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Ecophysiological aspects of the germination of *Physalis angulata* L. seeds

Aspectos ecofisiológicos da germinação de sementes de *Physalis angulata* L.

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ABSTRACT - *Physalis angulata* L. (Solanaceae), known as 'camapu', has pharmacological and agroindustrial potentials, but information on the ecophysiological aspects that influence germination is scarce. In this context, the objective was to evaluate the expression of the physiological potential of *P. angulata* seeds as a function of the maturation stage, temperature, substrate and light condition. Four experiments were conducted: in the first, the maturation stages (green calyx + fruit, yellow calyx + fruit and light brown calyx + fruit) were evaluated at temperatures of 25, 30 and 35 °C, separately; in the second, the interaction between the temperatures of 35, 40 and 45 °C and types of substrate (on paper, between paper and between vermiculite) was tested in a 3x3 factorial scheme (temperatures as the first factor and types of substrates as the second factor); in the third experiment, the effect of light (absence of light, white light, red and far-red light) was evaluated; and, in the fourth, increasing values of photoperiods (0, 8, 12 and 16 hours) were used. In all experiments, the design was completely randomized with four replicates of 50 seeds. Analysis of the results showed that there was no interaction between the temperatures and the substrates tested, demonstrating the isolated action of the factors. *P. angulata* seeds expressed better physiological potential when the calyx and fruit are yellow. In the germination test, the seeds should be sown between paper towels, under temperature of 35 °C and absence of light.

Keywords: Solanaceae. Photoperiod. Maturation. Seed analysis. Ecophysiology.

RESUMO - *Physalis angulata* L. (Solanaceae), conhecida por camapu, possui potenciais farmacológico e agroindustrial, porém são escassas as informações sobre os aspectos ecofisiológicos que influenciam a germinação. Neste sentido, objetivou-se avaliar a expressão do potencial fisiológico de sementes de *P. angulata* em função do estádio de maturação, temperatura, substrato e condição luminosa. Para isso, conduziram-se quatro experimentos: no primeiro, avaliou-se os estádios de maturação (cálice + fruto verdes, cálice + fruto amarelos e cálice + fruto marrom-claros) nas temperaturas de 25; 30 e 35 °C, separadamente; no segundo, testou-se a interação entre as temperaturas 35; 40 e 45°C e tipos de substrato (sobre papel, entre papel e entre vermiculita) em esquema fatorial 3x3 (sendo o primeiro fato as temperaturas e o segundo os tipos de substratos); no terceiro experimento, avaliou-se o efeito da luz (ausência de luz, luz branca, luz na frequência do vermelho e do vermelho-distante); e, no quarto, utilizaram-se valores crescentes de fotoperíodos (0; 8; 12 e 16 horas). Em todos os experimentos o delineamento foi o inteiramente casualizado com quatro repetições de 50 sementes. Na análise dos resultados foi visto que não houve interação entre as temperaturas e os substratos testados, demonstrando a atuação isolada dos fatores. As sementes de *P. angulata* expressaram melhor potencial fisiológico quando o cálice e o fruto estão amarelos. No teste de germinação, as sementes devem ser semeadas entre papel toalha, sob a temperatura de 35 °C e ausência de luz.

Palavras-chave: Solanaceae. Fotoperíodo. Maturação. Análise de sementes. Ecofisiologia.

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INTRODUCTION

Physalis angulata L., commonly known as 'camapu', is an herbaceous plant belonging to the Solanaceae family (SANTIAGO et al., 2019a). It is a rustic species and can be found in most tropical and subtropical countries, especially in South America (KRINSKI, 2013). In Brazil, it occurs mainly in the North and Northeast regions.

Although little exploited commercially, *P. angulata* fruits are appreciated for having a sweet and slightly acidic taste, as well as high pharmacological potential (SUN et al., 2017). Thus, there has been a growing interest of the pharmaceutical industry in using this species for technological purposes (SANTIAGO et al., 2019b).

Despite that, studies regarding ecophysiological aspects of *P. angulata* are still scarce (LEITE et al., 2018), especially regarding the germination of its seeds (SANTIAGO et al., 2019a; RAMOS et al., 2021). This need is observed both in national and international rules for seed analysis (BRASIL, 2009; ISTA, 2017), whose specific recommendations for the germination test are still non-existent. However, the recommendations suggested for *P. angulata* are the same as those used for the genus *Physalis* or for the species of the Solanaceae family.

