricinus communis* L.); however, the authors did not study these correlations in response to fertilization.

In the set 2/set 3 combination, the highest correlation coefficient in fertilization 1 was obtained for rootlet size. According to Tozzi & Takaki (2011), as germination occurs, the protein bodies identified in the cotyledons are degraded and almost completely consumed up to the moment when radicle protrusion occurs. Therefore, the faster the radicle protrusion process, the more vigorous the seedling is, because it shows greater capacity to transform the reserve supply into meristematic tissues, which may explain the great effect of the variable rootlet size on the coefficient of correlation.

In fertilization 2, the highest positive correlation was observed for the variables germination, first germination count, and seedling size; while, in fertilization 1, only the vigor variables FCAA, rootlet size, and GRI presented higher correlation values, with small contribution of the variable germination (Table 2).

In the combination of the production and of the seed germination and vigor traits (set 1/set 3), the variable number of fruits presented the greatest contribution to the effect of the variables seedling size and GRI, in fertilization 1 (Table 2). In fertilization 2, the variables productivity and fruit mass presented great contribution to the vigor variables fist germination count, rootlet size, and GRI.

Balkaya et al. (2011) found that the variable total number of fruits (set 1) and the variables fruit diameter and length (set 2) contribute the most to the explanatory capacity of canonical variables; therefore, they should be used in the selection of more productive pumpkin (*Cucurbita maxima* Duch.) genotypes. In the present study, the variable number of fruits also provided the greatest contribution to crop production in fertilization 1.

In the set 1/set 3 combination, the variable productivity was negatively correlated with the variables of set 3, which presented greater effect on fertilization 1 (seedling size and GRI); in fertilization 2, productivity was the most influential variable of set 1, while the GRI was the only variable with great effect on set 3, for both levels of fertilization (Table 2). This confirms that the GRI is a good vigor test to identify superior lots or genotypes (Nakagawa, 1999). In the same combination, the variable germination correlated negatively to the variables of set 1; and, in the set 2/set 3 combination, it correlated positively with the variables of set 2, regardless of the level of fertilization.

Table 1. Degrees of freedom and chi-square values, obtained by the canonical correlation analysis among sets of production variables (set 1) and of seed morphological (set 2) and physiological traits related to germination and vigor (set 3), for passion fruit genotypes (*Passiflora edulis*), in treatments with two levels of fertilization⁽¹⁾.

Combination	Degrees of freedom				Chi-square			
	1 st CP	2 nd CP	3 rd CP	4 th CP	1 st CP	2 nd CP	3 rd CP	4 th CP
Fertilization 1								
Set 1/Set 2	12	6	2	-	23.30 ^{ns}	6.13 ^{ns}	0.34 ^{ns}	-
Set 2/Set 3	21	12	5	-	89.59*	39.30*	9.01 ^{ns}	-
Set 1/Set 3	28	18	10	4	153.49*	56.78*	9.18 ^{ns}	3.93 ^{ns}
Fertilization 2								
Set 1/Set 2	12	6	2	-	12.8 ^{ns}	5.50 ^{ns}	0.59 ^{ns}	-
Set 2/Set 3	21	12	5	-	74.07*	24.64 ^{ns}	3.27 ^{ns}	-
Set 1/Set 3	28	18	10	4	156.41*	59.70*	7.48 ^{ns}	1.03 ^{ns}

⁽¹⁾Fertilization 1, 50% of the average dose recommended for the crop; and fertilization 2, dose 50% higher than the average recommended for the crop. *Significant at 5% probability. ^{ns}Nonsignificant. CP, canonical pair.

The stress resistance trait accelerated aging did not correlate positively – or did not exert great effect – on the set 2/set 3 and set 1/set 3 combinations. Therefore, its use is not a good alternative for the selection of superior genotypes of passion fruit.

The UPGMA method allowed the formation of three groups of genotypes, based on the differences between groups when considering 30% of the distance (Figure 1). In fertilization 1, group I was composed by five progenies (1, 2, 3, 4, and 5); group II, by two (8 and 9); and group III, by three (6, 7, and 10). In fertilization 2, three groups were also formed: I, by two progenies (2 and 10); II, by three (3, 4, and 6); and III, by five (1, 5, 7, 8, and 9).

