REVISTA DELOS
DESARROLLO LOCAL SOSTENIBLE

Photoperiod and temperature on seed germination of passion fruit 'SCS437 Catarina'

Fotoperíodo e temperatura na germinação de sementes de maracujá 'SCS437 Catarina'

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ABSTRACT
The passion fruit (*Passiflora edulis* Sims.) propagates by sexual reproduction and presents slow and uneven seed germination. This work has the goal of identifying a temperature and photoperiod that provide greater germination of the cultivar SCS 437 Catarina. We used an entirely randomized experimental design in a 2 x 3 + 1 factorial: two temperatures (alternating between 20/30 ºC and constant 25 ºC) and three photoperiods (8/16 – 8 hours of light and 16 hours of darkness; 12/12 – 12 hours of light and 12 hours of darkness; and absence of light), and for alternating temperature, an absence of light treatment was added, being classified as neutral photoblastic, and at a constant temperature of 25 ºC made germination unfeasible, while the 20/30 ºC alternating temperatures provided an average germination of 75%. The seeds showed an indifferent behavior to light, and the temperature alternated with 12/12 h photoperiod was the ideal condition to promote a higher percentage of germination.

Keywords: *Passiflora edulis* Sims, photoblastic, abiotic factors, germinability.

RESUMO
O maracujazeiro-azedo (*Passiflora edulis* Sims.) é propagado por via sexuada e apresenta germinação lenta e desuniforme das sementes. O objetivo do trabalho foi identificar a temperatura e o fotoperíodo que proporcionam maior germinação da cultivar SCS 437 Catarina. Foi utilizado o delineamento inteiramente casualizado em esquema fatorial 2 x 3 + 1: duas temperaturas (alternada de 20/30 ºC e constante de 25 ºC) e três fotoperíodos (8/16 - 8 horas de luz e 16 horas de escuro; 12/12 - 12 horas de luz e 12 horas de escuro; e ausência de luz), e para temperatura alternada foi adicionado um tratamento de ausência de luz, sendo classificadas como fotoblásticas neutras, e a temperatura constante de 25 ºC inviabilizou a germinação, enquanto a temperatura alternada 20/30 ºC proporcionou germinação de 75 %. As sementes apresentaram um comportamento indiferente à luz, e a temperatura alternada com fotoperíodo 12/12 h foi a condição ideal para promover maior percentual de germinação.

Palavras-chave: *Passiflora edulis* Sims, fotoblastica, fatores abióticos, germinabilidade.

1 INTRODUCTION

Brazil is the largest producer and consumer of passion fruit in the world, with more than 50 % of world production, with passion fruit (*Passiflora edulis* Sims.) responsible for 95 % of production (Petry & Marchesi, 2019). The propagation of passion fruit is carried out mainly through seeds.

Seed germination derives from a sequence of physiological events influenced by intrinsic factors and extrinsic factors or environmental factors (Kleczewski et al., 2010). Extrinsic factors can affect germination and seedling establishment, both at the laboratory level and under natural conditions in the field. Germination problems are common in the Passiflora genus, even for the passion fruit. According to Pereira e Dias (2000), the seeds present low and uneven germination, with a long period between the beginning and the end of germination, making it difficult to form uniform seedlings.

The temperature has a great influence both on the percentage of germinated seeds and on the speed of germination, in the state of quiescence and dormancy, affecting the speed of water absorption and the biochemical reactions that regulate the metabolism involved in these processes (Marcos Filho, 2005). In seed germination, the effects of temperature are mainly related to two processes: the transconformation of macromolecules, especially proteins, and the regulation of gene expression. The transconformation can mean, in the case of an enzyme, an alteration in its catalysis capacity. Therefore, it modifies the chemical reactions that will mobilize or degrade the stored reserves and the synthesis of several substances in the seed for the later growth of the seedlings. While harsh thermal fluctuations can prevent or repress specific genes, thus altering the epigenetic program of the seed (Cardoso, 2013).