Regarding the appropriate temperature for the germination of *P. angulata* seeds, there is still no consensus, and values ranging between 25 and 35 ºC are commonly used (LANNA et al., 2013; OLIVEIRA et al., 2013; SOUZA et al., 2014; GAIER et al., 2019). Temperature is one of the main abiotic factors that influence germination. The biochemical reactions involved in the germination process are dependent on enzymatic systems, which require adequate temperature ranges. In addition, it acts directly on the speed of imbibition, number of germinated seeds, and germination uniformity and speed (PEREIRA; SANTOS; MARTINS FILHO, 2011).

Light, despite not being an essential environmental factor for germination, can positively stimulate some species, especially small and wild seeds (LESSA et al., 2013). For *P. angulata* seeds, the influence of this factor on germination is not yet properly clarified, but there is evidence that it can occur both in the presence and in the absence of light (YILDIRIM; KARLIDA; DURSUN, 2011).

The expression of the physiological potential of seeds is associated with the maturation stage and is sensitive to environmental factors (MARCOS-FILHO, 2015). In this context, Santiago et al. (2019a) found that the maximum expression of the physiological potential of *P. angulata* seeds occurs around 35 days after anthesis, when the fruits are ripe and close to senescence. However, in practice it is difficult to ascertain exactly the age of the fruits of this species in the field. Therefore, the criterion based on the color of the fruit and fruiting calyx may be useful for the analysis of P. *angulata* seeds (CARVALHO et al., 2014).

The Rules for Seed Analysis (BRASIL, 2009) suggest that the germination test for *Physalis sp.* should be conducted on paper (on or between paper) or sand (on sand) substrates. In *P. peruviana*, in addition to the use of paper, germination between 60 and 80% was recorded with the use of vermiculite (DINIZ; CHAMMA; NOVEMBRE, 2020). For *P. angulata*, there are still no specific recommendations regarding the type of substrate to be used during this test.

Therefore, the objective of this study was to evaluate the expression of the physiological potential of *P. angulata* seeds as a function of the maturation stage, temperature, substrate and light condition.

MATERIAL AND METHODS

The study was carried out at the Federal Institute of Education, Science and Technology of Pará (IFPA), Castanhal campus (1°17′49″S and 47 55′19″W, at altitude of 41 m). *P. angulata* fruits were harvested manually in 20 spontaneously occurring plants at the IFPA campus, Castanhal – PA, Brazil. Fruits visibly damaged by pests and diseases or fermented were discarded.

Calyces with the fruits were stratified into three maturation stages: green calyx + fruit, yellow calyx + fruit and light brown calyx + fruit (Figure 1).

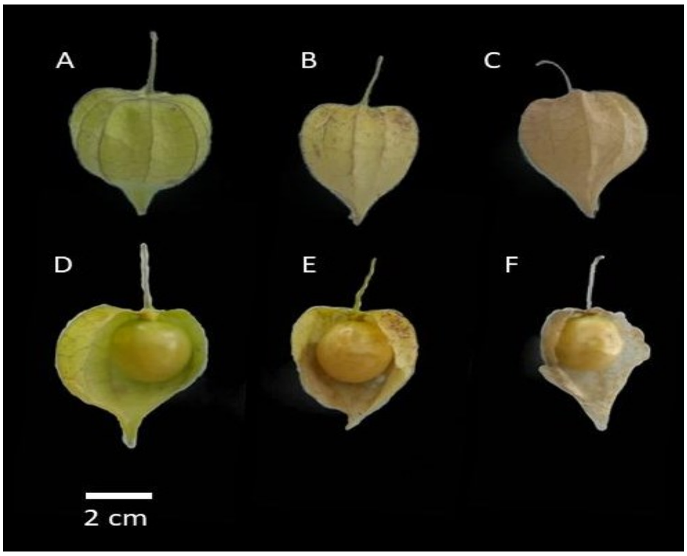

Figure 1. Corresponding colors between calyces and fruits of *Physalis angulata* L.: green calyx and fruit (A, D); yellow calyx and fruit (B, E); and light brown calyx and fruit (C, F).

