GROWTH VARIATION IN REPRODUCTIVE STRUCTURES OF PHYSALIS POPULATIONS

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SUMMARY

Physalis peruviana *L., a species native to the Andean region, is well-adapted to the soil and climatic conditions of Brazil, but studies on the genetic behavior of the species in Brazil are scarce. The purpose of this study was to characterize the phenotypic variation in traits of physalis populations related to the phenological cycle of the crop. The experiment consisted of six physalis populations, arranged in a randomized block design with two replications. The traits: number of flower buds, of flowers and of fruits were assessed 36, 43, 50, 57, 64 and 71 days after planting the seedlings in the field. Analysis of variance showed a significant effect of the population×time interaction, at 5% error probability. Regression analysis (P<0.05) showed that flower bud and flower formation was lower in the population of Vacaria, but fruiting was similar to the populations from Lages and Colombia, with a production of 13 fruits/plant. The population from Fraiburgo produced a higher number of fruits (16 fruits/plant), but the negligible divergence between this and other populations hampers the selection processes for the fruit yield trait. The results show limited variation for traits related to crop cycle, impairing the early selection of populations for flower bud formation. In this sense, techniques to expand and/or generate genetic variability are imperative for genetic progress.*

Introduction

The fruit species *Physalis peruviana* L., commonly known as physalis, belongs to the Solanaceae family, native to the Andean region. The species occurs mostly in tropical and subtropical regions, and can have an annual or perennial behavior, depending on the prevailing regional temperature conditions (Fischer and Melgarejo, 2014). The genus name *Physalis* is derived from the Greek 'Physa' (bladder or bulb), for having a fruit wrapped in a characteristic capsule. The flowers are hermaphroditic, with a yellow tubular corolla, which facilitates pollination by insects and wind (National Research Council, 1989).

Physalis is a production alternative for the economy of various countries in view of the nutritional traits and medicinal properties of the fruit (Briones-Labarca *et al.*, 2013). It is characterized by a sugary fruit, with an appealing visual appearance and considerable vitamin A and C contents (Barcia *et al.*, 2010). In Brazil, it is consumed as exotic fruit, sold at high prices ranging from 50 to 70 R$/kg (Lima *et al.*, 2009). Its high production cost is related to management factors, e.g., high labor requirement at harvest (Rodrigues *et al.*, 2013). However, another limiting factor for production is the incomplete understanding of the genetic behavior of this crop under conditions present

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VARIACIÓN EN EL CRECIMIENTO DE ESTRUCTURAS REPRODUCTIVAS EN POBLACIONES DE PHYSALIS
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RESUMEN

Physalis peruviana *L., especie originaria de la región andina, ha mostrado buena adaptación a las condiciones de cultivo brasileñas, pero los estudios sobre el comportamiento genético de la especie en las condiciones brasileras son incipientes. El objetivo del estudio fue caracterizar la variación fenotípica en poblaciones de physalis y caracteres relacionados con el ciclo fenológico del cultivo. El experimento consistió en seis poblaciones de physalis, realizado con un diseño de bloques al azar, con dos repeticiones. Los rasgos medidos fueron: número de botones florales, número de flores y de frutos, cuantificados a los 36, 43, 50, 57, 64 y 71 días después de la siembra las plántulas en el campo. El análisis de varianza mostró un efecto significativo para la interacción población×tiempo, al 5% de probabilidad. A través de los resultados del análisis de regresión (P<0,05), la población de Vacaria mostró menor aparición de brotes de flores y flores, pero fructificación fue similar a Lages y Colombia, con una producción de 13 frutos/planta. La población de Fraiburgo mostró mayor número de frutos (16 frutos/planta), pero la pequeña diferencia de esta población complica el proceso de selección para la producción de fruta. Los resultados muestran una variación limitada de rasgos relacionados con el ciclo del cultivo, no permitiendo seleccionar poblaciones con el ciclo temprano de la aparición de botones florales. En este sentido, la adopción de técnicas de expansión y/o creación de variabilidad genética son esenciales para obtener progreso genético.*