According to the Regras para Análise de Sementes (RAS) (Brasil, 2009), to evaluate the germination of *P. edulis* seeds, the ideal temperature can be either 20/30 ºC alternating or 25 ºC constant, in the absence of light. In the study by Pereira e Andrade (1994), testing the germination conditions of passion fruit, they recommended using an alternating temperature regime of 20/30 °C to favor germination.

The germinal response to photoperiod is associated with the phenology of the plant. Light can participate by inducing or overcoming dormancy, and in germination itself. Seeds can be divided into three groups, depending on their germinal response to white light: aphotoblastic or neutral, which would be indifferent to light; positively photoblastic, that have higher germinability and/or germination speed under light than in the dark; and negatively photoblastic that show a higher response to germination in the dark (Cardoso, 2013).

Maciel e Bautista (1997) obtained a higher percentage of germination in dark conditions, with reduced values during exposure to light, and concluded that passion fruit seeds are negatively photoblastic. Ribeiro et al. (2017) found that passion fruit seeds exposed to dark periods had higher emergence values compared to those exposed to continuous light or continuous dark, inferring that passion fruit seeds need periods in the absence of light to trigger the process of germination.

This indicates a high divergence related to the response to the presence or absence of light during the germination period, as well as the ideal temperature for germination tests of the passion fruit. Therefore, this work aimed to identify the influence of temperature and photoperiod on seed germination of the passion fruit, cultivar SCS 437 Catarina.

2 MATERIAL AND METHODS

The experiments were carried out at the Laboratório de Sementes do Departamento de Diagnóstico e Pesquisa Agropecuária, da Secretaria da Agricultura, Pecuária e Desenvolvimento Rural (DDPA/SEAPDR) of Rio Grande do Sul in Porto Alegre between October and December 2019, with seeds of passion fruit 'SCS437 Catarina', produced at the Estação Experimental Agronômica of the Universidade Federal do Rio Grande do Sul (EEA/UFRGS), Eldorado do Sul, RS – Brazil (30°06' S, 51°39' W, 58 meters of altitude). The soil where the passion fruit orchard is located is classified as a typical Dystrophic Red Argisol (Santos et al., 2018) and the climate of the region is characterized as humid subtropical, classified as Cfa by Köppen, with an average annual temperature and precipitation of 18.8 °C and 1,455 mm, respectively (Bergamaschi et al., 2013).

The seeds were extracted from fruits harvested on 06/28/2019 and stored for 15 days in a cold chamber at 7 °C, with relative humidity between 85 and 95 %. The fruits were cut in half, pulped and then the aril was removed by friction and rubbing the seeds with limestone over a sieve under running water. Later, the seeds were placed on paper to dry in shadow for about 2 days. After drying, they were placed in a transparent plastic bag and stored in a refrigerator at 4 °C until the beginning experiment.

The evaluation of the viability of the seeds was performed with a modified tetrazolium test. Due to the lack of recommendation of the tetrazolium test for passion fruit by the RAS, an adaptation of the routine methodology of other species from the Laboratório de Sementes DDPA/SEAPDR was carried out. It consisted of soaking 200 seeds in distilled water for 24 hours at 25 ºC, with a subsequent cross-section in the seed, and imbibition in the tetrazolium solution at a concentration of 1 % for 4 hours at 25 ºC in the absence of light. The embryos were visualized under a magnifying glass, being considered viable those that presented a pink color in the entire embryonic axis, radicle, and in more than 50 % of the cotyledon. The seed lot showed 90 % viability.

To avoid contamination by pathogens during germination, asepsis of the seeds was performed by immersion in 70 % alcohol for one minute and then in a 1 % sodium hypochlorite solution for three minutes, according to the adaptation of the methodology by Correia (2018) and DDPA/SEAPDR routine.