The fruits were processed for seed extraction, followed by washing in running water and drying on paper towels at room temperature for 24 hours. After this period, the water content of the seeds was determined by the oven method at 105 ± 3 °C for 24 h (BRASIL, 2009). The seeds, with 11% water content, were placed in a glass container sealed with a metal lid, wrapped with a plastic bag, and then stored at the bottom of the refrigerator (~10 ºC) until the beginning of the experimental phase. Four experiments were subsequently carried out, as described below:

In the first, seeds from fruits in the three maturation stages (green calyx + fruit, yellow calyx + fruit and light brown calyx + fruit) were subjected to germination tests. The experimental design was completely randomized with four replicates of 50 seeds. These were sown in Petri dishes (90 mm in diameter x 15 mm in height), having substrate consisting of two sheets of paper towel, moistened with distilled water in the volume equivalent to 2.5 times their dry weight. To prevent the substrate from drying, the Petri dishes were wrapped with PVC films and placed in transparent plastic bags. Subsequently, the Petri dishes were incubated in germination chambers with constant temperatures of 25, 30 and 35 °C and 12-hour photoperiod

The first count was performed at seven days after sowing and the germination test lasted 28 days, following the recommendations established in the Rules for Seed Analysis for *P. pubescens*, a species of the same genus as *P. angulata* (BRASIL, 2009). The criterion used to indicate germination was the emergence of the primary root and the results were expressed as percentage of germinated seeds. Normality and equality of variances of the data were tested by the Kolmogorov-Smirnov ($p = 0.01$) and Levene ($p = 0.01$) tests, respectively. Analysis of variance (ANOVA, $p = 0.05$) was performed, and the difference between means was assessed by Tukey test at 5% significance level.

For the second experiment, seeds from fruits that showed yellow calyx + fruit were used. The test followed a 3 x 3 factorial arrangement, with three temperatures (35, 40 and 45 °C) as the first factor and three types of substrates (on paper, between paper and between vermiculite) as the second factor. The experimental design was completely randomized, with four replicates of 50 seeds. Daily evaluations were performed to count germinated seeds until the 28th day to calculate the germination speed index (GSI) according to the recommendations proposed by Maguire (1962), considering seeds that produced primary root as germinated. Analysis of variance (ANOVA, $p = 0.05$) was performed, and the difference between means was assessed by Tukey test at 5% significance level.

The third experiment consisted of the evaluation of germination under constant temperature of 35 ºC and in four situations of exposure to light (absence of light, white light, red light and far-red light), as indicated by Alves et al. (2016). Seeds from yellow calyx and fruits were used, and the methods for conducting the germination test were the same as in experiment I. To simulate the conditions of absence of light, Petri dishes were covered with aluminum foil. For white light, four fluorescent lamps (2500 lux and 6000k) were used. for red light, Petri dishes were covered with two sheets of red cellophane paper. For far-red light, the Petri dishes were covered with four sheets of cellophane paper, two red and two blue. The experimental design was completely randomized with four replicates of 50 seeds.

At the end of the germination test, the following parameters were evaluated: percentage of germinated seeds; GSI; seedling length, measured from the end of the radicle to the insertion of the cotyledons, using a millimeter ruler; and seedling dry mass, following the oven method (65 ºC for 72 hours), according to the methodology proposed by Brasil (2009). After drying, the mass of the seedlings was determined on a precision analytical balance, and the results were expressed in $g.seedling^{-1}$. Analysis of variance (ANOVA, $p = 0.05$) was performed, and the difference between means was assessed by Tukey test at 5% significance level.

In the fourth experiment, the physiological response of the seeds subjected to the germination test using four different photoperiods (0, 8, 12 and 16 h) and temperature of 35 ºC was evaluated. The light used was white, obtained by means of four fluorescent lamps (2500 lux) fixed to the internal part of the door of the germination chambers. The seeds used were obtained from yellow calyx and fruits, and the test was conducted in the same way as in experiment I. At the end of the test, the germination percentage, GSI, seedling length and seedling dry mass, obtained as described in experiment III, were evaluated. Regression analysis ($p = 0.05$) was performed, considering the regression model with the highest coefficient of determination (R^2).