VARIAÇÃO NO CRESCIMENTO DE ESTRUTURAS REPRODUTIVAS EM POPULAÇÕES DE PHYSALIS
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RESUMO

A Physalis peruviana *L., originária da região Andina, apresentou boa adaptação às condições de cultivo brasileiras, porém os estudos sobre o comportamento genético da espécie em regiões brasileiras são incipientes. O objetivo do trabalho foi caracterizar a variação fenotípica em populações de physalis quanto a caracteres relacionados ao ciclo fenológico da cultura. O experimento consistiu de seis populações de physalis, conduzido em blocos ao acaso, com duas repetições. Os caracteres mensurados foram número de botões florais, número de flores e número de frutos, quantificados aos 36, 43, 50, 57, 64 e 71 dias após o plantio das mudas no campo. A análise de variância evidenciou efeito significativo para a interação população×época, a 5% de probabilidade de erro. Por meio dos resultados da análise de regressão (P<0,05), a população de Vacaria apresentou menor emergência de botões florais e flores, contudo a frutificação foi semelhante a Lages e a Colômbia, com a produção de 13 frutos/planta. A população de Fraiburgo demonstrou maior número de frutos (16 frutos/planta), porém a minuciosa divergência desta população em relação às demais populações dificulta os processos de seleção para o caráter produção de frutos. Os resultados apontam restrita variação para os caracteres relacionados ao ciclo da cultura, não possibilitando a seleção de populações precoces para a emergência de botões florais. Nesse sentido, a adoção de técnicas para ampliação e/ou criação de variabilidade genética são imprescindíveis para a obtenção de progresso genético.*

in Brazil, so that there are no officially registered commercial physalis varieties in Brazil (MAPA, 2015).

In the breeding of fruit trees, to characterize the season, number of buds and of flowers, and fruit set are critical data to identify populations with promising traits (Bernal-Parra *et al*., 2014), which allow for the exploitation of the phenological cycle and fruit yield. Determining the growth of reproductive structures enables the management of fruit supply according to the season and the adaptation of production technologies available in the region (Antunes *et al*., 2008). The use of cultivars with genetic variability in the trait production peak (early or late) contributes to the uninterrupted supply of the fruit and, consequently, increases sales and thus the farmers' income (Segantini *et al*., 2014).

In this sense, the morphological description of the population is the first step towards selection of superior parents (Singh *et al*., 2014). Breeding will only be successful in a selection program if the genetic variability in the traits of interest is high. Variations in genetic make-up between different populations can contribute to the formation of a genotypic constitution of physalis adapted to the particular soil and climatic conditions of regions with low winter temperatures.

The objective of this study was to characterize the phenotypic variation in physalis populations with regard to the traits related to crop cycles.

Materials and Methods

The study was conducted in an experimental field of the Institute of Breeding and Molecular Genetics (IMEGEM), Universidade Federal de Santa Catarina, in the municipality of Lages, state of Santa Catarina, Brazil (27º48'S, 50º19'W; mean elevation of 930masl). The regional climate is *Cfb* (temperate climate with cool summers), with annual means of 14.3°C and 1479.4mm of rainfall (Cardoso *et al*., 2003).

Data of an experiment consisting of six physalis populations were used. The populations were from the municipalities of Lages, Caçador and Fraiburgo, in the state of Santa Catarina, Brazil; from the municipality of Vacaria, Rio Grande do Sul, Brazil; and from local markets in Colombia and Peru. The fruits were randomly selected from plants of each population, the seeds were removed from the ripe fruits (Icontec, 1999), sown in trays and maintained in a greenhouse. After 47 days in the trays, i.e., when the seedlings had reached 10-15cm in height, they were planted in the field.

The experiment was arranged in a randomized block design with two replications and the experimental units consisted of 25 plants spaced 1m in rows

2m apart. Six plants of the evaluated area were assessed (counting of reproductive structures), repeatedly over time. The traits number of flower buds (NFB), number of flowers (NFL) and number of fruits (NFR) were assessed. The evaluation period for trait NFB was 36 to 71 days after field planting (dap) of the seedlings. For trait NFL, the counting was fron 43 to 71 dap. The number of fruits (NFR) was counted 50 to 71 dap.

