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Path analysis for physiological traits that influence seed germination of *Passiflora edulis* f. *flavicarpa* Deg

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ABSTRACT – *The quality of yellow passion fruit seed is determined by fruit storage and the duration of this period. Two or three harvest dates can thereby be defined to obtain maximum quality. This study aimed to obtain estimates of phenotype, genotype and residual correlation coefficients and evaluate the direct and indirect effects (path analysis) of genotype correlations in seed extracted from fruits stored for 7, 14 and 21 days at cooled (8 ºC) and at environment temperature (25 ºC). The variables accelerated aging and moisture content explained the higher germination percentage in the refrigerated environment. However, in natural conditions, the variables dry matter and electric conductivity influenced seed germination percentage strongly, evidencing that the indirect effects of accelerated aging, electric conductivity and weight reduction had the greatest influence on dry matter.*

Key words: multicollinearity, genotypic correlations, accelerated aging, direct and indirect effects, seed quality.

INTRODUCTION

The path analysis, developed by Wright (1921 and 1923), is the study of the direct and indirect effects of independent explanatory variables on a main dependent variable. The variables are estimated regression equations, after previous standardization.

In the assessment of direct and indirect effects of a trait set on a basic variable, path coefficients have to be estimated by regression equations in which the variables are previously standardized. The estimation of these coefficients can however be affected by effects of multicollinearity among the traits involved. Multicollinearity occurs when the sample observations of the explanatory variables or their linear combinations are correlated (Matsuo 1986 and Ferrari 1989). According to Carvalho (1995), in case of multicollinearity, the variances associated to the estimators of the path coefficients can reach overrated values, making them unreliable. Besides, the parameter estimates can assume values without any coherence to the biological phenomenon under study. Shrivastava and Sharma (1976), for example, found a direct negative effect on rice yield for the traits number of tillers, number of grains, grain weight and panicle length. To circumvent the adverse effects of multicollinearity, variables can be eliminated from the regression model. Shrivastava and Sharma (1976) excluded panicle length from the analysis to find that all other traits contributed positively. The authors therefore proposed to select the

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study variables very carefully and recommended caution in the use of this technique.

The technique is widely applied in the improvement of diverse crops such as common bean (Furtado et al. 2002), bell pepper (Carvalho et al. 1999), elephant grass (Daher et al. 2004), among others. As there are still hardly any studies on seed quality, this is a promising area of research. Seeds of the species *Passiflora edulis* were evaluated, which is commercially explored from north to south throughout Brazil, in tropical and subtropical regions that are favorable for its cultivation. A noteworthy limitation to the crop expansion is the means of propagation, via seeds. Fruit farmers mostly collect seeds from plants of their own orchards, without the due selection criteria. Consequently, fruits are highly variable in size, color, weight and juice yield (Meletti and Nagai, 1992). This propagation method, the self-incompatibility and consequent inbreeding originate heterogeneous populations and impair the establishment of genetic improvement programs and the selection of varieties. In spite of these drawbacks, the most widely used form of propagation is through seeds, due to the low cost and easiness in passion fruit seedling production.

If all explanatory variables influence the main variable (germination percentage), and the correlations between some of them are strong, it can be concluded that there is a system of interrelations among these traits and by which a particular variable could interfere with the main variable via another correlated trait. The most in-depth specification of how this could take place is given by the path analysis. It shows the different possibilities of how study variables can be related, and the consequent direct and indirect effects on the main variable. The part of variation in the germination percentage that cannot be attributed to any of the variables under study is computed as error or residue. Dewey and Lu (1959) emphasized that breeders must be aware of the relations of cause and effect among the variables, based on a priori determination or on experimental evidence when constructing the diagram.

This study aimed to estimate the coefficients of phenotypic, genotypic and residual correlations and partition the genotypic correlations in direct and indirect effects (path analysis) of the variables first germination count, electric conductivity, weight reduction, moisture and dry mass (independent explanatory variables) on germination percentage (main dependent variable) of seeds of yellow passion fruit extracted from fruits stored for 7, 14 and 21 days at refrigerated and environment temperature.

MATERIAL AND METHODS

The fruits were harvested from an orchard in Campos dos Goytacazes, state of Rio de Janeiro, between January and March 2004. The fruits of the population Yellow Master were hand- pollinated as follows: the anthers of a flower were touched to impregnate the fingers with pollen and to then gently touch the three stigmas of another flower.