RESULTS AND DISCUSSION

Analysis of the data obtained in experiment I indicates that germination was not satisfactory (between 0% and 11%) at temperatures of 25 and 30 ºC, suggesting that these conditions are not favorable to the germination of *P. angulata* seeds. At temperature of 35 °C, lower germination was observed in seeds obtained from fruits that showed green calyx + fruits (GCF), but there was no statistical difference for the results of first germination count (FGC) in the different stages of fruit maturation (Table 1).

Table 1. Means of germination (G) and first germination count (FGC) of *Physalis angulata* seeds as a function of the color of calyx and fruit at different temperatures.

Calyx color	Temperature (ºC)					
	25		30		35	
	G (%)	FGC (%)	G (%)	FGC(%)	G (%)	FGC (%)
GCF	1 a	1 a	11 a	3 a	37 b	25 a
YCF	0 a	0 a	4 b	0 b	64 a	33 a
LBCF	0 a	0 a	6 b	0 b	80 a	34 a
CV (%)	13.8	14.2	11.2	12.4	13.1	12.8

GCF: green calyx + fruit; YCF: yellow calyx + fruit; LBCF: light brown calyx + fruit. Means with equal letters in the column do not differ statistically by Tukey test at 5% probability level.

In a study conducted by Carvalho et al. (2014), the germination of *P. angulata* seeds was equal to 89% for green calyx + fruit and 23% for light brown calyx + fruit. These results differ from those obtained in the present study, because in addition to the experimental conditions having been different (eight hours of light at 30 ºC and 16 hours in the dark at 20 ºC), the fruits came from the southern region of Brazil, whose climate differs from the Amazon climate.

Under conditions similar to those of the present study, Santiago et al. (2019a) also found greater germination of *P. angulata* seeds in ripe fruits (yellow calyx + fruits), close to senescence. Also in this study, it was observed that the colors of calyx and fruit can serve as practical criteria for harvesting *P. angulate* seeds, since brown fruits tend to fall and/or ferment, so yellow fruits are recommended for cultivation in the Amazon region.

As for temperature, for most plant species of tropical climate, the optimal value for seed germination occurs in the interval between 15 and 30 ºC (TAIZ et al., 2015). For some species of the genus *Physalis*, such as *P. ixocarpa* and *P. peruviana*, or even for *P. angulata* sown in plant culture medium (*in vitro*), the constant temperature of 25 ºC promoted germination above 80% (LANNA et al., 2013; OLIVEIRA et al., 2013).

However, the requirement of temperatures above 30 ºC for the germination of *P. angulata* seeds is also observed in species of the same family. In their study, Diniz, Chamma, and Novembre (2020) found reduction in the germination of *P. piruviana* seeds, under temperatures of 15 to 20 ºC, while the alternating temperature of 20-30 ºC was more adequate, promoting germination of 70%.

Other studies have found germination percentages for *P. angulata* seeds close to 100% under constant temperature of 35 ºC (SOUZA et al., 2014). This shows that this temperature tends to be the most indicated for conducting the germination test of this species. The requirement of high temperatures for the germination of P. *angulata* seeds is consistent with the geographical distribution of this species, since it occurs spontaneously in a wide variety of habitats in tropical countries (KRINSKI, 2013), where daily temperatures can exceed 35 ºC.

As observed for *P. angulata*, Pereira, Santos, and Martins Filho (2011) also obtained, under temperature of 35 ºC, the highest values of germination and germination speed for seeds of 'cubiu' (*Solanum sessiliflorum* Dunal), a vegetable native to the Amazon. According to Maia et al. (2008), the requirement of high temperatures for germination is characteristic of species that establish themselves spontaneously and quickly in anthropized areas, as is the case of *P. angulata.*

Germination at temperature of 45 ºC was less than 1%, regardless of the substrate. Thus, this temperature was found to be inadequate to the germination process of *P. angulata*. For this reason, the data of this temperature were not considered for statistical analysis. There was no significant interaction between temperatures (35 ºC and 40 ºC) and substrates (OP, BP and BV), but there were individual effects of the factors (Table 2). Although germination occurred at the temperature of 40 ºC, it was lower than that found at 35 ºC, and consequently the expression of vigor was also affected.

Table 2. Means of germination (G), first germination count (FGC) and germination speed index (GSI) of *Physalis angulata L.* seeds, as a function of temperature and substrate.

