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Biometric characteristics of fruits and physiological characterization of seeds of *Physalis* species (Solanaceae)

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ABSTRACT

The *Physalis* genus includes species with nutraceutical uses and is typically identified by morphological characteristics. In Brazil and the world, the best-known species are *P. angulata*, *P. peruviana* and *P. ixocarpa* emerging more in the production of fruit used for various purposes. In this scenario, the physicochemical characteristics of mature fruits and seeds, are extremely important, especially for commercial purposes as well as the separation and identification of promising genotypes. Biometric analysis of ripe fruit, measuring the ºBrix, seed analyses were performed and compared four potential species *Physalis* grown in Feira de Santana, Bahia, Brazil. Principal component analysis showed two of the ten analyzed characteristics that represent about 98% of the total variance observed between species, namely: °Brix and E15 (Emergency in 15 days after sowing). *P. ixocarpa* fruit stood out in relation to other species, reaching the highest average for the biometric data.

Key words: ºBrix; emergency; *Physalis ixocarpa*; *P. peruviana*; *P. angulata*

Características biométricas de frutos e caracterização fisiológica de sementes de espécies de Physalis (Solanaceae)

RESUMO

O gênero *Physalis* inclui espécies com usos nutracêuticos e são identificadas basicamente por caracteristicas morfológicas. No Brasil e no mundo as espécies mais conhecidas são *P. angulata*, *P. ixocarpa* e *P. peruviana* despontando mais na produção de frutos utilizados para diversos fins. Neste cenário, as características físico-químicas dos frutos maduros e sementes são extremamente importantes, especialmente para fins comerciais bem como na separação e identificação de genótipos promissores. Análises biométricas de frutos maduros, a medição do ºBrix, análises de sementes foram realizados e comparados entre quatro espécies potenciais de *Physalis*, cultivadas em Feira de Santana, Bahia, Brasil. Análises de componentes principais mostraram duas das dez características analisadas que representam cerca de 98% da variância total observada entre as espécies, a saber: °Brix e E15 (Emergência em 15 dias após a semeadura). Frutos de *P. ixocarpa* se destacaram em relação às outras espécies, atingindo as maiores médias para os dados biométricos.

Palavras-chave: ºBrix; emergência; *Physalis ixocarpa*; *P. peruviana*; *P. angulata*

Introduction

The *Physalis* genus includes around 100 species and they are recognized in the family Solanaceae due to the presence permanent and inflated calyx in the fruit it can become yellow or orange when ripe (Whitson & Manos 2005; Silva et al. 2016a) and contain a large number of seeds, considered the main means of propagation. The seeds show high viability (Rufato et al. 2008; Souza 2015) and can be conserved for many years in appropriated conditions of the low humidity and temperature control (Souza et al., 2014).

Many *Physalis* species are native to the Americas and are distributed from the United States to South America, including the Antillean islands (Vargas-Ponce et al., 2011). The taxonomy of the genus can be confusing, and many species are not easily distinguishable, even by specialists, and many have been wrongly identified (Kindscher et al., 2012). Morphological characters are commonly used to identify *Physalis* species (Vargas-Ponce et al., 2011), but the in-depth knowledge concerning the interspecific genus differences, mainly by the inclusion and assessment of parameters productive and reproductive, can be another tool for their differentiation.

Although botanically this genus has been difficult to identify, floral and fruit characteristics have contributed to establish this group and different species or even subgroups of plants. Regarding the development of the plants, the physicochemical characteristics of mature fruits and biometric data of fruits and seeds, are extremely important, especially for commercial purposes as well as the separation and identification of promising genotypes. Since the environment, as well as the genotype x environment interaction, can interfere with the relative performance of genotypes (Borém & Miranda, 2013) is hypothesized that the *Physalis* development cycle, the biometric and physicochemical characteristics of fruits undergo alteration due to crop region, water regime, temperature, spectral light and other biotic factors.

Planting density and spectral light quality studies have been carried out to evaluate the potential and the success of cultivation in Brazilian subtropical areas with *P. peruviana* (Moura et al., 2016a, 2016b; Silva et al., 2016a, 2016b). The quality of fruits produced in open area systems and dense plantations resulted in better quality fresh weight, anthocyanins, vitamin C, soluble solids, and sugars such as sucrose and glucose (Moura et al., 2016a).

