Revista Ciência Agronômica, v. 50, n. 3, p. 431-438, jul-set, 2019
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Physiological maturity of *Physalis angulata* L. seeds[1]

Maturidade fisiológica de sementes de *Physalis angulata* L.

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ABSTRACT - Determination of seed physiological maturity allows predicting the most appropriate time for harvest, aiming to obtain seeds with high physiological potential. *Physalis angulata* (Solanaceae) has pharmacological and agro-industrial potential, but there is little information on its reproductive phenology. Thus, this study aimed to evaluate the physiological maturity of *P. angulata* seeds according to fruit age. Fruits were harvested at 7, 14, 21, 28 and 35 days after anthesis (DAA) and the following evaluations were conducted: fruit weight and diameter, seed moisture content, 100-seed dry weight, 1000-seed weight, electrical conductivity, germination, germination first count, emergence, and seedling dry weight and length. The experimental design was completely randomized. Fruit weight and diameter increased until 35 DAA and seed dry weight increased until 21 DAA. Highest seed physiological potential occurred at 35 DAA, with highest germination percentage (97%). The results allow concluding that *P. angulata* seeds become physiologically viable from 21 DAA, with highest physiological potential at 35 DAA. However, harvest should be carried out around 28 DAA, before fruits fall.

Key words: Solanaceae. Anthesis. Harvest. Germination. Physiological potential.

RESUMO - A determinação da maturidade fisiológica de sementes possibilita prever o momento mais adequado para a colheita, visando a obtenção de sementes com elevado potencial fisiológico. *Physalis angulata* (Solanaceae) possui potencial farmacológico e agroindustrial, todavia ainda há carência de informações quanto à sua fenologia reprodutiva. Nesse sentido, objetivou-se avaliar a maturação fisiológica de sementes de *P. angulata* em função da idade dos frutos. Estes, colhidos aos 7, 14, 21, 28 e 35 dias após a antese (DAA) e foram submetidos às seguintes avaliações: massa e diâmetro dos frutos, teor de água das sementes, massa seca de 100 sementes, peso de mil sementes, condutividade elétrica, germinação, primeira contagem de germinação, emergência, massa seca e comprimento de plântulas. O delineamento experimental foi o inteiramente casualizado. Houve incrementos na massa e diâmetro de frutos até aos 35 DAA e para a massa seca de sementes até aos 21 DAA. O maior potencial fisiológico das sementes ocorreu aos 35 DAA, com maior porcentual de germinação (97%). Os resultados permitem concluir que sementes de *P. angulata* se tornam fisiologicamente viáveis a partir de 21 DAA, com maior potencial fisiológico aos 35 DAA. Todavia, recomenda-se realizar a colheita em torno dos 28 DAA por conta da queda de frutos.

Palavras-chave: Solanaceae. Antese. Colheita. Germinação. Potencial fisiológico.

DOI: 10.5935/1806-6690.20190051
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Received for publication in 26/09/2017; approved in 14/01/2019
[1] Trabalho extraído da Tese do primeiro autor apresentada ao Programa de Pós-Graduação em Fitotecnia, Centro de Ciências Agronômicas e Florestais, Universidade Federal Rural do Semi-Árido; pesquisa financiada pela CAPES
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INTRODUCTION

Physalis angulata L. is a herb plant with annual cycle, belonging to the Solanaceae family, which occurs all over the national territory of Brazil, especially in its Northern and Northeastern regions (KRINSKI, 2013). It is an essentially wild species, whose nutritional and medicinal properties impart high pharmacological and agro-industrial potential (OLIVEIRA *et al*., 2011).

Although *P. angulata* can be propagated asexually (OLIVEIRA *et al*., 2015), its main form of multiplication is still through seeds (COMMONWEALTH AGRICULTURAL BUREAUX INTERNACIONAL, 2013), and there is lack of information regarding its reproductive phenology. As its fruits commonly show uneven maturation, this may result in seeds with different levels of physiological quality.

Physiological maturity of seeds encompasses morphological and functional changes which occur from ovule fertilization, especially in size, contents of dry matter and moisture, germination capacity and vigor (CARVALHO; NAKAGAWA, 2012; PEREIRA *et al*., 2014). Determination of criteria that make it possible to associate the physiological development of the seed with its physical aspects, and those of fruits, allows estimating the most appropriate time for harvest, so as to coincide with the point of physiological maturity (BEWLEY *et al*., 2013; MARCOS-FILHO, 2015).