The statistical model used was based on an experimental design with repeated measurements over time, as

$$Y_{ijk} = \mu + b_j + \alpha_i + \gamma_k + (\alpha\gamma)_{ik} + e_{ijk,}$$

where Y_{ijk}: observed value of the response variable in the j^{-th} block of the i^{-th} population in the k^{-th} estimated time, μ: observed overall mean, b_j: effect of block j, α_i: effect of population I, γ_k: effect of period k, $(\alpha\gamma)_{ik}$: effect of interaction between population i at time k, and e_{ijk}: random error associated with the observations of the k^{-th} time for the i^{-th} population in the j^{-th} block. The hypothesis of correlated error is a result of the continuous assessment of the same plants throughout the evaluation periods, in which observations closer in time tend to be more highly correlated than more distant observations (Gurevitch and Chester, 1996).

The data were structured by adjusting the matrices of variances and residual covariances, where the best structure was chosen based on Akaike's AIC criterion. The values are obtained by the equation AIC= -2logL+2d, where L is the maximum restricted likelihood value and d is the number of covariance parameters. For the analyses we used software SAS 9.2 and the procedure PROC MIXED (SAS, 2002). Although the statistical linear model used considers effects as fixed (people and time), the errors are random and correlated, from data with measures repeated in time (Littel *et al.*, 2006), which justifies the use of PROC MIXED. Univariate analysis of variance and regression analyses were performed for each trait, at 5% error probability. For these analyses the general linear model procedure (GLM) of SAS 9.2 was used.

Results and Discussion

From the matrices of variances and residual covariances (Table I) the following three were selected: unstructured (UN) for number of flower buds; heterogeneous compound symmetry (CSH) for number of flowers, and first-order antedeendence (ANTE (1)) for number of fruits. The choice was based on the lower values established by the Akaike information criterion than in the other matrices. The modeling of the matrices to obtain appropriate variance and covariance structures is essential to ensure the reliability of conclusions about treatment means (Xavier and Dias, 2001). This procedure satisfies an important assumption of the statistical model, so that errors are mutually independent (Silva, 2003).

TABLE I
VALUES MEASURED BY THE VARIANCE AND COVARIANCE MATRICES (MVC) BY AKAIKE'S INFORMATION CRITERION (AIC), CONSIDERING THE TRAITS NUMBER OF FLOWER BUDS, NUMBER OF FLOWERS AND NUMBER OF FRUITS

MVC	AIC		
	NFB	NFL	NFR
UN	2266	1593	1198
UN(1)	2391	1608	1210
AR(1)	2759	2524	1339
ARH(1)	2293		1200
TOEP	2711	2530	1341
TOEP(2)	2763	2524	1340
TOEPH	2283	1594	1203
CS	2749	2525	1337
CSH	2314	1590	1201
VC	2773	2527	1340
ARMA(1,1)	2743	2526	1337
ANTE(1)	2285	-	1193
HF	2602	2286	1221
FA(1)	2277	-	1195

UN: unstructured matrix, UN(1): banded unstructured, AR(1): first-order autoregressive, ARH(1): first-order heterogeneous autoregressive, TOEP: Toeplitz, TOEP(2): banded Toeplitz, TOEPH: heterogeneous Toeplitz, CS: compound symmetry, CSH: heterogeneous compound symmetry, VC: component of variance, ARMA(1,1): first-order autoregressive of mobile means, ANTE(1): first-order antedependence, HF: Huynh-Feldt, FA(1): first-order factor analytic model.

