

Variability and correlations between agronomic characters in mutant populations of *Physalis*

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Abstract

The *Physalis* populations cultivated in Brazil have a restricted genetic base, hampering the genetic progress of the species. The mutation and correlation studies are tools that boost the development of new cultivars. The study aimed to characterize the phenotypic variability of produced and mutant *Physalis* and obtain estimates of phenotypic correlations between agronomic relevant features. The treatments were the seeds of three *Physalis* populations (Colombia, Lages, and Fraiburgo), submitted to gamma-irradiation (Co^{60}) for three exposure times (0, 250, and 500 Grays). The experimental design used randomized blocks with three replications. The study assessed the pH, the titratable total acidity (TTA), the total soluble solids (TSS), maturation index (MI, TSS/TTA), the number of flowers (NOF) and fruits (NFR), the weight of fruits without boll (FWB, in g) and total productivity (PROD as $kg\ ha^{-1}$). The results prove variation in mutant populations when compared to their recognized populations (cultured populations) for the qualitative components (acidity and total solids) but not for quantitative components (number of flowers per plant, number of fruits per plant, fruit weight without boll and the estimated total productivity throughout the cycle). There is a positive correlation between acidity and total solids. Cross breedings between contrasting populations can produce genotypes with more equilibrated taste.

Keywords: cultivars, fruit, gamma irradiation, genetic improvement

Introduction

Physalis peruviana is of Andean origin, belonging to the Solanaceae family. The species has hermaphrodite flowers, preferably allogamous. The species' fruits have great flavor and aroma (Ramírez & Davenport, 2021). Thus, this fruit tree has great potential for family farming due to its good economic return (Fischer et al., 2014).

Physalis production in Brazil is insufficient to meet domestic demand due to inadequate management practices and the lack of improved cultivars (Goulart Junior et al., 2017; Trevisani et al., 2016). In addition, the limited genetic basis found in the populations cultivated in the country for the traits of agronomic importance hampers obtaining genetically superior cultivars (Trevisani et al., 2018; Lagos-Burbano et al., 2020).

According to Borém & Miranda (2013), the most used tool in plant breeding to overcome the lack of genetic resources is the induction of mutation by physical

agents. The advantage of physical agents through ionizing radiation, having cobalt⁶⁰ as a source, is their superior ability to penetrate plant tissue. Thus, it results in higher rates of chromosomal, chromatic aberrations, and single-base gene mutations (Ahloowalia & Maluszinsky, 2001; Antúnez-Ocampo et al., 2020).

The creation of new allelic forms by artificial mutation may allow the selection of plants with fruits with better organoleptic properties. According to Fischer (2014), the aim is also to reduce the significant post-harvest losses and high production yields. However, many of these characters are highly complex for selection, mainly due to polygenic control, with pleiotropic or/and epistatic genes demonstrating a strong environmental influence on their expression, making their quantification difficult (Allard, 1999). Thus, it is relevant to use multivariate techniques (path analysis) to help the breeder.

The multivariate path analysis allows the unfolding

of character correlation coefficients (explanatory variables) on the expression of a principal (basic) variable in direct and indirect effects (path coefficient). This analysis provides accurate estimates of cause and effect, being necessary to assess the relevance of one trait over another quantitatively. Furthermore, enabling such a correlation makes it possible to indirectly select a significant trait that has low heritability or is difficult to assess (Ferreira et al., 2012). Furthermore, studies with this purpose associated with mutation induction can accelerate obtaining new cultivars, increasing the genetic variability and the realization of crosses aimed at obtaining superior genotypes (Cruz et al., 2012).

The objective of this work was to characterize the variability in cultivated and mutant populations of *Physalis*, together with obtaining estimates of phenotypic correlations and their consequences in direct and indirect effects between characters of agronomic importance.

Materials and Methods

The work was performed at the Universidade do Estado de Santa Catarina (UDESC), located in the city of Lages-SC (27° 48' S and 50° 19' W), with an average altitude of 930 m. The municipality has a Cfb climate (temperate climate with cool summers) and an average annual temperature of 14.3° C, with an average annual rainfall of 1500mm.

For obtaining the mutant populations, three cultivated populations of *physalis* were selected according to their origin (Colombia, Lages, and Fraiburgo). Subsequently, these populations submitted to gamma irradiation (physical method) with the mutagenic agent Cobalto⁶⁰ at doses of 0, 250, and 500 Grays (Gy) (with a rate of 15.24 Gy per minute), which resulted in 9 populations or treatments. The determination of irradiation doses for the culture was performed according to Caro-Melgarejo et al. (2012). First, gamma irradiation gave rise to populations of the M1 generation. Then, from the M1 generation, this generation was self-fertilized to obtain the M2 populations. This process of self-fertilization can also be called generational advancement. Finally, the agronomic traits of interest were assessed in M2 populations.