The point of physiological maturity is the time when the seed expresses maximum germination and vigor, normally coinciding with maximum dry matter accumulation (MARCOS-FILHO, 2015). Several studies on the maturation of seeds of cultivated species have been conducted, for example by Nakada *et al*. (2011) with cucumber (*Cucumis sativus*), by Figueiredo Neto *et al*. (2014) with pumpkin (*Curcubita moschata*), and by Pereira *et al*. (2014) with pepper (*Capsicum baccatum).* In this context, some studies have also been conducted with species of the genus *Physalis* (BARROSO *et al*., 2017; SBRUSSI *et al*., 2014). However, specifically for *P. angulata*, there is lack of information on the physiological maturity of its seeds, since studies have adopted as the main criterion the color of calyces and fruits (CARVALHO *et al*., 2014), without considering the period of fruit maturity after anthesis.

The present study aimed to determine the physiological maturity of *P. angulata* seeds as a function of fruit age.

MATERIAL AND METHODS

The experiment was conducted at the Federal Institute of Education, Science and Technology of Pará (IFPA), in Castanhal, PA, Brazil (1°17′49″ S, 47°55′19″ W and altitude of 41 m). The climate of the municipality is humid megathermal, Ami, according to Köppen’s classification. Annual rainfall ranges from 2,000 to 2,500 mm, with mean annual temperature between 24 and 27 ºC, and relative air humidity between 78 and 90% (COELHO *et al*., 2003).

P. angulata seedlings were produced in a screened environment, on polystyrene trays with 128 cells, containing peat enriched with essential nutrients (Table 1). For that, seeds were collected in mature fruits, harvested from ten spontaneous plants located at IFPA. Three to four seeds were planted in each cell, and thinning was performed fifteen days after sowing, leaving one plant per cell, under daily irrigation.

The seedlings were manually transplanted to the field at 27 days after thinning, when they were on average 7 cm tall, with three to six true leaves. These seedlings were then planted in masonry beds (0.4 m high x 0.5 m wide x 10 m long), at spacing of 0.5 m between plants, totaling two beds with 20 plants each. Chemical and physical attributes of the soil are described in Table 2.

At 10 days after transplanting, initial symptoms of nutritional deficiency were visually observed in the plants. Because of that, they were sprayed with a commercial solution of essential nutrients (Table 3), at seven-day interval. Cultivation practices along the cycle consisted of manual weeding and irrigation, using an alternative sprinkler system (laser-microperforated polyethylene tape).

Forty days after transplanting, abundant flowering was observed and then flowers were tagged, always in the morning, after anthesis. Fruits were manually harvested at 7, 14, 21, 28 and 35 days after anthesis (DAA) and taken to the Laboratory of Seed Analysis, where they were selected, by discarding those visually attacked by pests, diseases or fermented. For each collection, the following evaluations were carried out:

Fruit diameter and fresh weight - determined using a digital caliper (0.01 mm), in three replicates of 10 fruits (without calyx), and the results were expressed in millimeters (mm). Then, fruits were weighed on a precision scale (0.0001 g) and the results were expressed in grams (g).

Seed moisture content - determined by the oven method at 105 ± 3 ºC, for 24 hours (BRASIL, 2009), in three replicates, and the results were expressed in percentage (wet basis).

100-seed dry weight - quantified in three replicates of 100 seeds, on a precision scale (0.0001 g), after drying in the oven at 105 ± 3 ºC for 24 hours, with results expressed in milligrams (mg).