The selected matrices clearly showed a random behavior of the correlation of errors throughout the evaluation periods; for example, a feature of the UN matrix is to express unequal variances and covariances. The variances and residual covariances were significant (5% error probability by Student's t test) in relation to the assessed traits (NFB, NFL, NFR). In addition to the variations related to the (uncontrolled) environmental effect, the variation of genetic order among and within populations (reproduction by cross-pollination) may have contributed to these results. The degree of homogeneity of variance and covariance at the different evaluation times must be modeled (Fortin *et al.*, 2007). The data are corrected by structuring the most appropriate matrix of variance and covariance for the experiment.

Analysis of variance revealed a significant effect of the factor population and the factor population×time interaction, at 5% error probability (Table II). The result indicated that the growth pattern of reproductive structures of the physalis populations was not the same throughout the study period. The genetic variability between populations is a promising genetic resource to be exploited. A plant breeder must be able to select, based on the phenotype, alleles with traits of agronomic interest (Herrera *et al.*, 2012), e.g., fruit yield in relation to the soil and weather conditions of the cultivation regions.

TABLE II
ANALYSIS OF VARIANCE FOR FIXED EFFECTS AMONG THE TRAITS NUMBER OF FLOWER BUDS, NUMBER OF FLOWERS AND NUMBER OF FRUITS, OF SIX PHYSALIS POPULATIONS

Traits	SV	NDF	DDF	F value
NFB	Block	1	65	1.81
	Population	5	65	3.68*
	Period	5	65	1000.65*
	Population×period	25	65	4.57*
NFL	Block	1	65	0.01
	Population	5	65	2.09ns
	Period	4	264	357.75*
	Population×period	20	264	2.32*
NFR	Block	1	65	0.09
	Population	5	65	1.44ns
	Period	3	198	798.03*
	Population×period	15	198	2.81*

* Significant at 5% probability by the F test, ns: not significant, SV: sources of variation, NDF: number of degrees of freedom of the numerator, DDF: number of degrees of freedom of the denominator.

Regression analysis performed for each population indicated a significant association between the growth of flower buds over the study period (Figure 1). There is an increasing and significant quadratic relationship by the F test ($p<0.05$), with an insignificant intercept, linear regression coefficient and significant quadratic regression coefficient for the six populations. The negative sign of the quadratic regression coefficient showed an intense production of flower buds in a certain evaluation period and subsequent decrease in the growth of these structures. The bud formation speed depends strongly on temperature (Wanjura and Buxton, 1972), but the decrease observed in bud production is due to the phenological cycle of the crop, with the transition of the floral bud to open flower stage.

When assessing the maximum bud formation, it was found that the population of Vacaria produced a lower number of these structures than the other populations (Figure 1). This population reached the production peak at 57 days after planting the seedlings in the field, with 31 buds per