The design used was completely randomized, in a 2 x 3 + 1 factorial, the first factor being temperature (alternate at 20/30 ºC and constant at 25 ºC) and photoperiod the second factor (8/16 - 8 hours of light and 16 hours of dark; 12/12 - 12 hours of light and 12 hours of dark; and absence of light). For alternating temperature, treatment with an absence of light was added due to temperature variation between the photoperiods (2 treatments with all dark periods: 8/16 - 8 hours of darkness at 30 °C and 16 hours of dark at 20 °C; 12/12 - 12 hours of dark at 30 °C and 12 hours of dark at 20 °C).

Seeking the homogeneity of the seed lot for the germination test, a total of 100 seeds distributed in 4 replications of 25 seeds per treatment were used. The germination test was carried out in a gerbox box with a double layer of blotting paper soaked in water, equivalent to 2.5 times the weight of the paper. The boxes were placed inside transparent polyethylene bags to maintain humidity, in germination chambers, at the temperature and photoperiod adequate for each treatment.

The variables evaluated were: germination percentage, mean germination time (MGT), vigor, and germination speed index (GSI). The values of the first germination count and the germination speed index started on the 7th day after sowing (DAS) as indicated in the RAS. The measures stopped when, after two counts, the seeds showed no more germination, which corresponded to 44 days. Intermediate counts were performed with an interval of 3 days, to reduce the risk of damaging the structures of the seedlings that were not well developed, the loss of moisture from the substrate, the contamination of the test and to define the GSI.

Seed vigor was evaluated by the first germination count, performed together with the germination test. The normal seedlings present after 7 days of sowing were counted and the results were expressed in the percentage of normal seedlings. For the GSI the formula of Maguire (1962) was used and for the MGT the formula of Edmond e Drapala (1958). The MGT was distinguished in MGT– physiological, considering the radicle protrusion; and the MGT – technological, which considers the development of all the structures of the embryo, and open cotyledons. This differentiation allowed us to follow the time that goes from the protrusion of the radicle to the appearance of all the structures considered essential.

In the germination test, according to the RAS, every seed that presents emergence and development of the essential structures of its embryo to produce a normal plant under normal and favorable field conditions is considered germinated. Thus, germinated seeds were those that presented all the structures and cotyledons exposed and open.

Seeds that did not germinate during the evaluated period were considered: hard or dormant when, at the end of the test, the seeds were not swollen, with the appearance of newly placed seeds in the substrate; or dead, when they were softened, attacked by microorganisms or when they formed deformed, deteriorated or damaged seedlings.

The results of the variables analyzed were submitted to analysis of variance and the means compared by Tukey's test ($p \geq 0.05$). The absolute values were subjected to tests of normality and equality of variances (ANOVA) with the aid of the computer software R v.3.6.1 (R Core Team, 2019) and polynomial regression using the SigmaPlot 14.0 software. The data of the variables dead seeds, 1st and 2nd count of germinated seeds needed to be transformed, aiming at the adequacy for the feasibility of the analysis of variance (ANOVA).

3 RESULTS AND DISCUSSION

The germination percentage of passion fruit seeds 'SCS437 Catarina' was influenced by the temperatures tested (Table 1). At a constant temperature of 25 ºC, seed germination did not occur regardless of the presence of light. This result contradicts the indication of the Regras para Análise de Sementes (RAS) (Brasil, 2009), which suggests the germination of *P. edulis* at both temperatures (25 ºC and 20/30 ºC). The result of the present study corroborates with Santos et al. (1999), who studied the germination of seeds of *P. edulis* Sims f. *flavicarpa* Degener, at alternating temperatures of 20/30 ºC and a constant temperature of 25 ºC, indicating that the alternating temperature is the most suitable for its germination.

Table 1: Germination of passion fruit seeds at constant temperatures of 25 ºC and alternating at 20/30 ºC.