Table 1 - Chemical and physical attributes of the substrate used to produce *Physalis angulata* seedlings

pH	CE (mS/cm)	Ds (kg/m^3)	U	CRA	N	P2O5	K2O	Calc.
			(%)					
5.8	0.7	260	55	60	0.04	0.04	0.05	1.5

pH: hydrogen potential; EC: electrical conductivity; Ds: Density on dry basis; U: Moisture; WRC: water retention capacity; N: nitrogen; P_2O_5: phosphate; K_2O: potassium; Calc.: calcitic lime

Table 2 - Chemical and physical attributes of the soil (0-20 cm) used to plant *Physalis angulata* seedlings

Sand	Silt	Clay	pHwater	P	K	Na	Ca	Mg	Al
(g/kg)				(mg/dm^3)			(cmol$_c$/dm^3)		
352	266	30	6.6	931	101	53	9.7	2.7	0.0

pH: hydrogen potential, P: phosphorus, K: potassium, Na: sodium, Ca: calcium, Mg: magnesium, Al: aluminum

Table 3 - Chemical and physical attributes of the foliar fertilizer applied on *Physalis angulata* plants

pH	CE (mS/cm)	N	P_2O_5	K_2O	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
		(%, x 10^{-3})											
2	150	75	50	50	10	10	3	0.6	0.5	3	0.7	0.5	1.5

pH: hydrogen potential, EC: electrical conductivity, N: nitrogen, P_2O_5: phosphate, K_2O: potassium, Ca: calcium, Mg: magnesium, S: sulfur, B: boron, Cu: copper, Fe: iron, Mn: manganese, Mo: molybdenum, Zn: zinc

1000-seed weight - determined in eight replicates of 1000 seeds, using a precision scale (0.0001 g), as recommended by the Rules for Seed Analysis (BRASIL, 2009), with results expressed in mg.

Germination - four replicates of 25 seeds were planted on two filter paper sheets, placed on Petri dishes (90 mm diameter x 15 mm height), moistened with 0.2% potassium nitrate (KNO_3) solution using a volume equivalent to 2.5 times the paper weight. To prevent the substrate from drying, the dishes were wrapped with PVC films and placed in transparent plastic bag. Then, they were taken to a germination chamber at constant temperature of 35 ºC in the absence of light. The substrate was moistened again with distilled water whenever needed. Evaluations were conducted at seven and 28 days after sowing, based on the recommendations for a species of the same genus, *P. pubescens*, established in the Rules for Seed Analysis (BRASIL, 2009), and the criterion adopted was the primary root protrusion (MARCOS-FILHO, 2015), with results expressed in percentage.

Electrical conductivity - four replicates of 50 seeds were immersed in 25 mL of distilled water and maintained in germination chamber at 25 °C for 24 h, according to Pereira *et al.* (2014). After this period, electrical conductivity values were measured using a benchtop conductivity meter and the results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

Seedling emergence - determined in treatments in which seeds germinated. Four replicates of 50 seeds were planted in sand, which was previously sieved (1-mm mesh) and sterilized at 150 ºC in forced air circulation oven, for three hours, and moistened with 0.2% KNO_3 solution. After that, they were maintained in environment under natural light, free from direct sunlight and with no temperature control. The substrate was moistened again with distilled water whenever needed. At 15 days after sowing, emerged seedlings were counted and the results were expressed in percentage.

Seedling shoot length - measured in the seedlings used in the emergence test, from the collar to the point of attachment of the cotyledons, using a ruler graduated in millimeters, with results expressed in cm.

Seedling shoot dry weight - quantified in the seedlings used in the emergence test. These seedlings were placed in paper bags, dried in forced air circulation oven at 65 ºC for 72 hours, and then weighed on precision analytical scale (0.0001 g).

The experiments were conducted in completely randomized design. Preliminarily, normality and equality of variances were assessed by the Kolmogorov-Smirnov ($P<0.05$) and Bartlett ($P<0.05$) tests, respectively. Linear and quadratic polynomial regression analysis ($P<0.05$) was then carried out, considering the regression model with highest coefficient of determination.

RESULTS AND DISCUSSION

Regular increments were observed in fruit fresh weight (FW) and fruit diameter (FD) along the evaluation period, even close to senescence (between 28 and 35 DAA), which allowed fitting a linear model for both variables (FW: F = 462.8; P = 0.000; FD: F = 666.4; P = 0.000) (Figure 1 A and B).

In general, both fruit weight and diameter were lower than the mean values obtained by Oliveira *et al.* (2011) (4.3 g and 18.6 mm), for *P. angulata* plants grown spontaneously in Belém-PA. The values were also lower than those found in other species of the genus *Physalis*, such as *P. peruviana* (LIMA *et al.*, 2012, 2013; SBRUSSI *et al.*, 2014) and *P. ixocarpa* (BARROSO *et al*., 2017).