Due the nutraceutical and ornamental characteristics of the fruits and the medicinal potential of parts of the plants make this crop commercially exploited (Rufato et al., 2008), such as *P. peruviana*, which has been considered the most economically important in subtropical regions (Silva et al., 2016a), and their growing conditions and productivity are being investigated by several research groups in Brazil. Still little known and used in Brazil, the *P. ixocarpa* species shows considerable agricultural importance due to the quality and diversity of the fruits produced, being enough consumed in Mexico, considered the great distribution center.

Give the variability between species and the importance of this genus as food and medicinal source need to identify parameters of intraspecific differentiation. The aim of this study was to compare the performance of the four species potential of *Physalis* through quantitative characteristics concerning the fruit and assess the germinative capacity of the seeds of these species.

Methods

The species evaluated in this study were *Physalis peruviana*, *P. angulata*, *P. ixocarpa* and *P. pubescens*. The seeds were sown in April 2013, in plastic containers of 200 ml filled with Plantmax® substrate and sifted topsoil (ratio 1:3), and kept in a greenhouse for 45 days. After this period, the seedlings were transplanted to the field in the Experimental Station Arboretum of the State University of Feira de Santana (UEFS), Feira de Santana, Bahia, Brazil, where they remained until the analyzes start. Only *P. peruviana* was transplanted to plastic pots with a capacity of 5 kg, filled with the same previous substrate remaining in the greenhouse throughout the experimental period. For each species, 50 fruits collected from 15 plants were used.

Fruits were harvested when ripe, based on the standard fruit color for each species and taken to the UEFS Germination Laboratory to perform the biometric analysis. The color varies from yellow-orange (*P. peruviana*) to green (*P. ixocarpa*). As the ripening of fruits proved to be gradual and different in each species, the difference between the first and last collection took on average 30 days. In the laboratory, fruits were separated from calyces and washed in running water. For each fruit, the following measurements were taken: weight (g), length and diameter (mm), determination of total soluble solids (expressed in Brix), number and weight of seeds per fruit. To obtain these measurements, the following equipment were used: digital scale, digital caliper and manual refractometer. To perform the germination test, moisture content and emergency, seeds were grouped by species, forming a pool, and selected regarding the presence of embryonic material using a stereomicroscope. The seeds were washed and left to dry in an incubator saturated with a calcium chloride solution at 20 °C for 24 hours until moisture equilibrium and then stored in the refrigerator until the time of the experiments.

For the analysis of germination, the seeds of each species were separated into four replicates of 25 seeds and placed into Petri dishes (5.5 cm diameter) containing two sheets of germitest paper moistened with distilled water, equivalent to 2.5 times the weight of the substrate. These were placed in a growth chamber with a photoperiod of 12/12 hours and alternating temperatures of 20/30 °C. The assessments were daily performed, considering as germinated those seeds protruding the radicle (1 mm). The moisture content of the seeds was measured by the method described by Brasil (2009), using an oven at 105 °C for 24 hours, with four replicates of 100 seeds. For the analysis of emergency, seeds were sown in polystyrene trays, kept in a greenhouse, containing Plantmax® and sifted topsoil (ratio 1:3) as a substrate, using four replications of 25 seeds. Emergency analyses were performed considering the appearance of the cotyledons above the soil line at 5 (E5), 10 (E10) and 15 (E15) days after sowing (DAS). Of all data collected arithmetic mean was taken and dissimilarity analysis and principal component using the statistical programs Genes (Cruz, 1998) and R (R Development Core Team, 2012) were performed. The parameters germinability (G%), average time (Ta), average speed (Sa), germination speed index (GSI) and germination uniformity coefficient (GUC) were analyzed as normality and homogeneity, being transformed with arcsine when necessary, and analyzed by Tukey test at 0.05 probability at Sisvar (Ferreira, 2011).