Temperatures (°C)	G (%)	FGC (%)	GSI
35	74 a	56 a	16.9 a
40	50 b	14 b	8.7 b
CV (%)	15.1	9.8	10.2
Substrates	G (%)	FGC (%)	GSI
OP	47 b	35 a	10.6 a
BP	66 a	41 a	13.7 a
BV	73 a	30 a	14.1 a
CV (%)	10.3	11.4	13.4

OP: on paper, BP: between paper, BV: between vermiculite. Means with equal letters do not differ statistically by Tukey test at 5% probability level.

In tropical regions, seed germination in a high temperature range generally favors faster establishment in the field, conferring survival advantages over other species that require lower temperature ranges (MAIA et al., 2008). However, very high temperatures, above optimum, tend to cause thermoinhibition of the germination process, since they cause negative effects on enzymatic activity, lead to disruption of cell membranes and impact the integrity of organelles (MARCOS-FILHO, 2015; TAIZ et al., 2015).

Regarding the effect of the substrates, it was observed that the substrates between paper (BP) and between vermiculite (BV) promoted higher germination at a temperature of 35 °C, since they allow a larger surface of contact between the substrate and the seeds and, consequently, higher efficiency in water supply. However, for *P. peruviana*, Diniz, Chamma, and Novembre (2020) recorded no statistical difference between the use of blotter paper (on paper) and vermiculite, with average germination percentages between 65 and 89%, respectively.

In this study, although the use of vermiculite promoted an average germination percentage higher than 70%, it was more difficult to perform the tests of first germination count and germination speed index under this treatment, due to the adherence of the radicle to the substrate particles, besides the difficulty of visualization during the evaluation.

The results regarding the light condition revealed that the absence of light (AL) promoted better results for all the variables studied in the experiment (Table 3). On the other hand, light at far-red frequency (FR) caused lower results for most of the variables analyzed.

Table 3. Means of germination (G), first germination count (FGC), germination speed index (GSI), seedling length (SL) and seedling dry mass (SDM) of *Physalis angulata* L. as a function of light quality.

Light quality	G (%)	FGC (%)	GSI	SL (cm. seedling^{-1})	SDM (g. seedling^{-1})
AL	91 a	89 a	13.7 a	5.2 a	0.5 a
WL	51 b	50 b	7.1 b	4.0 b	0.3 b
R	59 b	59 b	4.8 b	3.8 b	0.3 b
FR	34 c	32 c	5.8 b	3.9 b	0.2 c
CV (%)	13.1	9.89	10.04	11.2	14.3

AL: absence of light; WL: white light; R: red; FR: far-red. Means followed by the same letter do not differ statistically by Tukey test at 5% probability level.

With seeds of *P. angulata*, in the presence of light, Carvalho et al. (2014) and Oliveira et al. (2013) obtained germination above 80%. However, the results of this study do not allow the seeds of this species to be classified as positive photoblastic. It is observed that, although *P. angulata* seeds can germinate both in the presence and in the absence of light, the latter condition promoted the greatest expression of the physiological potential.

The percentage of germinated seeds in the first count (seven days) was 89% under absence of light, but under white light it was 50%. Based on these results, according to the recommendations established in the Rules for Seed Analysis (BRASIL, 2009) for the species *P. alkekengi* and *P. pubescens* indicating the presence of light as a promoter of germination, this light proved to be inadequate for *P. angulata.* Likewise, Yildirim, Karlida, and Dursun (2011) obtained, under the absence of light, high seed germination (~90%) for species of the genus *Physalis* (*P. ixocarpa* and *P. peruviana*).

The sensitivity of seeds to light is a characteristic that can be acquired as a result of the environmental conditions throughout maturation, not necessarily being a genetic factor (MARCOS-FILHO, 2015). This physiological response of seeds to light can vary according to their age, storage conditions, integrity of integuments, water potential, temperature and treatment to overcome dormancy (LESSA et al., 2013).

When evaluating the effect of light on the germination of seeds of native Amazon species (*Mimosa scabrella* Benth., *Chorisia speciosa* St. Hill., *Tabebuia avellanedae* Zhange, *Esenbeckia leiocarpa* Engl.), Dias, Kageyama, and Issiki (1992) obtained results similar to those of the present study. These authors found that germination was higher in the dark and decreased in the following order: red, blue, white and far-red light.