Besides the influence of the environment, such as temperature, luminosity, water availability and soil attributes, the weight and diameter of *P. angulata* fruits are also associated with the genetic variability of this species, because it is still an essentially wild plant. Thus, it is difficult to judiciously analyze the differences in the values of fruit weight and diameter obtained in the present study compared to the other studies cited.

100-seed weight significantly increased until 21 DAA, subsequently tending to stabilization, and the data fitted to a quadratic regression model (F = 167.4; P = 0.000), with maximum point at 28 DAA (Figure 1C).

According to Carvalho and Nakagawa (2012), dry weight is the variable that best describes seed maturation. Seeds act as a sink of products from photosynthesis, lipids, proteins and other materials, resulting in increase in dry matter content until the moment when translocation from the plant to the seed stops, indicating that the seed has reached physiological maturity.

For *P. angulata*, it became evident that dry matter translocation tends to stop after 21 DAA. However, Pereira *et al.* (2014) explain that maximum dry matter accumulation does not always coincide with the point of physiological maturity because biochemical and physiological alterations favorable to seed physiological potential may still occur at a later time.

The values of 100-seed weight were lower than those obtained by Souza *et al*. (2010) (~50 mg). For the species *P. ixocarpa*, Barroso *et al*. (2017) found dry weights between 2 and 10 mg seed^{-1} (average of 50 seeds, 15 to 55 DAA). It should be pointed out that seed dry weight is commonly proportional to the size of the seed, which is small in the case of *P. angulata* (~ 1.6 mm) (SOUZA *et al*., 2010).

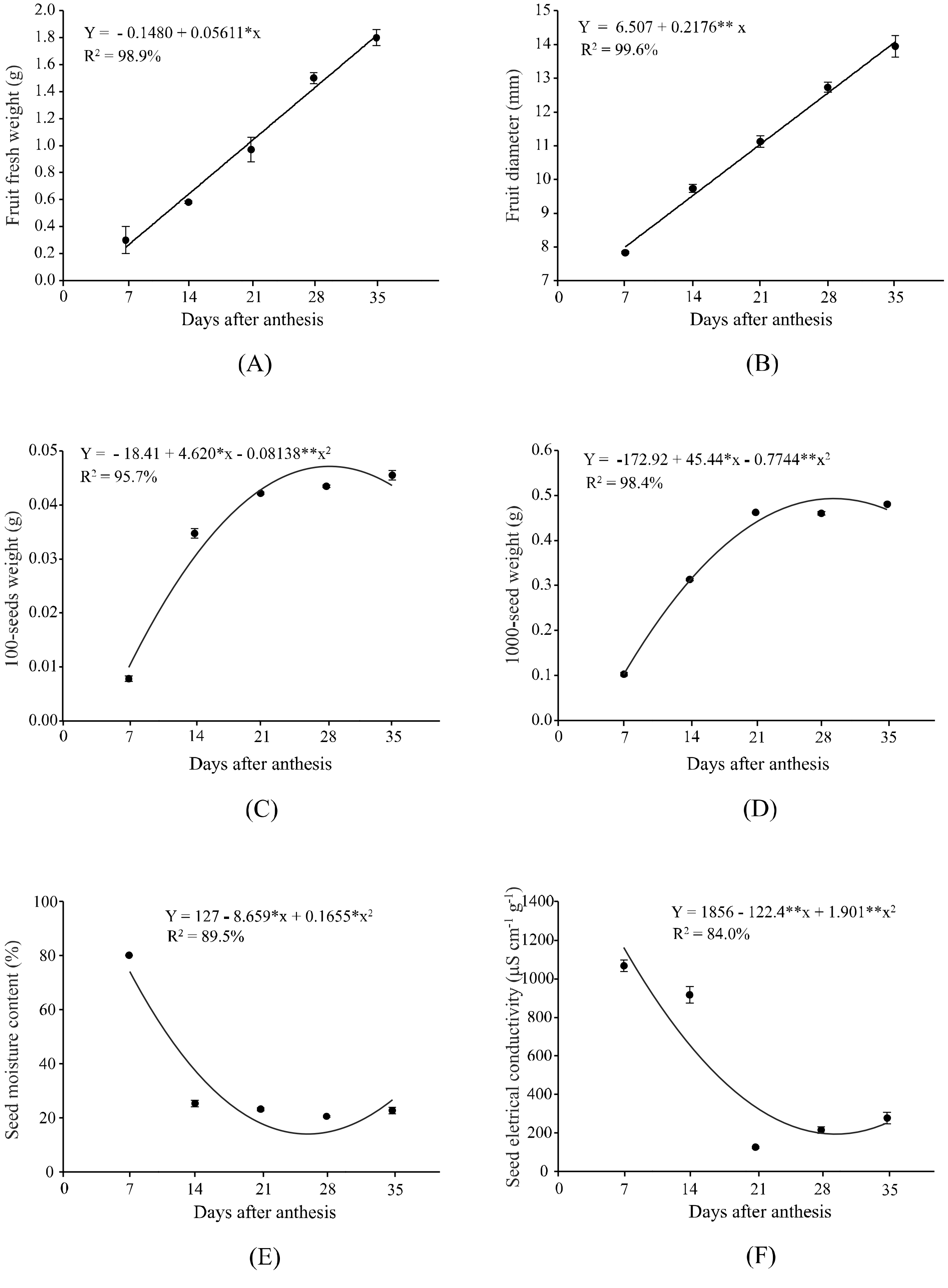
1000-seed weight showed a similar behavior to that of 100-seed weight, and its data also fitted to a quadratic regression model (F = 970.4; P = 0.000) (Figure 1D). According to the Rules for Seed Analysis (BRASIL, 2009), 1000-seed weight provides a notion of seed size, maturity stage and health condition, and it allows estimating sowing density or number of seeds per package.