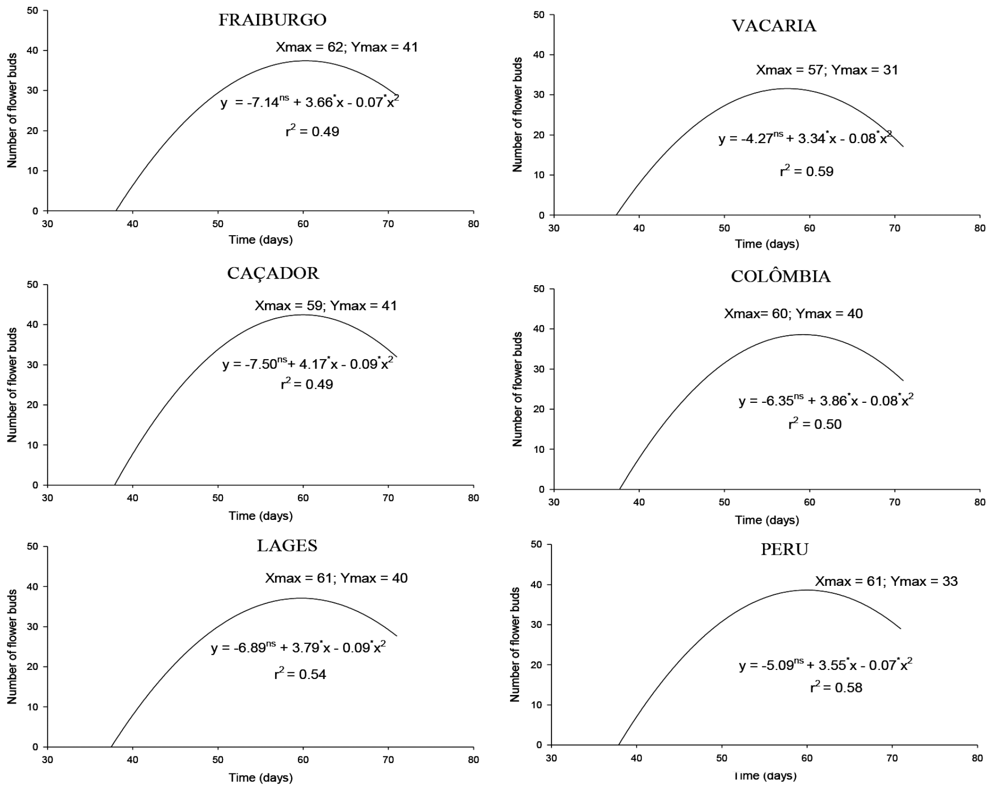

Figure 1. Diagram of estimates of second-degree polynomial equations for the trait number of buds and coefficient of determination (r^2) among the six physalis populations. Xmax: day of highest bud production. Ymax: highest number of formed buds.
*: significant at 5% probability by the F test, ns: not significant.

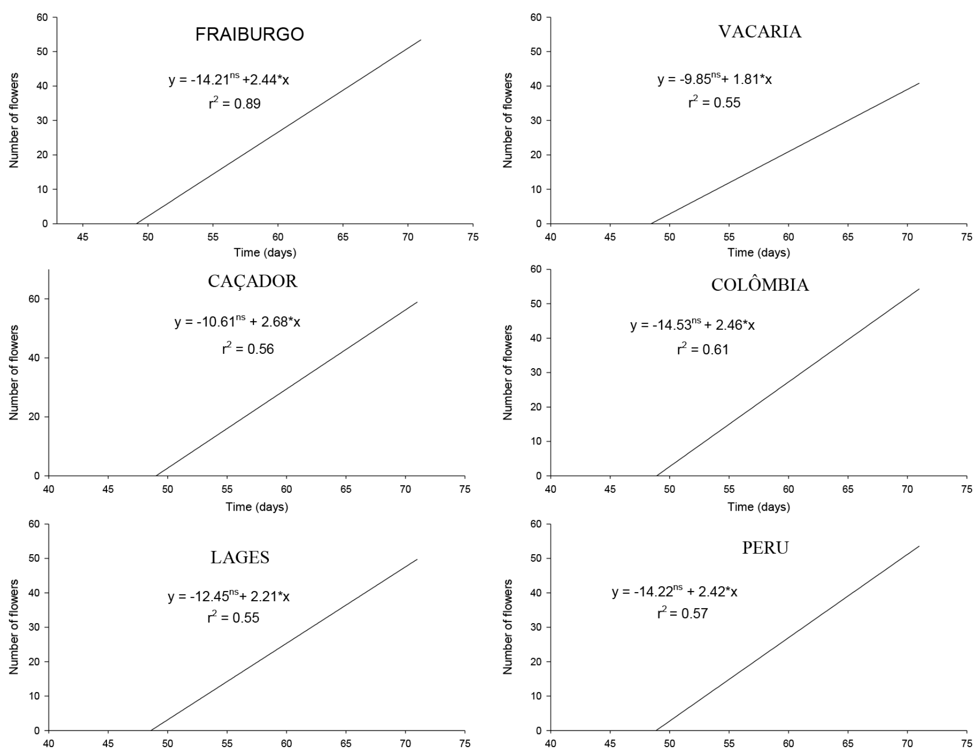

Figure 2. Diagram of estimates of first-degree polynomial equations for the trait number of buds and coefficient of determination (r^2) in six physalis populations
*: significant at 5% probability by the F test, ns: not significant.

plant. On the other hand, Fraiburgo reached peak production at 62 dap, with 41 buds. The population of Caçador produced the same amount of buds (41 buds), 59 dap.