Results and Discussion

Principal component analysis showed two of ten analyzed characteristics accounting for about 98% of the total variance observed among the species, namely: °Brix and E15 (Table 1). Ligarreto et al. (2005) working with the principal component analysis in *P. peruviana* and *P. philadelphica* noted that there is a significant dispersion separating these species, demonstrated in agronomic and morphological differences. According to the authors, these are due possibly to the fact that species have evolved in different habitats, separated by barriers that have prevented their interaction. Thus the difference found between species can also be due to the way of dispersion thereof.

The results of the cluster analysis allowed separating the species into two distinct groups (Figure 1), based on visual analysis and setting the cut-off point from the abrupt change of the dendrogram branches. Group 1 is formed only by the species *P. ixocarpa*, while group 2 is formed by remaining species (*P. angulata*, *P. pubescens,* and *P. peruviana*). *P. ixocarpa* stands out and differs from other species tested in most quantitative traits assessed in this experiment. According to Trillos Gonzalez et al. (2008), working with a collection of *P. peruviana* in Antioquia (Colombia), the selection of progenitors should be made based not only on qualitative morphological characters but also quantitative, taking into account specific demands of breeders, farmers, and consumers. The assessment of these characteristics should be performed in various production systems as the quantitative attributes can display great interaction with the environment, which will reflect the quality of the final product (Trillos Gonzalez et al., 2008).

Biometric data of fruit showed that *P. ixocarpa* stood out in relation to the other species, reaching the highest averages for the parameters length and diameter of fruits, 33.6 and 41.1 mm respectively (Figure 2A and 2B). These data are in agreement with those obtained by Rodríguez-Burgos (2010) who worked with five varieties of *P. ixocarpa*, obtaining an average of 32.4 mm long and 38.4 mm in diameter in fruits. According to Ponce Valerio et al. (2012), agricultural dynamics of *P. ixocarpa*, especially in Mexico, demands improved cultivars that fit the requirements of national and international markets.

Among the improved characteristics desired are the growth habit, income, color, shape and size of the fruit. *Physalis peruviana* had an average length of 14.0 mm and diameter of 13.4 mm and is considered a nearly round berry. For *P. angulata* and *P. pubescens*, which had 13.9 mm in diameter, that value was close to the data reported by Ligarreto et. al (2005), which reported that the fruits of both species have an average diameter of 13 mm.

The fruit weight followed the same pattern obtained from biometrics of fruits (Figure 2C), especially *P. ixocarpa*, which reached an average of 34.5 g. This value is also in agreement with Rodríguez-Burgos (2010), which had an average of 30.5 g for the tested varieties. Angulo (2005), working with seedlings of *P. peruviana* in two different conditions, greenhouse and open field, noted that the weight of the fruit of this species varies

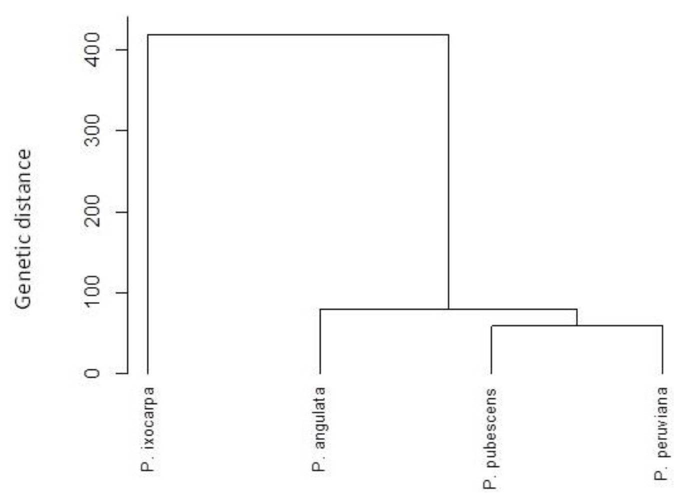

Figure 1. Dendrogram generated from an average Euclidean distance among four species of *Physalis*. Feira de Santana, Bahia, Brazil.

Table 1. Estimation of cumulative percentage variance (λ%), explained by the principal components (CPi) and the sets of eigenvectors associated with each principal component expressing the relative importance of 10 quantitative traits in 4 species of *Physalis*. Feira de Santana, Bahia, Brazil.