The values of 1000-seed weight were always low (100-500 mg). Pereira *et al*. (2014) obtained values around 9000 mg for seeds of pepper (*Capsicum baccatum*). However, for *P. peruviana*, Sbrussi *et al.* (2014) also obtained low values, always around 100 mg, during all seed maturation. It is worth mentioning that this variable is always associated with seed moisture content (BRASIL, 2009), which can greatly vary from species to species, being mainly influenced by storage conditions (MARCOS-FILHO, 2015).

In relation to seed moisture content (Figure 1E), highest percentage was recorded at seven DAA (~ 80%), when dry matter content was minimal (Figure 1C). After that, there was accentuated reduction in the percentages until 14 DAA and a trend of stabilization from this time, allowing a quadratic regression model to be fitted (F = 29.3; P = 0.002).

According to Marcos-Filho (2015), the moisture content of seeds at early maturation is normally high, above 80%, compatible with the result found here. For *P. ixocarpa*, Barroso *et al*. (2017) observed values around 90 to 54%, from 15 to 35 DAA. In the case of *P. angulata*, as the full development of fruits occurs in a short time interval, dehydration and stabilization of moisture content in the seeds also occurred rapidly (14 DAA), coinciding with the period of highest increment in dry matter (7 to 14 DAA) (Figure 1C).

Figure 1 - Fruit fresh weight (A), fruit diameter (B), 100-seed weight (C), 1000-seed weight (D), seed moisture content (E) and seed electrical conductivity (F) for *Physalis angulate* as a function of days after anthesis (n = 4, *p<0.001, **p<0.005)



According to Carvalho and Nakagawa (2012), the high moisture content, normally observed at the initial stages of maturation, is due to the constant water flow from the plant to the seed, because the latter requires abundant hydration to translocate, synthesize or metabolize reserve materials. Throughout maturation, the moisture content is substituted for dry matter, and seeds dehydrate until reaching stable values that are adequate for physiological maintenance (FIGUEIREDO NETO *et al*., 2014; NAKADA *et al*., 2011; PEREIRA *et al*., 2014).

Highest values of electrical conductivity were observed between 7 and 14 DAA, with a subsequent decrease to a minimum point at 28 DAA, and the quadratic model was the most representative for this variable (F = 40.7; P = 0.001) (Figure 1F). High values of electrical conductivity demonstrate high release of electrolytes in the solution, due to lower structuring and selectivity of plasmatic membranes, which are characteristic of seeds at early maturation (FIGUEIREDO NETO *et al*., 2014; PEREIRA *et al*., 2014). In the case of *P. angulata*, the present study showed that, from 21 DAA, the membranes are already well structured, capable of containing the cell material, minimizing leakage.