It is suggested that the high germination of *P. angulata* seeds in the dark is indicative of the non-limiting concentration of phytochromes in their active form, with no need for the presence of light to trigger the germination process. However, when the seeds are exposed to light, the ratio between the radiations at the red and far-red frequency decreases (R > WL > FR), because the phytochromes are inactivated (TAIZ et al., 2015). Therefore, the results indicate high sensitivity of *P. angulata* seeds to light at the far-red frequency.

For all variables, white light (WL) and light at the red frequency (R) promoted similar results, which is consistent with Marcos-Filho (2015), who emphasized that these two types of lights commonly provoke similar physiological effects on seeds. In studies on the germination of seeds of wild herbaceous species, such as *Coniza* sp. and *Emilia coccinea* Sims, a similar effect was also found between WL and R (LESSA et al., 2013).

The results of the germination speed index (GSI), under the absence of light, were not only higher than those obtained under the other light conditions, but was also higher than those obtained in other studies with *P. angulata* under white light (LANNA et al., 2013; OLIVEIRA et al., 2013). On the other hand, the GSI values obtained by Souza, Souza, and Pelacini (2011) and Oliveira et al. (2013) are consistent with those observed in this study under white light.

Seedling length under the absence of light was longer than under the other light conditions. Under conditions of shading or absence of light, it is common to observe etiolation, which is the cellular elongation as an ecological strategy of the plant in search of light and that usually also occurs in the seedling stage. However, the influence of light condition on seedling length and mass depends on other factors, such as the species and the environmental temperature (STEFANELLO et al., 2008).

As for the photoperiod, there was a significant fit of the regression curve for all variables (Figure 2). The absence of light not only stimulated greater germination and vigor of *P. angulata* seeds, but there was also an inverse relationship between the physiological potential of the seeds and the time of exposure to light. These results are consistent with those obtained in the light quality experiment (Table 3).