Temperature (ºC)	Germination (%)
25	0
20/30	75

The absence of germination at a constant temperature of 25 ºC indicates that the passion fruit 'SCS437 Catarina' needs thermal alternation to reach the ideal temperature range for germination and that the constant temperature of 25 ºC alone is not enough to supply this thermal demand. This is because quiescent seeds do not germinate unless they encounter a set of environmental factors that are not limiting to their needs, such as temperature (Cardoso, 2013).

According to Marcos Filho (2005), the lack of germination of passion fruit seeds can be explained because seeds of some species have different requirements concerning the daily fluctuation of temperature to germinate. The alternation of temperature alters the balance of substances promoting and inhibiting germination, reducing the amount of the latter during the low-temperature cycle and increasing the amount of promoting substances during the high-temperature phase. These promoter and inhibitor substances would be the phytohormones, of which, mainly gibberellin and cytokinin would act as germination promoters, while abscisic acid would act as a dormancy inducer (Taiz & Zeiger, 2017).

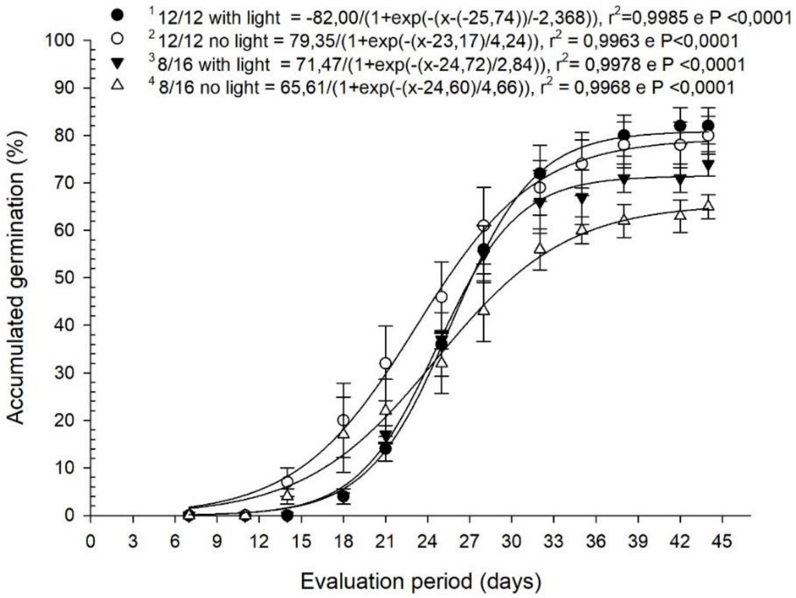
Zanini et al. (2016) also showed the absence of germination of P. edulis seeds at a constant temperature of 25 °C. In their study, only seeds that received immersion in a solution of 500 mg L^{-1} of gibberellic acid (AG3) for 10 seconds showed germination, ranging from 71 to 86 %. Santos et al. (2013) evaluated the action of AG3 on seed germination and vigor of *P. edulis* seedlings in a germinator at a temperature of 25 ºC with a photoperiod of 12 h and observed that AG3 stimulated the percentage of passion fruit seed germination. At the concentration of 100 mg AG3 L^{-1} of the solution, germination was 64 %, thus obtaining an increase of 42 % compared to the control. This is because gibberellin acts as a mediating hormone between environmental factors, such as light and temperature, and has action in the synthesis of specific proteins and RNA in germination, both in overcoming dormancy and by controlling the hydrolysis of reserves, on which the growing embryo depends (Taiz & Zeiger, 2017).

The germination response to thermal demand may also be variable according to the genetic material used, as observed by Osipi e Nakagawa (2005). These authors studied the effect of temperature on the germination of passion fruit (*P. alata* Curtis) seeds, using fruits from five second-year commercial plants as seed suppliers, and two germination temperatures in the absence of light, at 25 °C constant and 20/30 °C alternating with a photoperiod of 8/16 h. They found that germination ranged from 58 to 91 % at 20/30 ºC and 0 to 8.5 at 25 ºC. Interestingly, the plant that presented seeds with the highest percentage of germination at an alternating temperature of 20/30 ºC (91 %), showed 100 % of dormant seeds at 25 ºC, while the plant with lower germination at alternating temperature (58 %) was the which showed higher germination (8.5 %) at a constant temperature.