The experimental design used was randomized blocks with three replications. The experimental unit consisted of 10 plants, three plants per meter, spaced 3 m apart. The cultural treatments followed the technical recommendations for the culture (Muniz et al., 2014). The qualitative variables evaluated in the fruits were: i) the pH, ii) the titratable total acidity (% citric acid) by titration with 0.1 M NaOH (TTA), iii) total soluble solids (TSS, in °Brix), with

the aid of a manual refractometer, and iv) maturation index (MI, TSS/TTA ratio), and quantitative traits evaluated in the plants were: v) number of flowers (NOF) vi) number of fruits (NFR), vii) weight of fruits without boll (FWB, in g) and viii) total productivity (PROD in kg ha⁻¹).

The normality of errors was tested by Shapiro-Wilk and the homogeneity of variances by Levene. Data were submitted to analysis of variance (ANOVA), using general linear models (DFM procedure), using SAS University Edition 9.4 software, with the model: $Y_{ij} = m + b_i + t_j + e_{ij}$. Where: Y_{ij} is the observation of the i -th treatment in the j -th block; m is the overall average of the experiment; b_i is the effect of the i -th block; t_j is the effect of the j -th treatment; e_{ij} is the random effect of the error.

Univariate contrasts were also performed for each population at dose zero versus the respective population at doses of 250 and 500 Gy, according to the following scheme: C1: Colômbia01_0 vs. Colômbia01_250; C2: Colômbia01_0 vs. Colômbia01_500; C3: Lages02_0 vs. Lages02_250; C4: Lages_0 vs. Lages_250; C5: Lages_0 vs. Lages_500; C6: Fraiburgo_0 vs. fraiburgo_250; C7: Fraiburgo_0 vs. Fraiburgo_250; C8: Fraiburgo_0 vs. Fraiburgo_500.

Heritability in the broad sense and the ratio between genetic and environmental variation coefficient (gVC/eVC) were estimated from the variance components. The phenotypic correlations between all characters were estimated and measured using Pearson's correlation coefficients at 5% probability. A causal diagram was adopted for path analysis, considering the direct and indirect effects, including the primary variable, the total fruit yield (PROD).

The degree of multicollinearity of the X'X matrix between the independent variables of the regression model was evaluated according to the criteria indicated by Montgomery & Peck (1981). The number of conditions corresponding to the ratio between the highest and lowest eigenvalues to the correlation matrix was adopted. The crest regression method was used to circumvent the effects of multicollinearity. Statistical analyzes were performed and processed using the computational resources of the genes program (Cruz, 2016).

Results and Discussion

Table 1 displays the variance analysis values for the assessed variables. The results point out significant differences in the treatments of the variables total titratable acidity and soluble solids. On the other side, the other characters did not show a significant differential behavior for the populations evaluated.

Table 1: Summary of analysis of (Mean squares) for the variables, total titratable acidity (TTA), maturation index (MI), amount of soluble solids in Brix degree (TSS), number of flowers per plant (NOF), number of fruits per plant (NFR), fruit weight without boll (FWB), estimated total yield throughout the cycle (PROD), Lages-SC, 2022.

VS	DF	Medium Squares						
		PH	MI	TTA	TSS	NOF	FWB	PRODT
Blocks	2	0.007	0.319	0.069	0.64	689.13	931.44	2,398,433.33
Treatments	8	0.008	0.722	0.047*	3.14*	11.73	557.98	413,175.00
Residue	16	0.005	0.439	0.018	1.024	179.15	346.29	365,125.00
VC(%)		2.03	10.71	7.14	8.66	20.89	19.91	29.22
gVC/eVC		0.49	0.46	0.74	0.83	-0.99	0.45	0.21
Herdability (%)		41.74	39.09	61.89	67.43	52.68	37.94	11.63
Average		3.53	6.19	1.89	11.68	20.26	93.47	2067.78

*VS- Variation Source; DF – Degrees of Freedom; VC –Variation coefficient; gVC/eVC- genetic variation/environmental variation. *Significant at $P \leq 0.05$ by the F test.

