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ASSOCIATIONS BETWEEN TRAITS IN FISÁLIS: A TOOL FOR INDIRECT SELECTION OF SUPERIOR PLANTS[1]

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ABSTRACT - Knowledge about associations between traits is fundamental for plant breeding, since indirect selection can accelerate the development of promising genotypes. This study assessed the magnitude of associations between agronomically important traits in fisális (*Physalis peruviana* L). The experiment was conducted in Lages, Santa Catarina, and the treatments consisted of six fisális populations. The experiment was arranged in a randomized block design, with two replications and seven plants per plot. The correlations between traits were estimated based on the Pearson correlation coefficients and partitioned into direct and indirect effects through path analysis. Fruit weight was positively correlated with number of seeds (0.874), equatorial fruit diameter (0.738) and polar fruit diameter (0.672). By path analysis, number of seeds was identified as the trait with the highest direct contribution to fruit weight. The high phenotypic correlations between the equatorial and polar fruit diameters with fruit weight were mainly due to the indirect effect via number of seeds (0.505 and 0.459). The selection for heavier fisális fruits was strongly influenced by number of seeds, i.e., this trait should be taken into account for selection.

Index terms: phenotypic correlation, path analysis, plant breeding, *Physalis peruviana* L.

ASSOCIAÇÃO ENTRE CARACTERES EM FISÁLIS: UMA FERRAMENTA PARA SELEÇÃO INDIRETA DE PLANTAS SUPERIORES

RESUMO - O conhecimento da associação entre caracteres é de fundamental importância no melhoramento de plantas, uma vez que a seleção indireta pode acelerar a obtenção de genótipos promissores. Este trabalho teve como objetivo quantificar a magnitude da associação entre caracteres de importância agronômica na cultura da fisális. O experimento foi conduzido em Lages-SC, e os tratamentos foram constituídos de seis populações de fisális. O delineamento experimental foi em blocos casualizados, com duas repetições e sete plantas por parcela. A correlação entre os caracteres foi estimada com base nos coeficientes de correlação de Pearson e particionados em efeitos diretos e indiretos por meio da análise de trilha. Houve associação positiva entre a massa do fruto e o número de sementes (0,874), diâmetro equatorial do fruto (0,738) e diâmetro polar do fruto (0,672). Por meio da análise de trilha, foi possível verificar maior contribuição direta do caráter número de sementes sobre a massa do fruto. A elevada magnitude de correlação fenotípica entre diâmetro equatorial do fruto e diâmetro polar do fruto com a massa do fruto deve-se, principalmente, ao efeito indireto via número de sementes (0,505 e 0,459). A seleção de frutos de fisális com maior massa é fortemente influenciada pelo número de sementes, de maneira que este caráter deve ser considerado no momento da seleção.

Termos para indexação: correlação fenotípica, análise de trilha, melhoramento de plantas, *Physalis peruviana* L.

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INTRODUCTION

The increasing cultivation and sales of fisális (*Physalis peruviana* L.) fruits are basically the result of the market acceptance by consumers owing to the peculiar fruit traits, particularly the high nutrient and vitamin contents and antioxidant capacity (FISCHER et al., 2014; LUCHESE et al., 2015), as well as the exotic appearance and organoleptic properties of the fruit. In Brazil, more specifically in the southern region, fisális is cultivated by small and medium producers, although the national production is insufficient to meet the demands of the consumer market, causing import dependence of this fruit. In this scenario, breeding programs are addressing the selection of superior genetic constitutions to improve fruit yields and quality (TREVISANI et al., 2016).

Yield assessments are essential in plant breeding programs. However, in species of the small fruit group, the quality of the fresh product is decisive for acceptance in the consumer market (DI VAIO et al., 2014). The quality is conditioned by several traits, particularly by fruit size, color, flavor, and the sweet/acid taste (SALLA et al., 2015). Similar to strawberry, blackberry and raspberry, fisális has traits that should be prioritized in selection. The development of plants with larger fruits is a primordial property in breeding of these fruit species. However, this quantitative trait is controlled by a high number of genes, strongly influenced by the environment and resulting from multiplicative effects of other essential traits (FERREIRA et al., 2016). Faced with the challenge of meeting these requirements, competence is required to identify the best genotypes in the breeding programs.

Analyzing and determining inter-trait correlations is a strategy that facilitates and contributes to the selection of promising genotypes (LESSA et al., 2012). Knowledge about the phenotypic correlations is important when a simultaneous selection of traits is desired as well as in cases of low heritability and/or difficulty of evaluation of the target trait (SILVA et al., 2016). Moreover, when relations between traits of complex nature are analyzed, e.g., fruit weight and its components, information about the direct and indirect effects of each trait on the basic trait (fruit weight) is required.