The variable response of germination to the condition considered ideal and from genetic material was also evidenced in the study by Souto et al. (2017). The authors evaluated five alternating temperature ranges (5/15 °C, 10/20 °C, 15/25 °C, 20/30 °C, and 25/35 °C) and three cultivars of passion fruit ('BRS Sol do Cerrado', 'BRS Gigante Amarelo' and 'FB-200 Yellow Master') and obtained different responses from the materials tested. The alternating temperature of 5/15 °C and 10/20 °C inhibited seed germination of all cultivars. For the cultivar BRS Sol do Cerrado, both temperatures of 20/30 °C and 25/35 °C stimulated germination (above 94 %), with no statistically significant difference between the two temperatures. For the cultivar BRS Gigante Amarelo, the alternating temperature of 25/35 °C stimulated greater germination, reaching above 98 %, while the other temperatures reduced germination, 20/30 ºC (88 %). And for the cultivar FB-200 Yellow Master, the seed emergence values remained below 65 %, which may be related to the genetic difference between the materials of passion fruit plants and their thermal requirements.

According to the accumulated germination curves (Figure 1), the alternating temperature of 20/30 °C (12/12 h) was more satisfactory for the germination percentage of the passion fruit 'SCS437 Catarina', both in photoperiod and in the absence of light. On the other hand, the temperature of 20/30 °C (8/16 h) proved to be less satisfactory, especially in the absence of light, where germination was 65 %, with a higher amount of hard seeds.

Figure 1: Percentage of accumulated germination, for the four photoperiods tested for the alternating temperature of 20/30 ºC. [1]12/12 h light (12 h light at 30 ºC and 12 h dark at 20 ºC), [2] 12/12 h dark (all period in the dark, varying 12 h at 20 ºC and 12 h at 30 ºC); [3] 8/16 h light (8 h light at 30 °C and 16 h dark at 20 °C) and [4] 8/16 h dark (all period in the dark, with varying exposure time to temperature, 8 h at 30 °C and 16 h at 20°C).



The light requirement is often observed in small seeds, where sunlight would help to ensure that the seedlings become photosynthetically self-sufficient before their reserves are exhausted (Taiz & Zeiger, 2017). There was no significant difference in germination concerning the presence or absence of light. In the first count of germinated seeds, as shown in Table 2, the seeds that did not receive light showed higher germination, however, with the course of the evaluation time, the effect of light was no longer visible, thus inferring a behavior indifferent to light.

Table 2: Percentage of hard or dormant seeds (D), dead seeds (M), 1st and 2nd count of germinated seeds (with 7 and 14 days, respectively), germination speed index (GSI), mean physiological germination time (MGT-p), and average time of technological germination (MGT-t) at 44 days as a function of photoperiods at temperature 20/30 ºC.

Treatments	D (%)	M (%)	1ª (%)	2ª (%)	GSI	MGT-p (days)	MGT-t (days)
12/12 Light[1]	16,00 b	2,00 ^{ns}	1,00 b	8,00 ^{ns}	0,76 ^{ns}	19,80 ^{ns}	27,85 ^{ns}
12/12 Dark[2]	17,00 b	3,00 ^{ns}	8,00 a	26,00 ^{ns}	0,86 ^{ns}	18,25 ^{ns}	25,46 ^{ns}
8/16 Light[3]	23,00 ab	3,00 ^{ns}	2,00 ab	6,00 ^{ns}	0,71 ^{ns}	19,72 ^{ns}	27,32 ^{ns}
8/16 Dark[4]	31,00 a	4,00 ^{ns}	9,00 a	19,00 ^{ns}	0,67 ^{ns}	19,98 ^{ns}	26,44 ^{ns}
CV (%)	26,14	42,71	38,12	37,94	14,62	12,06	7,93