The traits that provided the greatest contribution to divergence in fertilization 1 (Table 3) were: pulp mass, 37.30%; seedling size, 17.46%; and fruit mass, 12.71%, which totaled 67.47%. In fertilization 2, the best traits were: fruit mass, 31.34%; pulp mass, 13.48%; accelerated aging, 12.58%; productivity, 10.50%; and rootlet size, 10.13%, totaling 78.03%.

Of the variables of set 1 with relative contributions for the genetic divergence of the passion fruit progenies, pulp mass and fruit mass were more expressive in fertilization 1; and fruit mass, pulp mass, and productivity, in fertilization 2. For the variables of set 3, a lower contribution was observed, and, for those of set 2, the contribution was non-expressive (Table 3).

Table 2. Coefficients of canonical correlation among sets of production variables (set 1) and of seed morphological (set 2) and physiological traits related to germination and vigor (set 3), for passion fruit genotypes (*Passiflora edulis*), in treatments with two levels of fertilization⁽¹⁾.

Variable	Fertilization 1	Fertilization 2
	Set 2	
Seed length/width ratio	0.2876	-0.1043
Seed thickness (cm)	0.6703	-0.3519
1,000 seed weight (g)	-0.7261	0.8214
Set 3		
Germination (%)	0.5579	2.0100
First germination count (%)	-0.7355	0.9945
First count of the accelerated aging test (%)	1.0768	-0.8246
Accelerated aging (%)	-2.4011	-1.1491
Seedling size (cm)	-0.4740	1.5421
Rootlet size (cm)	1.9895	-0.7226
Germination rate index	1.5699	-1.9372
Total correlation	0.9994	0.9993
Set 1		
Productivity (Mg ha ⁻¹)	-0.8244	2.1200
Fruit mass (g)	0.3546	1.3835
Pulp mass (g)	0.3841	-1.7709
Number of fruits	1.4747	-1.5966
Set 3		
Germination (%)	-3.8440	-0.2897
First germination count (%)	-2.9676	6.3712
First count of the accelerated aging test (%)	-1.3806	-4.4471
Accelerated aging (%)	-0.4360	0.2574
Seedling size (cm)	1.7479	-9.8177
Rootlet size (cm)	-0.2192	4.1004
Germination rate index	3.7576	2.8057
Total correlation	1.0018	1.0022

⁽¹⁾Fertilization 1, 50% of the average dose recommended for the crop; and fertilization 2, dose 50% higher than the average recommended for the crop.

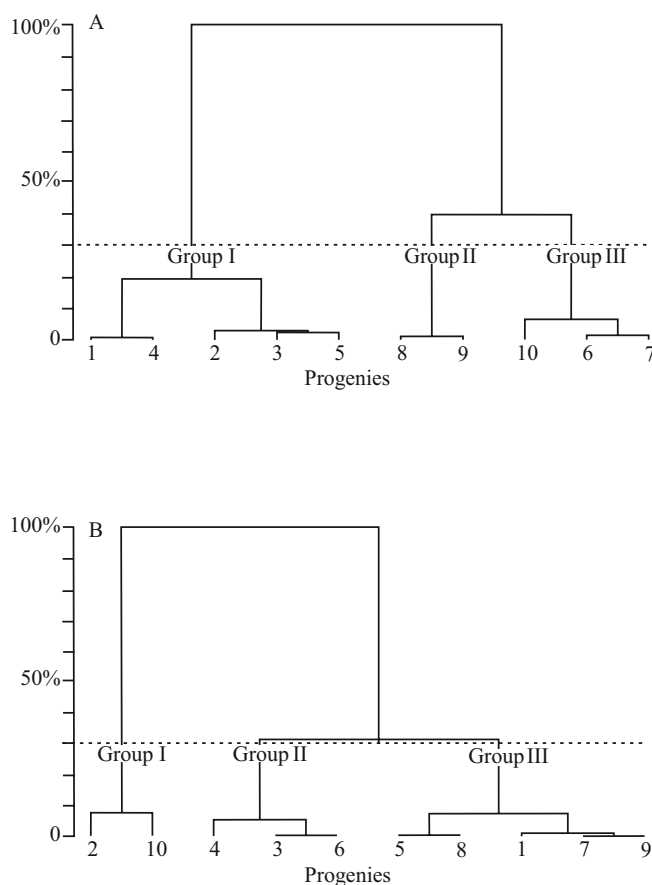


Figure 1. Dendrogram of genetic dissimilarities among ten progenies of passion fruit (*Passiflora edulis*), according to 12 variables evaluated at the levels of fertilization 1 (A, 50% of the average dose recommended for the crop) and 2 (B, dose 50% higher than the average recommended for the crop).