Fruits of the three maturation stages 55, 60 and 65 days after anthesis (DAA) were stored at temperatures of 8 ºC (cooled temperature – CT) and 25 ºC (environment temperature– ET) for 7, 14 and 21 days (DS) and an additional treatment of fruits without storage (NS) was conducted. The maturation stages were determined before fruit abscission, based on observations in the plantation, while the storage periods referred to Gamarra Rojas et al. 1994.

After each storage period (7, 14 and 21 days) the seeds were extracted with the removal of the mucilage by a depulper. The seeds were dried in the shadow at environment temperature. The arils were totally removed by rubbing the seeds against the wire mesh of a sieve. Nine treatments were evaluated at cooled temperature: T_1 - 55 DAA, 7 DS, 8º C; T_2 - 60 DAA, 7 DS, 8 ºC**;** T_8 - 65 DAA, 7 DS, 8 ºC; T_4 - 55 DAA, 14 DS, 8 ºC; T_5 - 60 DAA, 14 DS, 8 ºC; T_6 - 65 DAA,14 DS, 8 ºC; T_7 - 55 DAA, 21 DS, 8 ºC; T_8 - 60 DAA, 21 DS, 8 ºC; T_9 - 65 DAA, 21 DS, 8 ºC DS and 9 treatments at environment temperature: T_1 - 55 DAA, 7 DS , 25ºC ET; T_2 - 60 DAA,7 DS, 25ºC ET; T_3 -65 DAA, 7 DS, 25 ºC ET; T_4 - 55 DAA, 14 DS, 25 ºC ET; T_5 - 60 DAA, 14 DS, 25 ºC ET; T_6 - 65 DAA, 14 DS, 25 ºC ET; T_7 - 55 DAA, 21 DS, 25 ºC ET; T_8 - 60 DAA, 21 DS, 25 ºC ET and T_9 - 65 DAA, 21 DS, 25 ºC ET. The experimental design was completely randomized, with 9 treatments and 4 four replications, in each environment.

The following determinations and tests were used to evaluate the seed quality: moisture content (M) - measured by the oven method, using two 3 g samples that were separated immediately after the removal of the arils and oven-dried at 100 ºC for 24 hours according to the recommendations of the Seed Analysis Standards (Brazil 1992); seed dry mass (DM) – two sub-samples of 3 g were weighed, oven-dried at 100 ºC for 24 hours,

weighed again after drying, and the weight of the seed dry matter determined by the difference; mean fruit weight before and after storage (FWB and FWA, respectively), fruit weight reduction (WR); first germination count (FGC) – number of normal seedlings counted on the 14th day after sowing; final germination percentage (GP) - four sub-samples of 25 seeds were removed from the corresponding treatments and placed between germitest paper sheets (moistened with 9 ml distilled water) and transferred to a BOD incubator (20-30°C, 16 hours of dark and 8 hours of light), according to the species-specific recommendation of the Seed Analysis Standards (Brazil 1992). After 14 days (first count) and after 28 days (final count), the results were expressed in percentage of normal seedlings.

For the test of accelerated aging (AA) four replications of 100 seeds per treatment were evenly distributed on wire mesh and placed in germination boxes with 40 ml distilled water on the bottom. The germination boxes were transferred to a BOD incubator (42 °C, 72 hours). After this period, 25 seeds of each replication were placed to germinate according to the test procedures of germination and electric conductivity (EC). Each 25-seedsubsample was previously weighed on a precision scale (0.01g) and filled in a plastic cup (200ml) with 75 ml distilled water (constant 25 ºC, 24 hours); after this period, the samples were removed and the electric conductivity of the imbibition solution measured by a Thermal conductivimeter (CD-2P, model digimed) and the results expressed in $\mu S\ cm^{-1}\ g^{-1}$. The tests were carried out at the Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, from April to May 2004.