Regarding the experimental precision, the variable total titratable acidity, maturation index, and total soluble solids presented low coefficients of variation; for the number of flowers, fruit weight, and total fruit productivity, average values of 20 to 30%; and the number of fruits, a slightly higher value of 33%. High values of coefficient of variation for the character number of fruits show the nature of these traits, which have complex genetic control or possible effects of mutation induction that may interfere with the normal distribution of the data.

The decomposition of treatments by contrasts was also performed. The results showed that one of the

combinations (Fraiburgo population at the dose of 500 Gys) presented higher values than the other treatments for the variables total soluble solids (13.23%) and total titratable acidity (2.09%), at a 5% probability by the T-test (Table 2). Thus, part of this variation is genetic (gVC/eVC: 0.83 and 0.74, respectively), with high heritability, and can be used in crop improvement programs to develop new cultivars. In the mutant populations evaluated, the vegetative and production features did not present beneficial effects or physiological damage, in agreement with Antúnez-Ocampo et al. (2020).

Table 2: Average contrasts between the Fraiburgo population at the dose of 500 Gys vs. other treatments.

Comparison	Estimated	T value	Pr > t
-----TTA-----			
Fraiburgo 500 vs. other treatments	2.09	2.84	0.0119
-----TSS-----			
Fraiburgo 500 vs. other treatments	13.23	2.82	0.0124

The ploidy degree (four copies of the alleles) may be related to the mutation inefficiencies with irradiation for the quantitative traits evaluated in the present study (Pelé et al., 2018). In other words, the greater the degree of ploidy of a species, the greater the amount of genome. As mutations are random and casual, there are difficulties in obtaining mutant populations. According to Allard (1999), in polyploids, an allele can manifest its dominance, masking single base mutations (neutral mutations) and preventing the expression of other phenotypic classes. The species may have efficient genomic repair mechanisms, or the large number of genes involved in the expression of this character may mask its effects. There may need to be adjustments between dose-response of the mutagen. The environment can also buffer gene blocks, preventing gene expression and reducing variation, requiring genetic acclimatization of the species in Brazil (Allard, 1999).

Thus, new mutation inductions should be

performed with a more significant load or exposure time of irradiation and the introduction of new sources of genetic variation in the country, mainly from Colombia (Miranda & Fischer., 2021). In Colombia, the acquisition of new accessions from Guatemala and the Holanda allowed, through crosses with Colombian populations, made possible the development of two varieties, the Andean Copoica and the Dourado (Ramírez & Davenport., 2021). Promising new strains are being evaluated by Agrosavia (a Colombian agricultural company) or populations maintained by Colombian universities that can adapt to Brazil or be used for the development of new cultivars (Lagos-Burbano et al., 2020). There are also many native species of the genus *physalis* in the country. The introgression of the genes of these species has made it possible to expand the genetic base (Silva Júnior et al., 2022).

Among the characters evaluated using Pearson's

correlation matrix, five variables: pH, total soluble solids, maturation index, number of fruits, and fruit weight, presented significant phenotypic coefficients at a 5%

error probability. However, only the variables, number of fruits (0.41) and weight of fruits (0.40), resulted in a positive correlation with productivity (Table 3).

Table 3: Pearson's simple correlation coefficient between the characters' total titratable acidity (TTA), maturation index (MI), amount of soluble solids in Brixo degree (TSS), number of flowers per plant (NOF), number of fruits per plant (NFR), yield per plant (FWB), estimated total yield over the cycle (PROD)

	pH	TTA	MI	TSS	NOF	NFR	FWB	PROD
pH		-0.008	0.389	0.439*	0.019	-0.249	0.061	-0.305
TTA			-0.124	0.483*	0.001	-0.258	0.279	0.195
MI				0.456*	0.301	-0.066	-0.067	-0.032
TSS					0.211	-0.258	0.137	-0.195
NOF						-0.338	-0.160	-0.212
NFR							0.403*	0.418*
FWB								0.404*
PROD								

* significant at 5% probability of error, by the T-test.

^{ns} Not significant.

The maturation index (ratio between soluble solids and total titratable acidity), which is an important character in the definition of fruit flavor, presented a lower correlation with total titratable acidity (-0.12) than with soluble solids. (0.49). According to the results, despite the negative values and with low to medium scores for the total titratable acidity, with the other variables, this character must be evaluated with caution since it is related to the post-harvest of the fruits, being responsible for the prolongation of the shelf time. The shelf-life extension is due to preventing deterioration caused by fungi and enabling industrialization, easing the amount of sugar added to its processing (Giles et al., 2016; Morgado et al., 2010). On the other hand, the positive correlation between the maturation index and total soluble solids demonstrates that fruits with higher sugar contents can be selected, which is desirable for consuming the fruit *in natura*.