[1]12/12 h light (12 h light at 30 ºC and 12 h dark at 20 ºC), [2] 12/12 h dark (all period in the dark, varying 12 h at 20 ºC and 12 h at 30 ºC); [3] 8/16 h light (8 h light at 30 °C and 16 h dark at 20 °C) and [4] 8/16 h dark (all period in the dark, with varying exposure time to temperature, 8 h at 30 °C and 16 h at 20°C). *Means followed by the same letter in the column do not differ significantly from each other, by Tukey's test ($p \geq 0.05$).

Passos et al. (2004) obtained similar results when studying the in vitro germination of *P. nitida* Kunth seeds, they did not verify a significant effect of light/dark. Marques e Ferreira (2009) observed the behavior of *P. setacea* DC. seeds do not depend on light and dark conditions for germination percentage. And the result is also reinforced by the study by Ribeiro et al. (2017), who evaluated three periods of light exposure (0, 12, and 24 h) in *P. edulis* seeds set to germinate at an alternating temperature of 20/30 ºC, obtained a significant difference in germination between the tested periods of light exposure. The average percentage of emergence was 83, 92, and 86 %, respectively. Thus they concluded that seeds exposed to a period of 12 hours of light/dark presented higher values of emergence compared to those exposed to continuous light or dark, and allowed the development of more vigorous seedlings, both for sprouting and root.

In the study by Balsalobre et al. (2006), there was an inhibitory effect of light on germination in *P. edulis* seeds, once they were submitted to germination in the absence and presence of light at a temperature of 25 ºC with a photoperiod of 12 h, germination was 5 % when exposed to light and 34 % in the absence of light. Zucareli, Henrique e Ono (2015), studying the influence of light and temperature on the germination of *P. incarnata* Linnaeus, also observed that light has an inhibitory effect on seed germination.

Seeds that have the highest germination speed index (GSI) are considered more vigorous seeds (Oliveira et al., 2009). In the present study, there was no significant difference between treatments for IVG (Table 2), as well as for the average time of physiological germination (MGT-p) and the average time of technological germination (MGT-t). The MGT is a test of vigor correlated with the GSI, since the MGT is the average time required to reach maximum germination (days), and the shorter this time, the greater the germination speed (Oliveira et al., 2009). The MGT was controlled for 44 days and had the maximum average germination between 25 and 28 days, which is not in accordance with the RAS (Brasil, 2009), when indicating the last evaluation of seed germination at 28 days, since this was the moment of maximum germination peak and not its stabilization (Table 3), where germination at 28 days had not yet shown a significant difference between the photoperiods tested.

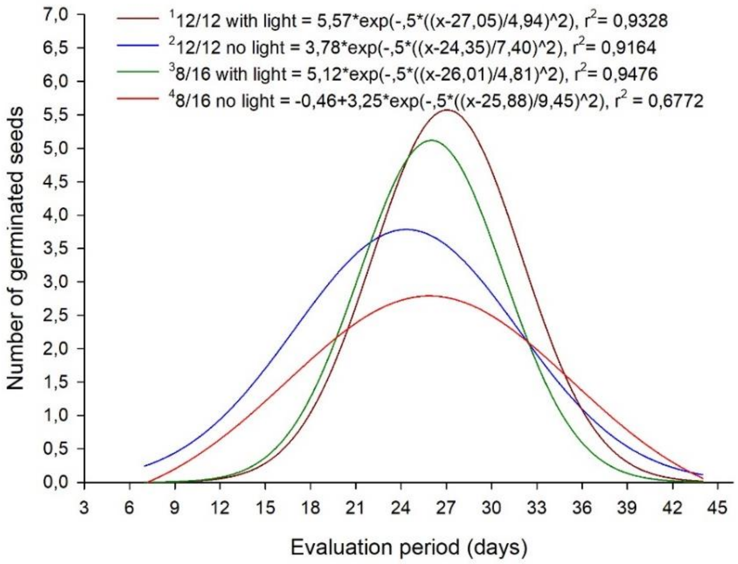
Table 3: Percentage of seed germination at 28 and 44 days as a function of photoperiods at 20/30 ºC.