In view of the regional climate conditions, with frosts at certain times of the year, particularly on the high plateau of Santa Catarina, early flower bud formation may be a promising trait in the selection of populations. The period for physalis cultivation is restricted in the regions with harsh winters in the Santa Catarina state (mainly Lages, Urupema, Fraiburgo), with production concentration in the warmer months of the year. Frosts lead to premature plant death, and the effect is harmful, especially when flower buds are affected (Borges *et al*., 2012). The population of Vacaria showed early bud formation; however, it expressed a lower bud production (number of buds) per plant than the other populations. For a profitable agricultural system, the earliness of the crop cycle must be directly linked to productivity (Guedes *et al*., 2013).

For flower formation, the beginning of the evaluations (43 days after planting the seedlings in the field) was marked by a high number of buds formed and small number of open flowers, with intense flowering in the subsequent periods (Figure 2). For flower formation, linear regressions were established to further explore the behavior. After 71 dap, the population of Caçador had produced 64 flowers per plant. The population of Vacaria obtained lower values again, with a production of 41 flowers in the last evaluation period. The other populations (Lages, Fraiburgo, Colombia, Peru) performed similarly.

Fruit formation is characterized by a quadratic response in the populations from Fraiburgo, Caçador and Peru, and linear for those from Vacaria, Lages and Colombia. The population of Fraiburgo formed up to 16 fruits per plant, unlike Peru, which produced 11 fruits per plant, 71 days after seedling