For the path analysis, the number of conditions for diagnosing the degree of multicollinearity in the path analysis among the independent variables presented values above the recommended (NC=5045.82) for the data set. According to Belsley et al. (1980), multicollinearity is considered severe when conditions exceed 1000. Therefore, crest regression (path analysis under multicollinearity) was used to circumvent the effects of multicollinearity, adopting a value of k=0.11. In the crest path analysis, a minor effect of the residual variable (0.43) and a higher coefficient of determination (R²: 0.82) were observed, proving to be more efficient in minimizing the effects of multicollinearity and explaining the model for the character effects on the base variable.

The path analysis (Table 4) observed that the

characters' number of fruits and weight presented high and positive direct effects, with the primary variable (total productivity, PROD). This relation is in agreement with the high values of simple linear correlation between these characters, already displayed in table 3.

According to Carvalho et al. (2004), determining which variables present more significant correlations with the primary variable is of fundamental importance. In addition, due to their easy measurement, these variables are good predictors, thus promoting the selection of populations with more significant numbers or weight of fruits and obtaining genetic gains through indirect selection for productivity or fruits of better quality. Soares et al. (2017) found similar results in pepper, with a direct correlation of 0.98 and a direct value of 0.36. Maga et al. (2013) found a direct positive effect on the number of fruits per plant (0.91) and fruit weight (0.28). Lúcio et al. (2013) also reported that the number of fruits traits had a more significant direct effect on the total weight of sour passion fruit than total soluble solids, fruit weight, length and diameter, skin thickness, and pulp yield.

As observed in the path analysis, the evaluation of Pearson's correlation coefficients between these two characters showed a significant correlation. However, the simultaneous selection of both would lead to the error of their use. This error confirms the limitation of the linear correlation coefficient since its decomposition presented a high negative indirect effect between the number of fruits and fruit weight, which can cause difficulty in increasing productivity and obtaining fruits that meet export standards. On the other hand, it is possible to select fruits with greater weight without affecting productivity (Wright, 1921; Cruz et al., 2012). Therefore, the number of

fruits, an essential production component, resulted in a negative correlation (-0.20) and a low direct effect on the primary variable. According to Pandya et al. (2016), this result is justified due to the presence of dominant genes

and pleiotropic effects, conferring high phenotypic plasticity because of the influence of the environment on this trait and the low heritability of one of the traits evaluated.

Table 4: The phenotypic direct and indirect effect of the explanatory independent variables pH, total titratable (TTA), maturation index (MI), amount of soluble solids in Brixo grade (TSS), number of flowers per plant (NOF), number of fruits per plant (NFR), fruit weight (FWB), on the primary dependent variable (PROD) Lages-SC, 2022.

Variables	Effects	Path Coefficient
pH	Direct effect on PROD	-0.073
	Indirect effect via MI	-0.029
	Indirect effect via TTA	0.003
	Indirect effect via TSS	0.058
	Indirect effect via NOF	0.005
	Indirect effect via NFR	-0.376
	Indirect effect via FWB	0.094
	Total	-0.325
MI	Direct effect on PROD	-0.058
	Indirect effect via pH	-0.036
	Indirect effect via TTA	-0.006
	Indirect effect via TSS	0.068
	Indirect effect via NOF	-0.007
	Indirect effect via NFR	-0.154
	Indirect effect via FWB	-0.188
Total	-0.387	
TTA	Direct effect on PROD	0.026
	Indirect effect via pH	-0.008
	Indirect effect via MI	0.013
	Indirect effect via TSS	0.056
	Indirect effect via NOF	0.001
	Indirect effect via NFR	0.286
	Indirect effect via FWB	-0.003
Total	0.374	
TSS	Direct effect on PROD	0.102
	Indirect effect via pH	-0.042
	Indirect effect via MI	-0.039
	Indirect effect via TTA	0.014
	Indirect effect via NOF	-0.006
	Indirect effect via NFR	0.050
	Indirect effect via FWB	-0.141
Total	-0.051	
NOF	Direct effect on PROD	-0.018
	Indirect effect via pH	0.021
	Indirect effect via MI	-0.023
	Indirect effect via TTA	-0.001
	Indirect effect via TSS	0.033
	Indirect effect via NFR	-0.040
	Indirect effect via FWB	-0.172
Total	-0.203	
NFR	Direct effect on PROD	0.841
	Indirect effect via pH	0.032
	Indirect effect via MI	0.011
	Indirect effect via TA	0.009
	Indirect effect via TSS	0.006
	Indirect effect via NOF	0.001
	Indirect effect via FWB	-0.347
Total	0.643	