CPi	Variance λi (%)	Set of associated eigenvectors									
		FL	FD	FW	NSF	WSF	Brix	MC	E5	E10	E15
CP1	71.4223	0.358	0.361	0.361	0.354	0.361	-0.364	0.183	0.360	0.212	0.13
CP2	98.1730	-0.180	-0.159	-0.162	0.061	-0.163	-0.010	0.527	-0.169	0.500	0.57
CP3	99.9999	-0.063	-0.019	-0.004	0.717	-0.015	0.547	0.306	-0.006	-0.245	-0.17
CP4	99.9999	-0.132	0.114	0.091	-0.317	-0.047	-0.270	0.735	-0.039	-0.337	-0.36
CP5	99.9999	0.617	0.532	-0.398	-0.129	-0.233	0.210	0.087	-0.234	0.012	0.02
CP6	99.9999	0.002	0.006	-0.002	-0.457	0.491	0.596	0.159	0.392	0.082	0.10
CP7	99.9999	-0.029	-0.029	-0.029	0.020	-0.029	0.068	0.023	-0.029	0.718	-0.69
CP8	99.9999	0.017	-0.017	0.003	-0.036	-0.676	0.040	0.011	0.734	0.011	0.01
CP9	99.9999	-0.294	0.152	-0.790	0.166	0.273	-0.268	-0.027	0.290	-0.053	-0.06
CP10	100.0000	0.592	-0.723	-0.227	0.031	0.116	-0.137	0.142	0.086	-0.081	-0.09

FL: fruit length, FD: fruit diameter, FW: fruit weight, NSF: number of seeds/fruit, WSF: Weight of seeds/fruit, Brix: Brix degrees, MC: Moisture content, E5, E10, and E15: Emergency at 5, 10 and 15 days after sowing, respectively.

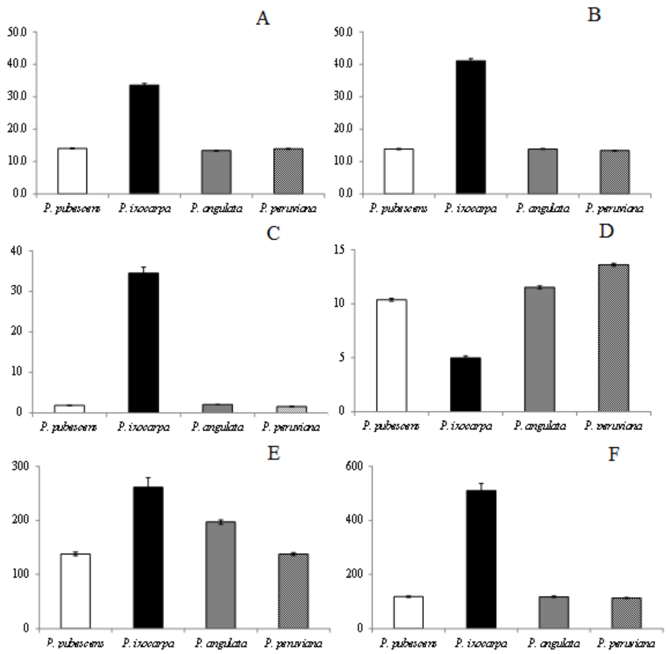

Figure 2. Length (A), diameter (B), weight of fruits (C), °Brix (D), number (E) and weight of seeds (F) of the fruits of six different species of *Physalis* grown in the Experimental Station Arboretum of the State University of Feira de Santana (UEFS), Feira de Santana, Bahia, 2014.

with climate, however, without suffering major influences if grown in a greenhouse or open field, being only slightly higher for the latter. The author reported values of 5.7 g and 5.4 g for open field and greenhouse, respectively, for seedlings planted in Colombia, higher than 1.6 g found in this study, in semi-arid conditions of Feira de Santana - Bahia, Brazil.

The measurement in Brix degree is the amount in grams of soluble solids existing in 100 ml of solution. In general, it represents a measure of the concentration of sugars and other solids diluted in juice or pulp of the fruit and is considered an indicator of quality since it provides data on the organoleptic characteristics of fruits (Silva et al., 2002; Leão et. al., 2006). The highest value for total soluble solids was observed in ripe fruits of *P. peruviana*, with 13.6 °Brix (Figure 2D). This value is according to the value found by Galvis et al. (2005), in which the ripe uchuva fruit (*P. peruviana*) showed between 13.0 and 15.0 °Brix. Also according to these authors, the fruits of this species, when unripe, show high levels of starch, which is hydrolyzed during the ripening process, increasing the soluble solids content. Most of the sugar consists of sucrose, and its content is up to 2.5 times higher than the glucose and fructose (Galvis et. al, 2005).