Other species have also shown high initial values of electrical conductivity, which tended to decrease along seed maturation. However, the values may vary considerably from one species to another. In seeds of West Indian gherkin (*Cucumis anguria*), Medeiros *et al*. (2010) found values between 2,321 and 1,556 µS $cm^{-1}g^{-1}$ (15 to 40 DAA). For cucumber (*C. sativus*), the values were only 71 to 16 µS $cm^{-1}g^{-1}$ (30 to 55 DAA) (NAKADA *et al*., 2011), and for pepper (*Capsicum baccatum*), the electrical conductivity varied between 800 and 300 µS $cm^{-1}g^{-1}$ (15 and 45 DAA) (PEREIRA *et al*., 2014).

Values of germination, germination first count, seedling emergence, and seedling shoot length and dry weight are presented in Figure 2.

Germination only occurred from 21 DAA, with increments until 35 DAA, reaching maximum percentage of 97%, which allowed a quadratic model to be fitted for germination (F = 91.93; P = 0.000) (Figure 2A) and germination fist count (F = 136.29; P = 0.000) (Figure 2B).

According to Pereira *et al*. (2014), null germination is commonly expressed by seeds which have not yet reached physiological maturity. In the case of *P. angulata*, seeds are still further from the point of physiological maturity until 14 DAA. For the species *P. ixocarpa*, germination only occurred around 20 DAA, reaching maximum value after 35 DAA (100%) (BARROSO *et al*., 2017). Nonetheless, in the study of Sbrussi *et al*. (2014) with *P. peruviana*, germination occurred along the entire time, regardless of maturity stage, with values ranging from 72 to 81%.

In general, germination percentages were consistent with those obtained in other studies (> 80%) with mature fruits of *P. angulata* (SOUZA *et al*., 2014; SOUZA; SOUZA; PELACANI, 2011). However, the results differ from those found by Carvalho *et al*. (2014), also for *P. angulata*, in which highest germination percentages were obtained in seeds of fruits still unripe (83%), whereas in mature fruits the value was only 40%.