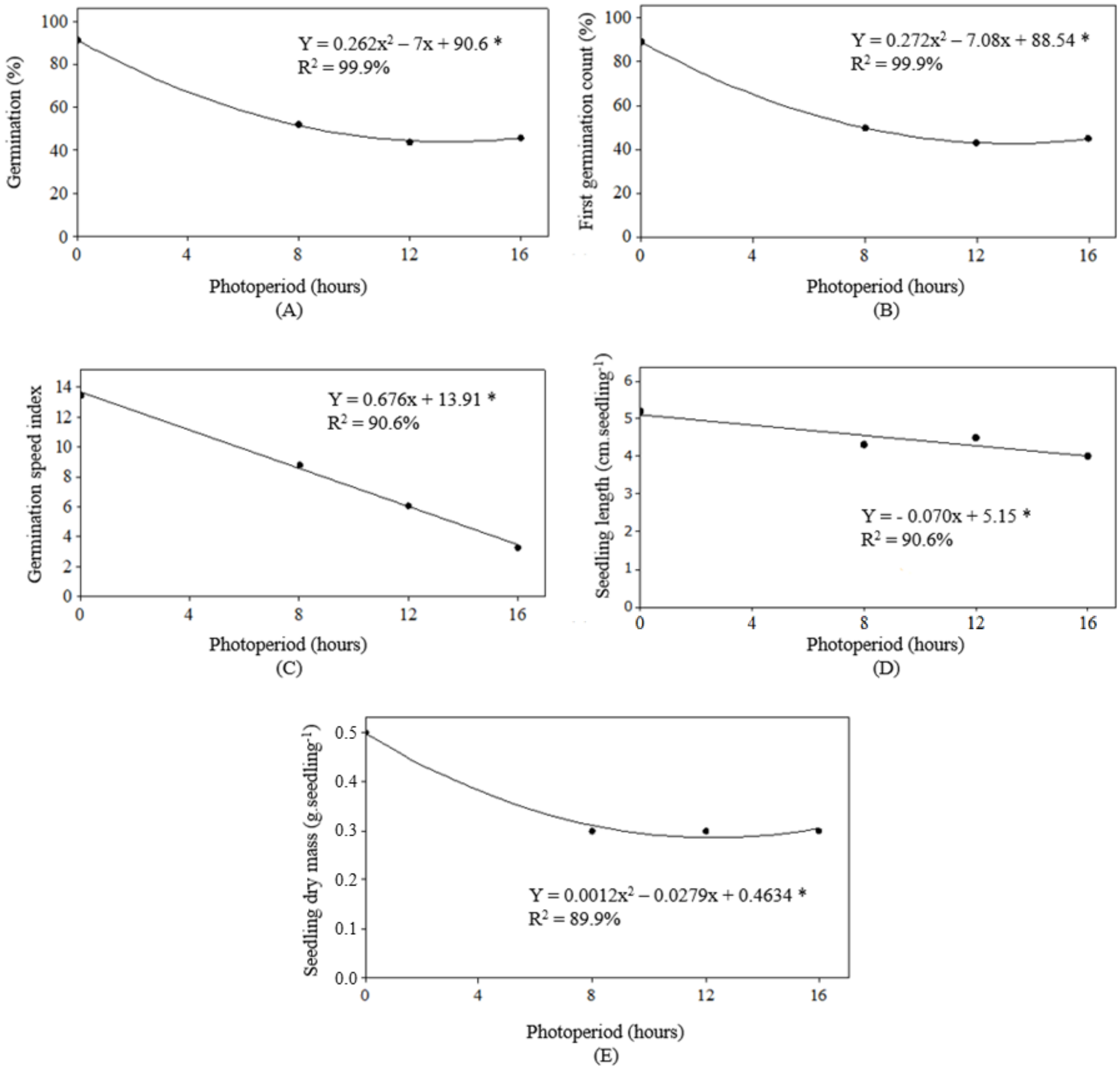

Figure 2. Germination (A), first germination count (B), germination speed index (C), seedling length (D) and seedling dry mass (E) of *Physalis angulata* L. as a function of photoperiod. *P < 0.001.

Most of the research with germination of *P. angulata* was conducted under a 12-h photoperiod, obtaining percentages above 80% (SOUZA et al., 2010; SOUZA; SOUZA; PELACINI, 2011; SOUZA et al., 2014). However, Oliveira et al. (2013) obtained, *in vitro*, germination above 80%, under the 16-h photoperiod, at 25 ºC, while Carvalho et al. (2014) obtained germination of 89% with 8-h photoperiod, under the alternating temperature of 20-30 ºC. For *P. peruviana*, Diniz, Chamma, and Novembre (2020) consider that the 8-h photoperiod is a more adequate condition than the absence of light during the germination test.

The results of this study contradict those obtained by Carvalho et al. (2014) and Oliveira et al. (2013) and allow deducing that *P. angulata* is a neutral photoblastic species and may acquire high sensitivity to light, possibly as a physiological response and adaptation to the environment where the plant develops (MARCOS-FILHO, 2015). Although *P. angulata* has characteristic behavior of pioneer species, quickly establishing itself in anthropized areas, this species can suffer strong environmental stress when exposed to sunlight for a prolonged time, due to its herbaceous consistency (SANTIAGO et al., 2019a). In this context, Freitas, Rodrigues and Osuna (2006) found that *P. angulata* plants tend to express higher growth in height and number of leaves when grown under 20 to 50% shading, respectively. Similarly, Rufato et al. (2008) also found that *P. peruviana* plants tend to show better development under shading.

Information on the ecophysiology of *P. angulata* seeds is useful, not only for plant management purposes, but also to promote the standardization of the germination test, since there are no specific recommendations for this species in the national and international Rules for Seed Analysis.

Throughout the study, it was found that, after the first count, at seven days, there was little or no increase in germination. Therefore, the period of 28 days to perform the germination test for the species *P. pubescens*, recommended by the Rules for Seed Analysis (BRASIL, 2009), is relatively long for *P. angulata* seeds. In this context, it was observed that the period of 14 days for performing the germination test of *P. angulata* seeds is the most appropriate, and the first count can be carried out between the third or fourth day after sowing. The same applies to *P. peruviana*, for which the period of 14 days is also the most indicated (DINIZ; CHAMMA; NOVEMBRE, 2020).

CONCLUSION

P. angulata seeds express better physiological potential when the calyx and fruit are yellow. In the germination test, the seeds should be sown between paper towels, under the temperature of 35 °C and in the absence of light.

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