Path analysis, as proposed by Wright (1921), allows the partitioning of the phenotypic correlation coefficient into direct and indirect effects on a trait of greater economic relevance. The resulting estimates ensure greater reliability in the selection and, consequently, accelerate the breeding and release of new cultivars. Therefore, in view of the importance of fisális in some regions of Brazil and the need for expansion of the crop, the study of the relationship between the traits related to fruit weight is fundamental for the development of higher-quality genotypes, with a view to establishing the self-sufficiency of fruit production in the country.

The purpose of this study was to quantify the magnitude of the relationship between the agronomically important traits of the crop, contributing to the selection of superior plants.

MATERIAL AND METHODS

The study was carried out in an experimental area of the Instituto de Melhoramento e Genética Molecular (IMEGEM) of the Universidade do Estado de Santa Catarina (UDESC), in Lages SC (lat. 27°48', long. 50°19' W; 916 m asl). The regional climate is temperate with cool summers (Cfb) and mean annual temperature of 14.3°C, with mean annual rainfall of 1479.4 mm.

The experiment consisted of six fisális populations from several regions of the South of the country, i.e., from Lages, Fraiburgo, and Caçador in the State of Santa Catarina, Vacaria in Rio Grande do Sul, and two populations purchased from Colombia and Peru. The seedlings were grown in styrofoam trays filled with commercial substrate for solanaceous seedlings, placed in a protected environment until reaching a height between 15 and 20 cm, and then transplanted to the final field site. The experiment was arranged in a randomized block design, with two replications. The experimental units consisted of seven plants, spaced 1 m apart in rows spaced 2 m apart, with evaluations of five plants per plot.

Six traits related to the morphological fruit quality were evaluated: fruit weight – FW (g), fruit capsule weight CW (g), 1000-seed weight ThSW (g), number of seeds NS, equatorial fruit diameter - ED, polar fruit diameter – PD, Stem diameter StD (mm), height of the first bifurcation of the plant BH (cm), plant height PH (cm), and number of fruits NF counted 71 days after planting the seedlings in the field.

For statistical analysis, the data were subjected to multivariate analysis of variance to estimate the general effects of variation ($p < 0.05$) and investigate possible differences between the fisális populations, according to the significance of the mean vectors. The results were generated by the SAS 9.2 GLM procedure.

For the inferences regarding the association between traits, Pearson's phenotypic correlation coefficients were estimated and partitioned into direct and indirect effects by path analysis. To this end, fruit weight was considered as the main and the others as explanatory (secondary) traits. Multicollinearity was diagnosed based on the number of conditions (NC), by the ratio of the highest by the lowest eigenvalue of the matrix. According to Montgomery and Peck (1981), if NC <100, multicollinearity is considered weak and not a problem for analysis; if <NC <1000, it is considered severe. The hypothesis that the phenotypic correlation coefficient is equal to zero (H_0: 0) was evaluated by the Student's *t*-test, at 5% probability. These analyses were carried out with GENES software (CRUZ, 2001).

RESULTS AND DISCUSSION

The multivariate analysis of variance showed differences for the population factor (Table 1), evidencing differences in at least two fisális populations for the set of traits evaluated. This result is promising and highly relevant for breeding programs addressing the selection of genetic constitutions with superior traits, where genetic variability is the basis underlying plant selection processes (ALLARD, 1960).

The estimates of phenotypic correlation coefficients revealed the traits strongly correlated with fruit weight: NS (0.874), SD (0.738) and SD (0.672) (Table 2). The correlation between fruit diameters and weight corroborated results of Rodrigues et al. (2014), who obtained a positive and significant correlation for ED (0.915) and PD (0.896) in a greenhouse experiment. Possibly, the positive correlation between fruit weight and diameter is not affected by the environment in which the genetic constitutions are evaluated (field or greenhouse). The results indicate the possibility of performing indirect selection via fruit diameter to consequently obtain heavier fruits.

The correlation between NS and FW and with the diameters is not surprising, since the seed has a direct influence on fruit formation. According to Kumar et al. (2014), the hormones synthesized in the seeds play a decisive role in fruit growth, since the hormonal activity is related to the expression of the genetic information, enzyme activity and functionality of the membranes. In this sense, if there is an increase in the number of seeds per fruit, there is also an increase in the production of auxins and, consequently, in fruit size.

The traits DE (0.729) and PD (0.663) were positively associated with NS (Table 2). As previously mentioned, seeds are a source of hormones that have a direct influence on fruit formation and size. According to Ryugo (1993) and Anzanello et al. (2013), fruit size is directly proportional to the number of viable seeds in developing fruits, and this fact is usually observed in fruits that produce many seeds such as kiwi, blackberry, strawberry, and fig.