Treatments	Germination (%)	
	28 days	44 days
12/12 Light[1]	56,00 ns	82,00 a
12/12 Dark[2]	61,00 ns	80,00 a
8/16 Light[3]	55,00 ns	74,00 ab
8/16 Dark[4]	43,00 ns	65,00 b
CV (%)	24,14	8,78

[1] 12/12 h light (12 h light at 30 ºC and 12 h dark at 20 ºC), [2] 12/12 h dark (all period in the dark, varying 12 h at 20 ºC and 12 h at 30 ºC); [3] 8/16 h light (8 h light at 30 °C and 16 h dark at 20 °C) and [4] 8/16 h dark (all period in the dark, with varying exposure time to temperature, 8 h at 30 °C and 16 h at 20°C). [*]Means followed by the same letter in the column do not differ significantly from each other, by Tukey's test ($p \geq 0.05$).

There was a reduction in the average number of new seeds germinated from days 28 and 32 for all treatments (Figure 2), and it is possible to observe a different behavior for seeds set to germinate in the presence and absence of light. The seeds of the treatments with the presence of light, presented an initial delay in the germination, as already mentioned, starting from 14 days with stabilization between 38 and 42 days. And they showed a higher concentration of seed germination, reducing asynchronism and unevenness in the formation of the stand. For seeds conditioned in the dark, it is possible to observe a less accentuated germination curve, with greater asynchronism, starting at 7 days, and stabilizing between 42 and 44 days. However, for all treatments, it is possible to observe from 32 days, that the increment in the number of germinated seeds was low, around 1.5 seeds per day.

Figure 2: The average number of germinated seeds, for the four photoperiods tested for the alternating temperature of 20/30 ºC. [1]12/12 h light (12 h light at 30 ºC and 12 h dark at 20 ºC), [2] 12/12 h dark (all period in the dark, varying 12 h at 20 ºC and 12 h at 30 ºC); [3] 8/16 h light (8 h light at 30 °C and 16 h dark at 20 °C) and [4] 8/16 h dark (all period in the dark, with varying exposure time to temperature, 8 h at 30 °C and 16 h at 20°C).



The different results obtained by the authors mentioned above and the results found in germination regarding the thermal and luminous requirements, show the existence of a variable behavior for the species of the genus, being able to even distinguish between the cultivars of the same species. This is probably due to this species still being in the process of domestication, and for the cultivar SCS437 Catarina being the result of genetic improvement by the method of mass selection and not made from matrices, which can provide greater variation regarding the requirements of the environment for greater germinability of the seeds.

According to IN42 of MAPA (2019), in the identity and quality standards for the production and marketing of *P. edulis*, a minimum germination of 75 % was established. Using the temperature of 20/30 ºC with the photoperiod 12/12 h in the presence or absence of light, this pattern was achieved with the cultivar 'SCS437 Catarina'.

4 CONCLUSION

According to the results obtained and under the conditions in which the experiment was carried out, it can be concluded that the constant temperature of 25 ºC does not promote the germination of seeds of *P. edulis* 'SCS437 Catarina. The seeds of 'SCS437 Catarina' show an indifferent behavior to the use of light, being considered aphotoblastic or neutral. The presence of light favored the concentration of seed germination, promoting greater uniformity of the stand.

The alternating temperature of 20/30 ºC with photoperiod 12/12 h is the most suitable environmental condition to express the highest germination values of *P. edulis* 'SCS437 Catarina' seeds and is indicated for carrying out germination tests of seeds.

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