The estimators of the coefficients of phenotypic, genotypic and environmental correlations were obtained by the expressions:

a) Phenotypic correlation

$$r_F = \frac{GMP_{xy}}{\sqrt{RMS_x . RMS_y}}$$

b) Genotypic correlation

$$r_G = \frac{(PMG_{XY} - RMP_{XY})/r}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{g(Y)}}} = \frac{\hat{\phi}_{g(XY)}}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{g(Y)}}}$$

c) Environmental correlation

$$r_A = \frac{RMP_{xy}}{\sqrt{RMS_x . RMS_y}}$$

where:

GMP_{xy}: mean product of the genotypes for the traits X and Y;
RMP_{xy}: mean product of the residues for the traits X and Y;
GMP_x: mean square of the genotypes for the trait X;
GMP_y: mean square of the genotypes for the trait Y;
RMS_x: mean square of the residues for the trait X;
RMS_y: mean square of the residues for the trait Y;
$\hat{\phi}_{g(XY)}$: estimator of the genotypic covariance;

$$\phi_{g(x)} = \frac{GMP_x - RMS_x}{r}$$

$$\phi_{g(y)} = \frac{GMP_y - RMS_y}{r}$$

where $\hat{\phi}_{g(XY)}$ and $\hat{\phi}_{g(Y)}$: estimators of the square components associated to the genotypic variability for traits X and Y, respectively.

The multicollinearity was diagnosed in the matrix of genotypic correlations involving the nine variables for cooled and environment temperature conditions. The results of the path analysis were interpreted based on Singh and Chaudhary (1979), and Vencovsky and Barriga (1992), as follows: i) high correlation coefficients and direct effects (path coefficients) indicate that these independent variables explain great part of the variation of the basic variable and ii) the correlation coefficients are positive (or negative) but the direct effect has a different or dispensable signal – in this case, the variables with greatest indirect effects must be considered simultaneously to explain the variation in the basic variable.

The direct and indirect effects of the explanatory variables on the basic variable are shown in an illustrative diagram (Figure 1). The software Genes (Cruz 1997) was used for all analyses.

RESULTS AND DISCUSSION

The multicollinearity involving the nine variables was diagnosed as weak. At cooled temperature, the variables DM, FWB and FWA presented moderate to severe multicollinearity (determinant of the matrix X'X = -0.035388 and number of condition NC (max /min) = 43.467561). At environment temperature, the variables M, FGC, FWB and FWA presented moderate to severe multicollinearity (determinant of the matrix X'X = - 0.00061 and number of condition NC (max /min) = 84.473395). According to Hill and Thompson (1978), it is important to bear in mind that when NC is below 100, negative and the determinant matrix is negative as well, there may be problems in the analysis.

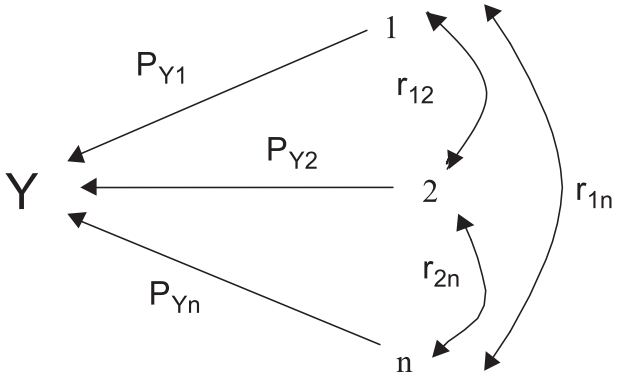

Figure 1. Illustrative diagram of the direct and indirect effects of the independent explanatory variables (Y1, Y2 and Yn) on the basic variable germination percentage (Y - GP). P_{Yi} :direct effect of each one of the three variables on the basic variable. r_{ij} : coefficient of genotypic correlation among the explanatory traits

The estimates of the genotypic correlations exceeded the phenotypic and environmental correlations for all combinations among variables evaluated at cooled temperature (Table 1) and for most of the combination pairs at environment temperature, aside from the pairs GP x FGC, GP x EC and EC x WR (Table 2). In these combinations, the genotypic correlation was not significantly different from zero by the t test (P> 0.05) (Table 1). With exception of pair WR x M, the phenotypic correlation estimates surpassed the environmental correlations, and were therefore lower than the genotypic correlations (Table 1).

Variations in the genotypic correlation estimates (and their significance) were observed in both environments (Tables 1 and 2). The genotypic correlations between GP x AA and GP x EC, for example, were positive (P<0.01) at cooled temperature (Table 1) and absent (P>0.05) in natural conditions (Table 2), unlike the combination GP x WR; in this case, no genotypic correlations were observed at refrigerated temperature and positive ones in natural conditions (Tables 1 and 2).