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FWB	Direct effect on PROD	0.685
	Indirect effect via pH	-0.010
	Indirect effect via MI	0.016
	Indirect effect via TTA	0.000
	Indirect effect via TSS	-0.021
	Indirect effect via NOF	0.005
	Indirect effect via NFR	-0.425
	Total	0.323
R ²		0.82
	K VALUE USED IN ANALYSIS	0.11
	EFFECT OF THE RESIDUAL VARIABLE	0.43

According to Montardo et al. (2003), the reason for the low correlation between the number of fruits character and the primary variable can also be explained by the occurrence of the restricted genetic base since this type of analysis seeks to identify a possible association in the variation of the characteristics under study. Fruit size is perhaps more related to productivity than the number of leaves (García-arias et al., 2018). In addition, the indeterminate habit can also contribute to the production of a high number of flowers, and there can be a high rate of abortion due to the source-drain relationship. In this way, the translocation of photoassimilates to fruits and not to flowers occur when competition for photoassimilates is high. Thus, not representing the effectiveness of this character on the primary variable, these values were found in soybean, canola, jaboticaba, and papaya (Salla et al., 2015; Ferreira et al., 2012; Oliveira et al., 2010; Araújo et al., 2007).

Another observation was that in addition to the associations with fruit weight and the number of fruits, there were other direct positive correlations between the characteristics evaluated with the main character. For example, total soluble solid presented a positive direct effect (0.10). However, the total correlation was negative and low magnitude (-0.05), indicating that indirect effects caused the association. On the other hand, the direct effect of total titratable acidity presented a higher association (0.37) and low magnitude (0.03) for the direct effects. Thus, indirect effects are also responsible for the lack of correlation. Thus, the best strategy for total soluble solids and total titratable acidity to provide good gains in the primary variable is the simultaneous selection of variables, emphasizing those whose indirect effects are significant.

On the other hand, there are positive correlations of indirect effects between total soluble solids, total titratable acidity, and some variables. These values corroborate Pearson's correlation analysis demonstrating that genotypes with greater balance in fruit flavor can be selected. Total soluble solids were the character

that presented results for the most significant number of variables regarding the adverse indirect effects. Carvalho et al. (2004); Coimbra et al. (2005) highlight that when a character correlates in a truncated way, that is, it has positive indirect effects with some variables and adverse effects with others, as observed in the case of total soluble solids, there is an indication to be extra careful, because, when selecting this character, it may provoke undesirable changes in other quantitative characters of interest.

Nevertheless, the literature has many studies showing that generally, with increasing productivity, there is a tendency in fruit trees to reduce fruit quality because it is easy to obtain an increase in carbohydrates than other elements due to lower energy expenditure (De Almeida et al., 2018; Taiz & Zeiger, 2017). The other characters did not present direct and indirect effects with interesting magnitudes, indicating that, in a physalis breeding process, the selection should occur by character, independent of the other characters. This fact of independence between the characters is essential for the breeder, as it is possible to obtain simultaneous genetic gains on important productive characters or obtain an increase for a given character without altering the others.

Although authors determined the importance of the analysis to accelerate and facilitate the selection of genotypes through the morphological characteristics of the evaluated fruits, other characteristics must also be analyzed, such as fruit dimensions, seed number per fruit, the thickness of the fruit peel which are defining characteristics for the primary variable (total productivity) (García-Arias et al., 2018). This need is due to the existing possibility of several unknown indirect effects that mask the effect of essential characteristics in the expression of the primary variable.

Conclusions

Physalis mutant populations of show variability for qualitative and not for quantitative components.

The number of fruits presented the most significant importance as the primary variable, while the number of flowers presented negative results. Crosses between contrasting populations, followed by selection, can help the breeders' selection of genotypes with more balanced flavor fruits with higher acidity and total solids values.

Acknowledgments:

The authors wish to thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil), the National Council for Scientific and Technological Development (CNPq, Brazil), the Santa Catarina University Scholarship Program by the Production and Development Support Fund da Higher Education (UNIEDU/FUMDES) for granting the scholarships. We are also grateful for the financial resources received from the Research and Innovation Support Foundation of Santa Catarina State (FAPESC).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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