Table 3. Estimates of the contribution of each variable (S,j) to the genetic divergence in passion fruit (*Passiflora edulis*) progenies, as well as their relative contribution values, in treatments with two levels of fertilization⁽¹⁾.

Variável	Fertilization 1		Fertilization 2	
	S,j	Relative contribution (%)	S,j	Relative contribution (%)
Productivity (Mg ha ⁻¹)	941,241.2	1.68	55,031,864.0	10.50
Fruit mass (g)	7,100,997.9	12.71	164,312,411.7	31.34
Pulp mass (g)	20,848,750.3	37.30	70,651,220.1	13.48
Number of fruits	3,053,676.3	5.46	43,567,026.0	8.31
Seed length/width ratio	786,984.0	1.41	40,393.9	0.01
1,000 seed weight (g)	1,682,845.9	3.01	1,749.7	0.00
First germination count (%)	5,790.1	0.01	11,972,802.8	2.28
Germination (%)	4,405,938.8	7.88	26,590,878.8	5.07
Accelerated aging (%)	3,145,093.8	5.63	6,593,9140.9	12.58
Seedling size (cm)	9,758,668.4	17.46	32,472,009.9	6.19
Rootlet size (cm)	2,083,041.4	3.73	53,081,644.1	10.13
Germination rate index	2,078,905.7	3.72	619,161.8	0.12

⁽¹⁾Fertilization 1, 50% of the average dose recommended for the crop; and fertilization 2, dose 50% higher than the average recommended for the crop.

Viana et al. (2006) were able to identify and separate the superior genotypes from the lower ones by studying the genetic diversity in passion fruit populations in two environments. The authors observed a greater group of genotypes with lower performance in the Campos dos Goytacazes and Macaé environments in the state of Rio de Janeiro, Brazil.

Increased nutrient availability (fertilization 2) caused the progenies to respond with increases in some variables. Therefore, it was possible to identify superior progenies for sets 1, 2, and 3.

Most progenies allocated in group II, in fertilization 2, showed increase in all production variables (set 1). In the case of the variable number of fruit, there was an increase in all progenies of the group, with an increase in fruit production of 31, 25, and 67%, in progenies 3, 4, and 6, respectively. Group III of progenies showed increases only in the variables production and number of fruit, among which stood out progeny 7, with increases of 216 and 186%, respectively. Therefore, the progenies of this group did not respond to increased fertilization, for the variables fruit mass and pulp mass.

The variables of set 2 presented a very low increase in some progenies, of 7 and 3%, on average, for the seed length/width ratio and 1,000 seed weight, respectively. This result shows that the genotypes were not responsive to the greater availability of nutrients, regarding these variables.

For the variables of set 3, the responsiveness to increased nutrient availability was also low, mainly for the variable first germination count, which presented an average of 26% increase in the responsive progenies. However, although its effect was not as great on the formation of groups (Table 3), this variable allowed the identification of progenies – allocated in group III of fertilization 2 – that responded to the increased nutrient availability in this set of variables.

Conclusions

1. The contributions of the studied variables to the selection of passion fruit (*Passiflora edulis*) progenies depend on the availability of nutrients in the environments.

2. The variable accelerated aging is not adequate for the selection of passion fruit genotypes responsive to increased nutrient availability.

3. The progenies most responsive to increased nutrient availability were grouped according to the production variables evaluated under conditions of high soil fertility.

4. The variables of the production set contribute more expressively to the genetic divergence in passion fruit progenies, regardless of the availability of nutrients.

5. Nutrient availability affects the relative contribution of the variables to the evaluation of the genetic divergence of the passion fruit progenies.

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