According to Falconer (1987) the causes of genetic and environment variation can affect the traits through different physiological mechanisms. For example, the different environments of passion fruit storage could influence one of these mechanisms.

The estimates of the direct and indirect effects of the independent explanatory variables on the final germination percentage (GP) at cooled and environment temperature are displayed in Tables 3 and 4, respectively.

Considering the environment mean, the coefficient of determination of the path analysis model (R^2) in question was 72.76%, (75.32% at cooled and 70.20% at environment temperature); it may be concluded that the variations of the basic variable can be partly explained by this causal scheme. Considering the path diagram as

Table 1. Estimates of the phenotypic (r_F), genotypic (r_G) and environmental correlation coefficients (r_A) among six traits of *Passiflora* seed evaluated at cooled temperature

	r	FGC[1]	AA	EC	WR	M
GP	F	-0.04	-0.34	-0.56	-0.07	-0.38
	G	-0.05	-0.61**	-0.71**	-0.09	-0.44**
	A	-0.03	-0.04	0.07	0.009	0.04
FGC	F		-0.32	0.50	-0.47	0.52
	G		-0.41*	0.60**	-0.78**	0.70**
	A		-0.25	0.29	0.11	-0.17
AA	F			0.22	-0.02	-0.11
	G			0.33*	-0.05	-0.21
	A			0.12	0.008	0.06
EC	F				-0.09	0.57
	G				-0.11	0.63**
	A				-0.04	0.01
WR	F					0.10
	G					0.13
	A					-0.10

** P<0.1; * (P<0.05);
[1] FGC - First germination count, GP - Germination percentage, AA- accelerated aging, EC - Electric conductivity, WR - Weight reduction, M - moisture content

Table 2. Estimates of the phenotypic (r_F), genotypic (r_G) and environmental correlation coefficients (r_A) among five traits of Passiflora seed evaluated at environment temperature

	r	AA[1]	EC	WR	DM
	F	-0.04	-0.16	-0.26	-0.26
GP	G	-0.03	-0.16	-0.36*	-0.30
	A	-0.11	-0.21	0.31	0.14
	F		0.26	-0.50	-0.37
AA	G		0.29	-0.64**	-0.38*
	A		-0.03	0.05	-0.26
	F			-0.13	-0.77
EC	G			-0.14	-0.82**
	A			-0.22	-0.03
	F				0.38
WR	G				0.44**
	A				0.11

** P<0.1; * (P<0.05);
[1] FGC - First germination count, GP - Germination percentage, AA- accelerated aging, EC - Electric conductivity, WR - Weight reduction, M - moisture content

Table 3. Path analysis: partitioning of the genotypic correlations in components of direct effect and indirect involving the main dependent variable (GP - germination percentage) and the independent explanatory variables FGC, AA, EC, WR, and M of Passiflora seed at cooled temperature, in Campos dos Goytacazes-RJ

Variable	Components	
FGC		
Direct effect on GP	-0.0744	
Indirect effect via AA	0.2724	
Indirect effect via EC	-0.1496	
Indirect effect via WR	0.1398	
Indirect effect via M	-0.2442	
TOTAL		-0.0559
AA		
Direct effect on GP	-0.6497	
Indirect effect via FGC	0.0312	
Indirect effect via EC	-0.0843	
Indirect effect via WR	0.0103	
Indirect effect via M	0.0741	
TOTAL		-0.6183
EC		
Direct effect on GP	-0.2487	
Indirect effect via FGC	-0.0447	
Indirect effect via AA	-0.2201	
Indirect effect via WR	0.0196	
Indirect effect via M	-0.2199	
TOTAL		-0.7140
WR		
Direct effect on GP	-0.1772	
Indirect effect Via FGC	0.0587	
Indirect effect Via AA	0.0378	
Indirect effect Via EC	0.0275	
Indirect effect Via M	-0.0463	
TOTAL		-0.0995
M		
Direct effect on GP	-0.3453	
Indirect effect via FGC	-0.0526	
Indirect effect via AA	0.1395	
Indirect effect via EC	-0.1584	
Indirect effect via WR	-0.0237	
TOTAL		-0.4406
Coefficient of determination (R^2)	0.7532	
Residual effect	0.4966	