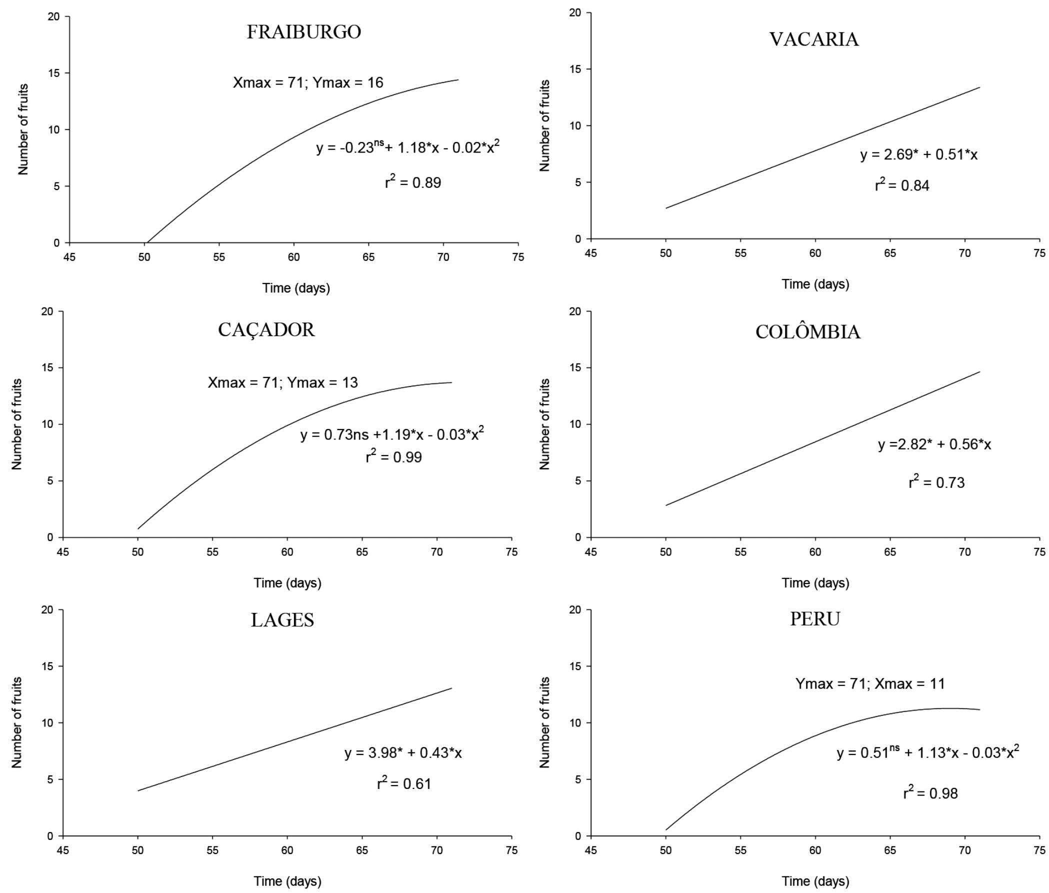

Figure 3. Diagram of estimates of first and second-degree polynomial equations for the trait number of fruits (NFR) and coefficient of determination (r^2) in six physalis populations. Xmax: day of highest fruit production. Ymax: highest number of produced fruit.

planting. Populations of Vacaria, Caçador, Colombia and Lages produced 13 fruits per plant. In comparison, the population of Vacaria, which had previously performed worse in terms of bud and flower formation and the population of Caçador, with higher production of buds and flowers, did not show this growth pattern in fruit formation. Therefore, the populations of Vacaria and Caçador, which differed in the other traits, performed similarly for fruit formation.

In fact, the commercial product is the fruit, which is the main criterion in the selection of promising genotypes (Fachinello *et al*., 2011). However, other criteria, such as bud and flower formation, cannot be disregarded in selection programs of genotypes. These traits are fundamental in the study of genotype behavior under the soil and climatic conditions of a given region (Rodrigues *et al*., 2006) and to establish official recommendations of management techniques for cultivation of physalis.

In selection for fruit production, the population of Fraiburgo appears promising. This population produced 16 fruits per plant at its peak in the evaluated period. However, taking into consideration that a physalis plant produces between 60 to 70 fruits per plant in the production cycle (Rodrigues *et al*., 2013), the differences between populations observed in this study can be considered negligible. In addition, the population of Fraiburgo was the only one with divergent behavior for this trait.

It is noteworthy that one of the most critical points in the beginning of a breeding program is the choice of the plants to be used directly or as parents in crossings, requiring a broad genetic base (Allard, 1971). Breeding usually leads to a narrowing of the genetic variability, resulting in selection gain. However, the genetic base must initially be broad to ensure an effective selection process (Ceccarelli, 2015). The limited variation in agronomic traits becomes a major constraint to breeding of *Physalis peruviana* L., which is a concerning factor for the cultivation of a species with a strong upward trend. The restriction of the genetic basis hampers and even impairs the development of new varieties, aside from accelerating genetic erosion due to the use of a small number of distinct genotypes in artificial hybridizations (Stebbins, 1971).

In Brazil, there are no improved and recorded physalis varieties. Farmers often initiate cultivation with seeds purchased commercially without knowledge about the genetic potential they offer. Thus, physalis populations grown by local producers in southern Brazil may originate from a single location. Still, it is possible to believe that the selection pressure (natural and artificial) occurred similarly in the genetic make-up of individuals of such populations. Another important aspect is the reproduction mode of the species and, in this regard, several scientific papers emphasize cross-pollination (Fischer *et al*., 2014). Allogamous populations are characterized by high genetic heterozygosity and heterogeneity between plants (Souza, 2011). However, crosses between plants with a high degree of parentage may be responsible for our results.

Therefore, some strategies should be adopted in physalis breeding programs. In cases where the genetic variability is limited, artificial hybridization and mutation induction are strategies used in plant breeding to broaden the genetic variability (Fehr, 1987; Shu, 2009). Also, the introduction of accessions derived from broad-based germplasm appears to be a source of variation available to the breeder, ready to be exploited (Bonilla and Espinosa, 2003).

CONCLUSION

Physalis populations differ in the growth pattern of reproductive structures, but the limited variability among the six physalis populations for the assessed traits is evident. Thus, the adoption of strategies that increase the genetic variability by the introduction of new accessions or through induced mutation technique is required.

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