Both *P. angulata* as *P. pubescens* showed values close to those obtained for *P. peruviana*, with respectively 11.5 and 10.4 °Brix (Figure 2D). The average soluble solids content found by Oliveira et al. (2011) for *P. angulata* was 12 °Brix, corroborating the value discovered in this work. Ripe fruits of *P. ixocarpa*, contrary to other parameters assessed, presented the lowest value among all species tested (4.98 °Brix), being the only species to present a result below 5.0 °Brix. As most of these species are used for consumption, often *in natura*, this parameter has high relevance for marketing.

All species showed a high number of seeds per fruit (Figure 2E) and the species with lower values were *P. pubescens* (138) and *P. peruviana* (138). *P. angulata*, despite having lower values for biometrics and fruit weight, presented an average of 197 seeds/fruit. For *P. peruviana* the obtained value (138) is below 241 seeds/fruit, the number found by Lagos et al. (2008). However, the same authors, working under greenhouse conditions obtained a maximum number of 123 seeds/fruit.

P. ixocarpa showed the highest values for both traits, noting that in addition to producing the highest number of seeds per fruit (261), this species presented the largest seeds, which was reflected in seed weight/fruit (510.35 mg) (Figure 2E). Rodríguez-Burgos (2010) found similar results for two varieties of *P. ixocarpa* (queretaro and mahone) with 0.555 g and 0.515 g, respectively. Although *P. angulata* is among the species with a higher number of seeds, it showed values similar to *P. pubescens* and *P. peruviana* regarding seed weight/fruit with 117.4 mg, 117.7 mg, and 112.9 mg, respectively. This can be explained by the small size of the seeds of this species, one of the lowest observed as noted by Souza et al. (2010).

The moisture content of seeds showed that all species tested presented contents ranging from 5.3% (*P. peruviana*) to 7.6% (*P. angulata*), suggesting that these may be classified as orthodox seeds. However, studies related to the conservation must be developed to test the period and the best way those seeds should be stored. As the protocol for seed conservation is species-specific, it must be developed for each species separately. Experiments were conducted aiming the storage in different environments with seeds of *P. angulata* (Souza et al., 2014).

Studies related to the physiology of seed germination in species of *Physalis* have shown that these seeds have high germinability (Pérez Camacho et al., 2008; Rufato, 2008; Souza et al., 2014). In this work, germinability was the only germination parameter that was not significantly different among the species (Table 2). It was found that all species tested showed more than 95% germination (Figure 3), which according to Pérez Camacho et al. (2008) is an acceptable level of the seed trade.

However, the kinetics of germination was different among the studied species, and this fact was demonstrated by the time needed for germination to occur (Figure 3). *P. ixocarpa* showed high germination percentage on the second day of evaluation, reaching values above 80% and average germination time of 2.1 days (Table 2). Seeds of *P. angulata* presented a delay in germinability when compared to *P. ixocarpa*, requiring an average duration of 3.8 days. However, it was the only species which reached 100% germination, data that are consistent with those obtained by Souza et al. (2014). This period was almost half of the time needed by *P. pubescens* (6.2 days) to achieve

Table 2. Germinability (G), Average Time (Ta), Germination Speed Index (GSI) and Coefficient of Uniformity of Germination (CUG) of four species of *Physalis*.

Species	G (%)	Ta (days^{-1})	GSI	CUG
P peruviana	95^{ns}	8,27D	2,98D	0,42B
P. pubescens	96^{ns}	6,19C	4,15C	0,44B
P. angulata	100^{ns}	3,81B	6,90B	1,47B
P. ixocarpa	99^{ns}	2,11A	12,15A	8,50A

Means followed by the same letter do not differ by Tukey test, p ≥ 0,05, ^{ns}= not significant.