FGC - First germination count, GP - Germination percentage, AA-accelerated aging, EC - Electric conductivity, WR - Weight reduction, M - moisture content

Table 4. Path analysis: partitioning of genotypic correlations in components of direct and indirect effect involving the main dependent variable GP and independent explanatory variables AA, EC, WR and DM at environment temperature of *Passiflora* seed,

Variable	Components	
AA[1]Direct effect on GP	-0.2705	
Indirect effect via EC	-0.3799	
Indirect effect via WR	0.0520	
Indirect effect via DM	0.5652	
TOTAL		-0.0331
EC		
Direct effect on GP	-1.2954	
Indirect effect via AA	-0.0793	
Indirect effect via WR	0.0115	
Indirect effect via DM	1.1980	
TOTAL		-0.1653
WR		
Direct effect on GP	-0.0812	
Indirect effect via AA	0.1733	
Indirect effect via EC	0.1836	
Indirect effect via DM	-0.6403	
TOTAL		-0.3646
DM		
Direct effect on GP	-1.4498	
Indirect effect via AA	0.1054	
Indirect effect via EC	1.0704	
Indirect effect via WR	-0.0358	
TOTAL		-0.3099
Coefficient of determination (R^2)	0.7020	
Effect of the residual variable	0.5458	

FGC - First germination count, GP - Germination percentage, AA-accelerated aging, EC - Electric conductivity, WR - Weight reduction, M - moisture content

well as the multiple linear regression model of Y in function of X1, X2,..., Xn. The determination coefficient represents the percentage of variation in Y explained by the proposed model.

Of the estimates of direct and indirect effects of the independent variables FGC, AA, EC, WR, and M, the genotypic correlation of variable AA was high (-0.6183) and had a high direct effect on the basic variable GP (-0.6497) at cooled temperature (Table 3). This value was higher than the effect of the residual variable (0.4966), indicating that seed vigor is closely related with germination percentage, i.e., in spite of the AA stress, the seed germination potential was high, evidencing that the seed vigor was preserved in the cooled environment.

It is worth emphasizing that of the five, AA was the only explanatory variable that had a good correlation and combination of path coefficient, with high values in both cases. These results indicate that at cooled temperature, there is marked influence of AA on the seed germination potential and the high correlation value of AA occurs due to the high direct effect on GP.

In spite of the high genotypic correlation with GP (-0.7140), variable EC had a reduced direct effect (-0.2487), which was even lower than the residual effect (0.49669), indicating that AA and M are more important.

Contrarily, at environment temperature (Table 4) DM was the explanatory variable of the four with high correlation and combination of the path coefficient, with a consequently stronger influence on seed germination. The high correlation of this variable is explained by the high direct effect on GP.

Although the direct effect on GP (-0.3453) and genotypic correlation (-0.4406) was lower than the value of the residual effect (0.4966), these values are similar for moisture. In this case seed moisture can be considered an important factor to evaluate seed germination, since an excess of humidity may affect the germination of passion fruit seed at cooled temperature. The indirect effect of moisture via AA (- 0.1395) was higher than the direct effect on GP itself.

The direct effect on GP of the variables FGC, WR and EC presented low values (-0.074, -0.177 and –0.248), and the respective genotypic correlations (-0.05, -0.09 and –0.714) were below the value of the effect of the residual variable (0.496), thus indicating that the variables AA and M are more important.

The variable dry mass (Table 4) exerted a strong effect on seed germination potential as well (-1.449), as this value exceeded the residual effect. The indirect effects of the variables AA (0.5652), EC (1.1980) and WR (-0.6403) were significant and of strongest expression in dry mass, since these values were higher than the residual effect (0.5458).

At environment temperature the variables dry mass and electric conductivity had greatest influence on the seed germination potential, evidencing that the indirect effects of the variables accelerated aging, electric conductivity and weight reduction influenced dry mass most.

CONCLUSIONS

The estimates in the two environments were greatly different. Nevertheless, the following conclusions could be drawn:

1) The variable accelerated aging is more relevant to explain seed germination power at cooled temperature, while at environment temperature, seed dry mass conditions have a stronger influence;

2) The variables accelerated aging and dry mass are highly useful in the evaluation of seed physiological quality of yellow passion fruit.

3) The path analysis proved to be an appropriate and precise procedure of easy application in the specification of the correlations among the variables studied.

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