In a study of Peña et al. (2010) on *P. peruviana*, a lower seed index was found in fruits of an accession known as "Kenia", due to a higher pulp weight and lower number of seeds/fruit. This indicates advantages of this accession for agroindustry and fresh consumption, a purpose for which larger fruits with a lower number of seeds are desirable. The fruits of the accession "Sudáfrica" on the other hand contained a higher quantity of seeds than pulp, indicating their usefulness for breeding programs and production of sexually propagated fisális seedlings. However, it is worth emphasizing that the indirect selection of fruits based on NS may not be the best breeding strategy, due to the difficulty of counting seeds.

Almost all correlations between NF and the other traits were negative and, for the traits FW, NS, StD, and PH, these values were negative and significant (Table 2). The result shows that the breeding of plants with higher fruit yield can on the other hand cause losses in important fruit and plant traits. According to Fischer et al. (2011), there is an inverse relation between number of fruits and mean fruit weight, i.e., a higher number of fruits results in lower fruit weight, since the availability of photoassimilates produced by the plant for the fruit is limited. Therefore, just as breeders prioritize higher fruit yield/plant or in selection, they should also take an important component of the morphological quality into consideration, the fruit weight, because size-related aspects define the consumer preference, as mentioned above.

The trait PH was significantly and positively correlated with FW (0.155), NS (0.191), ED (0.221), and StD (0.206) (Table 2). This result indicates the possibility for indirect selection in important fruit traits based on other, more easily measureable morphological traits. This aspect is promising in the selection of fisális plants: taller plants imply larger fruits. In this context, it is important to emphasize that in fisális production systems, short plants (less than 1.5 m) are an obstacle for crop management and, consequently, for fruit harvest. Plants with medium to tall height (between 1.5 and 1.8 m) should be prioritized in the selection.

For a better understanding of the direct and indirect relationships between traits, path analysis was performed. The multicollinearity diagnosis of the phenotype correlation matrix indicated NC <100, characterized as weak multicollinearity, which is not a problem for path analysis. The coefficient of determination of the model of path analysis (R^2 =0.817) indicates a good fitting of the model to the explanation of the genetic effects related to fruit weight.

Estimates of the direct and indirect effects of fruit weight components showed a high positive correlation (0.874) of NS, with a higher direct effect of this trait with FW (0.692), that is, 80% of the total correlation is explained by the contribution of NS (Table 3). There was an indirect contribution of trait ED (0.120), but since the value is low, indirect selection for ED may fail to provide satisfactory gains in FW, and is not the best selection strategy for larger fruits. This finding indicates that selection for NS would be a direct way to select for heavier fruits. However, as already pointed out, seed counting becomes too costly and therefore unfeasible.

The analysis of the traits ED and PD, which also had higher correlation values than the other traits, showed that trait NS provided the greatest indirect contribution to FW in both diameters (Table 3). Thus, of the total correlation, the direct effect of ED corresponds to only 22%, whereas of the same total correlation, NS contributed indirectly with most of the correlation (69%). For PD, the direct contribution of the total was only 15%, and the indirect contribution via NS was 80%. This clearly shows the strong influence of NS on the development of larger fruits. The intensified selection pressure for ED and PD may not provide satisfactory genetic gains, as this phenotypic correlation is mainly caused by indirect effects, and the cause-effect relationship is not observed. Indirect selection via ED and PD will only be efficient in increasing FF if concomitant indirect effects via NS are taken into consideration. Traits with a high favorable correlation, but with a low direct effect indicate that truncated selection for the trait may not provide satisfactory gains in the basic variable (AGUIAR et al., 2015). In this case, the best strategy should be the simultaneous selection of traits, including the traits with significant indirect effects.

In a blackberry breeding program carried out at the Experimental Station of Embrapa in Pelotas, RS, the researchers reported that fruit weight is the primary criterion in the selection for morphological fruit quality (ANTUNES et al., 2014). In fisális, it was possible to show that selection for heavier fruits would provide fruits with larger diameter and a higher seed content. As the number of seeds in a fruit is determined by genetic factors (PATTISON et al., 2014; CZEREDNIK, et al., 2015) and has a strong influence on the fruit growth rate, final size and shape (GRANGE, 1993), this characteristic will possibly be maintained throughout the selection generations.

The correlations of StD, BH, PH, and NF with FW were the lowest of all traits (Table 3). Trait PH had a low and negative direct effect on the basic trait (-0.067). However, the indirect effect via NS was higher, contributing with 86% of the total phenotypic correlation. In other words, the selection of taller plants may not lead to a significant reduction in fruit weight, but will reduce the number of seeds per fruit. For NF, the contribution of the direct effect to the total phenotypic correlation was also lower (-0.079) and the indirect contribution via NS higher (-0,128). In this sense, the selection of plants with higher fruit yield will have a greater effect on the reduction of NS, rather than on a reduction in FW, as observed here.