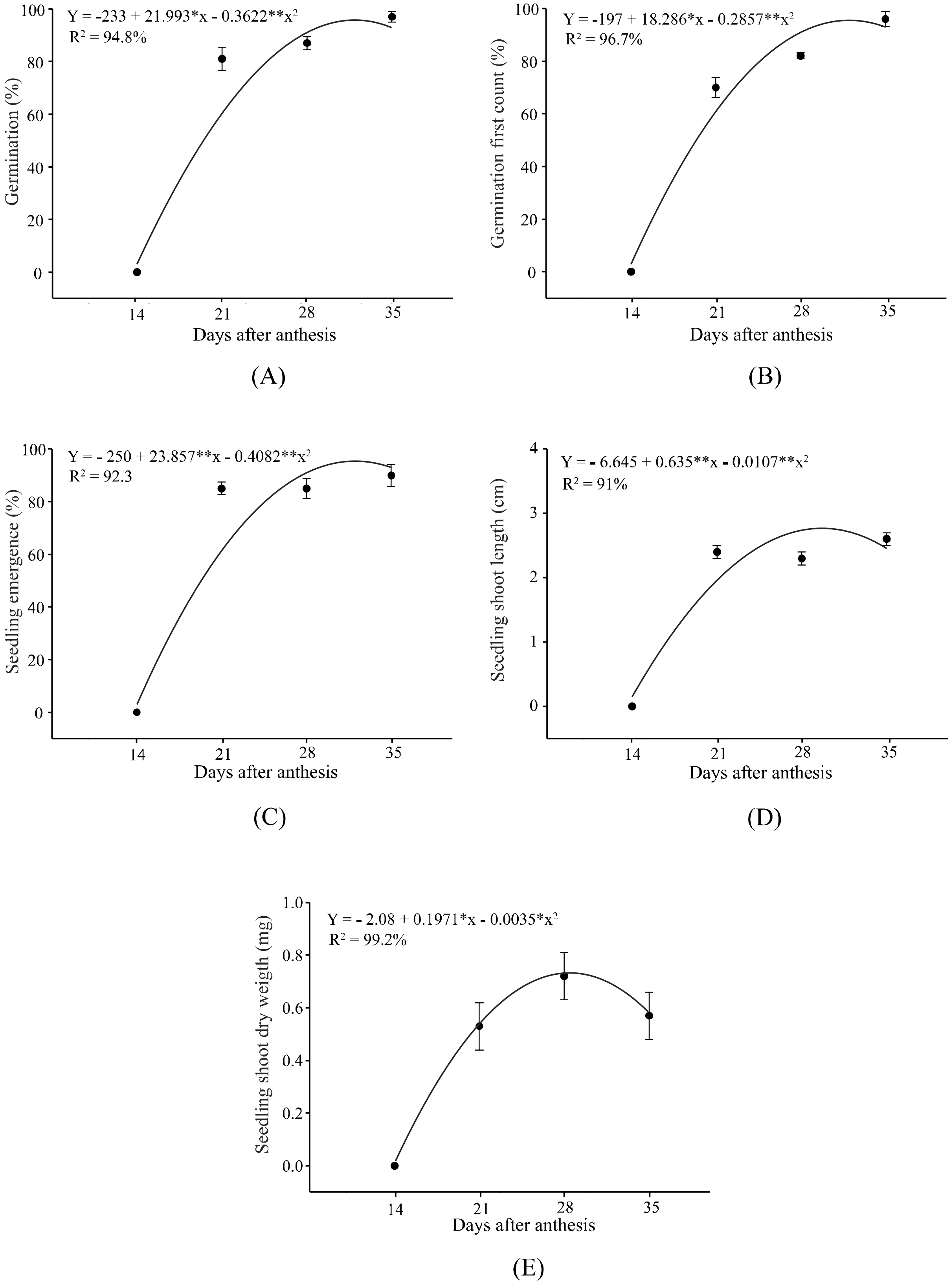
For germination first count, the values were always above 70%, considered high compared to other species of the Solanaceae family, such as tomato (*Solanun lycopersicum*) (53 to 67%) (VIDIGAL *et al*., 2006) and pepper (*C. baccatum*) (~10%) (PEREIRA *et al*., 2014). In the present study, the values for first count demonstrate that *P. angulata* seeds tend to express high vigor already at 21 DAA.

In relation to seedling emergence (Figure 2C), it was evident that seeds had high vigor from 28 DAA, with values always above 80%, and the data fitted to a quadratic model (F = 65.69; P = 0.000). However, the values were slightly lower than those obtained by Lanna *et al*. (2013) for *P. angulata* and *P. peruviana*, whose percentages varied between 92 and 100%, in organic substrates. Piva *et al*. (2013) found values between 53 and 95% in *P. peruviana* seeds, in different combinations of substrate. By contrast, in the study conducted by Sbrussi *et al*. (2014), emergence of *P. peruviana* seeds in sand varied from 67 to 81%.

As observed for emergence, quadratic responses were also observed in shoot length (Figure 2D; F = 58.68; P = 0.000) and shoot dry weight (Figure 2E; F = 40.08; P = 0.000). According to Marcos-Filho (2015), seedling length and biomass are also indicators of seed vigor. The more vigorous the seeds, the greater the length and biomass of the seedlings.

Joint analysis of all variables allowed observing that, although the moisture content and dry matter accumulation in *P. angulata* seeds tend to stability already from 14 DAA, these seeds become physiologically viable only from 21 DAA. Nevertheless, highest physiological potential occurs at 35 DAA, with highest germination percentage. However, at the harvest carried out at 35 DAA, increased fruit fall was observed, due to senescence and action of winds. Because of that, fruit harvest, to obtain seeds, should be carried out around 28 DAA.

Figure 2 - Germination (A), germination first count (B), seedling emergence (C), seedling shoot length (D) and seedling shoot dry weight (E) of *Physalis angulata* as a function of days after anthesis (n = 4, $*p<0.001$, $**p<0.005$)



CONCLUSIONS

P. angulata seeds become physiologically viable from 21 DAA, with highest physiological potential at 35 DAA. However, harvest should be carried out around 28 DAA, before the fruits fall.

ACKNOWLEDGMENTS

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial aid, to the Federal Rural University of the Semi-Arid Region (UFERSA) and to the Federal Institute of Education, Science and Technology of Pará (IFPA), for the logistic support.

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