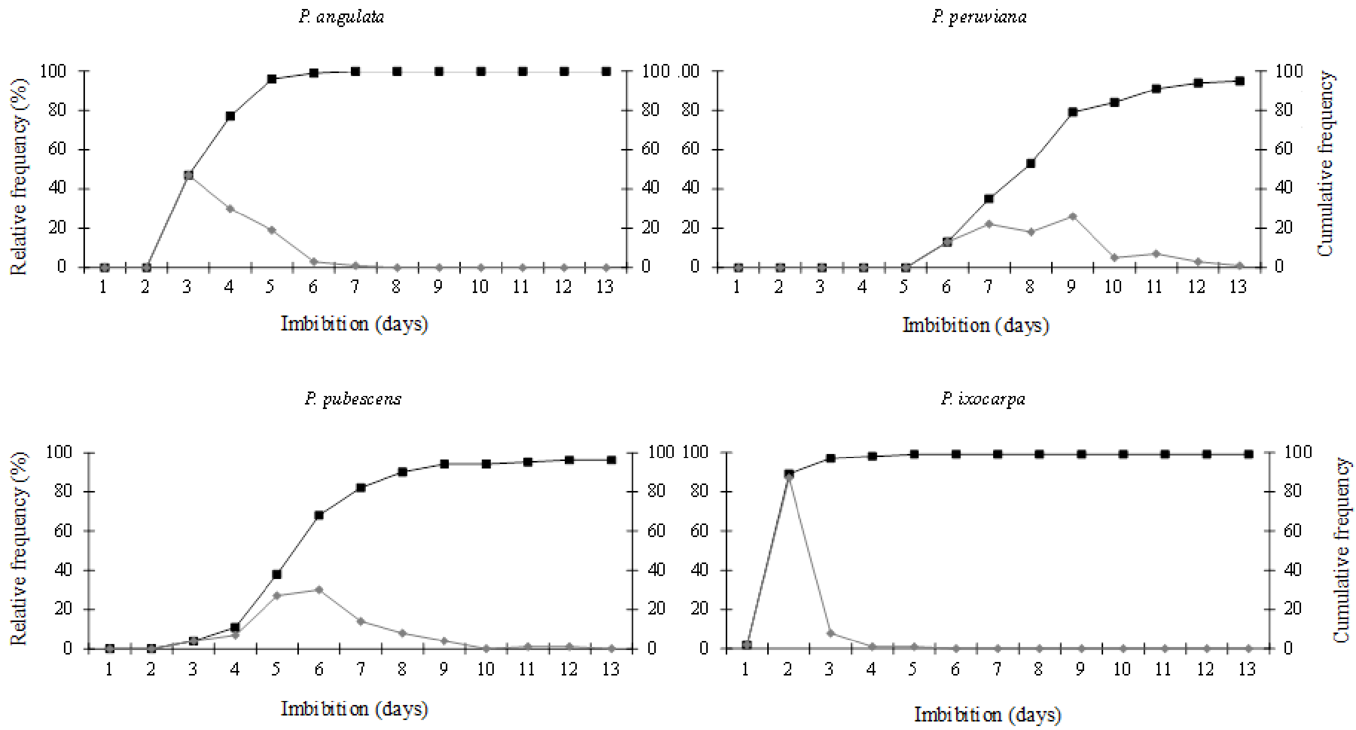

Figure 3. Relative and cumulative frequency of germination of four species of *Physalis* grown in the Experimental Station Arboretum of the State University of Feira de Santana (UEFS), Feira de Santana, Bahia, Brazil, 2014.

its maximum germination pool. In contrast, *P. peruviana* only started germination after six days and the average time was 8.3 days (Figure 3), the longest delay in germination demonstrated in this work. Seeds of *P. ixocarpa* again stood out among the other species not only by germinating in less time but also by showing a higher vigor, which reflected in the high coefficient of uniformity.

Conclusion

It was observed that the studied species are distinct for the majority of analyzed characteristics and E15 e ºBrix can become an important tool for differentiation of species of *Physalis*. Also as complementary, results of germinability and emergency reflect the need to monitor the seed when it is aimed at conservation of species or genotypes of interest. This can be explained because the cultivation of *Physalis* usually occurs where environmental conditions are more favorable and good conservation in the short or medium time are key to ensuring the vigor

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