Once again, the number of seeds evidently had a strong influence on FW. This result is promising for fisális breeding, when taller plants are desired, without however reducing fruit weight and yield. The results of path analysis were very useful to identify the true cause-effect relationships and to highlight possible changes when the associations between two traits are a consequence of the combined action of several factors acting simultaneously (CRUZ et al., 2004). In biology, the relationship between agronomic traits is rather complex, so that plant breeders must know whether the selection of one trait of interest has a negative or positive influence on another.

TABLE 1- Multivariate variance analysis at 5% error probability for six fisális populations, using Wilks's lambda distribution (λ) for the factors block and population. The evaluated traits were capsule weight, number of seeds, 1000-seed weight, equatorial fruit diameter, polar fruit diameter, stem diameter, bifurcation height of the plant, plant height, and number of fruits. UDESC, Lages-SC.

Sources of variation	NGL	DGL	Value λ
Block	7	107	0,76*
Population	35	452,54	0,35*

* * Significant at 5% probability of error by test F.
NGL: Numerator degrees of freedom.
DGL: Denominator degrees of freedom.

TABLE 2- Estimates of the Pearson phenotypic correlation coefficients among the 10 agronomic traits capsule weight - CW, number of seeds - NS, 1000-seed weight - ThSW, equatorial fruit diameter - ED, fruit diameter - PD, stem diameter - StD, bifurcation height of the plant - BH, plant height - PH, and number of fruits - NF, evaluated in six fisális populations. UDESC, Lages-SC.

Character	CW	NS	ThSW	ED	PD	StD	BH	PH	NF
FW	0,017	0,874*	0,062	0,738*	0,672*	-0,039	-0,111	0,155*	-0,197*
CW		-0,047	0,105	-0,002	0,006	0,140	-0,181*	-0,070	0,068
NS			-0,059	0,729*	0,663*	-0,070	-0,003	0,191*	-0,184*
ThSW				-0,002	0,0036	-0,040	-0,030	-0,040	0,005
ED					0,657*	0,007	-0,091	0,221*	-0,099
PD						0,019	-0,039	0,158	-0,077
StD							0,042	0,206*	-0,221*
BH								-0,119	-0,103
PH									-0,364*

* Significant at 5% probability of error by test t.

TABLE 3-Coefficients of path analysis, involving the basic trait fruit weight FW and the explanatory traits: capsule weight - CW, number of seeds - NS, 1000-seed weight - ThSW, equatorial fruit diameter - ED, fruit diameter - PD, stem diameter - StD, bifurcation height of the plant - BH, plant height - PH, and number of fruits - NF; partitioning of the phenotypic correlation coefficients into direct effects (main diagonal, bold) and indirect effects (above and below the main diagonal) in six fisális populations. UDESC, Lages-SC.

Character	CW	NS	ThSW	ED	PD	StD	BH	PH	NF	FW
CW	**0,021**	-0,033	0,009	-0,001	0,001	0,002	0,018	0,005	-0,005	**0,017**
NS	0,001	**0,692**	-0,005	0,120	0,067	0,001	0,001	-0,012	0,015	**0,874**
ThSW	0,002	-0,041	**0,093**	0,001	0,003	0,001	0,003	0,002	0,001	**0,062**
ED	0,001	0,505	0,001	**0,164**	0,066	0,001	0,090	-0,014	0,007	**0,738**
PD	0,001	0,459	0,003	0,108	**0,101**	0,001	0,004	-0,010	0,006	**0,672**
StD	0,002	-0,048	-0,004	-0,001	-0,002	**0,011**	-0,004	-0,014	0,017	**-0,039**
BH	-0,003	-0,002	-0,003	-0,015	-0,004	0,001	**-0,100**	0,008	0,008	**-0,111**
PH	-0,001	0,133	-0,004	0,036	0,016	0,022	0,012	**-0,067**	0,029	**0,155**
NF	0,001	-0,128	0,001	-0,016	-0,008	-0,002	0,010	0,024	**-0,079**	**-0,197**
$R^{2/*}$	0,817									
Er	0,427									

/* R^2 Determination coefficient. Er - residual effect of the variable.

CONCLUSIONS

The selection of heavier fisális fruits will produce fruits with larger diameters and higher seed content. Due to the strong influence of the number of seeds on fruit weight, it is of paramount importance for the success of selection to take the expression of this trait into account. However, indirect selection based on number of seeds proved unfeasible, due to the difficulty of measuring this trait.

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