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RESEARCH NOTE

Influence of light and temperature on the germination of *Passiflora incarnata* L. seeds[1]

Valdir Zucareli[2*], Luma Alana Vieira Henrique[2], Elizabeth Orika Ono[3]

ABSTRACT – The study aimed to assess the influence of temperature and light on germination of *Passiflora incarnata* L seeds. The treatments consisted of five temperature conditions (25, 30, 35, 20-30 and 30-20 °C) and two light conditions (constant light and constant darkness). The experimental design was completely randomized in a 2 x 5 factorial arrangement (light x temperature). The seeds were kept in a germination chamber during the period of 30 days. The germinated seeds were counted daily. At the end of the experiment, the percentage of seed germination (PSG), germination speed index (GSI), the germination rate (GR) and relative frequency of germination (RFG) were calculated. The data obtained were subjected to analysis of variance and means were compared by Tukey test at 5% probability. The relative frequency of germination was represented as graphs. It was observed that light has an inhibitory effect on seed germination. The constant temperature of 35 °C and the alternating temperatures of 30/20 °C promoted a higher germination seed index for *Passiflora incarnata.*

Index terms: passion fruit, medicinal plants, seed propagation.

Influência da luz e da temperatura na germinação de sementes de *Passiflora incarnata* L.

RESUMO – O trabalho teve como objetivo avaliar a influência da temperatura e da luz na germinação de sementes de *Passiflora incarnata* L. Os tratamentos foram constituídos de cinco condições de temperaturas (25, 30, 35, 20-30 e 30-20 ºC) e duas condições de luz (luz constante e escuro constante). O delineamento utilizado foi o inteiramente casualizado, em esquema fatorial 2 x 5 (luz x temperatura). As sementes foram mantidas em câmera de germinação durante o período de 30 dias. A contagem das sementes germinadas foi realizada diariamente. Ao final do experimento foram calculados a porcentagem de germinação, o índice de velocidade de germinação – IVG, a velocidade de germinação – VG e a frequência relativa de germinação. Os dados obtidos foram submetidos à análise de variância e as médias comparadas pelo teste Tukey a 5% de probabilidade de erro. A frequência relativa de germinação foi representada na forma de gráficos. Observou-se que a luz tem efeito inibitório sobre a germinação das sementes. A temperatura constante de 35 °C e a alternada 30/20 °C promoveram maior índice de germinação das sementes de *Passiflora incarnata*.

Termos para indexação: maracujá, plantas medicinais, propagação.

Introduction

The Passifloraceae family has twelve genera. From these, the one with the largest number of representatives is the *Passiflora* genus, comprising approximately 500 species (Junqueira et al., 2005).

Passion fruit (*Passiflora incarnata*) is used as a medicinal plant by the peoples of Europe, America and Asia due to its pharmacological characteristics, especially the benefits for the nervous system and it is used to relieve anxiety, depression and insomnia states (Lorenzi and Matos, 2002).

The species *Passiflora incarnata*, also known as purple passion fruit, has edible fruit (Dhawan et al., 2001) in use since the colonization of the Americas due to its sedative, soothing and antispasmodic effects (Lorenzi and Matos, 2002), being present in the formulation of many pharmaceutical medicaments and in

[1]Submitted on 03/12/2015. Accepted for publication on 06/16/2015.
[2]Centro de Ciências Agronômicas, Universidade Estadual de Maringá, Caixa Postal 65, 87501970- Umuarama, PR, Brasil.
[3]Departamento de Botânica, Universidade Estadual Paulista Júlio de Mesquita Filho, Caixa Postal 510, 18618000 - Botucatu, SP, Brasil.
*Corresponding author <valdirzucareli@yahoo.com.br>

combination with other drugs (Tankanow et al., 2003).

The passion fruit tree can be propagated by means of seeds or asexually by means of grafting, cuttings or tissue culture in vitro, and the propagation by seeds is the most used (Pantano, 2007). However, the seeds of most Passifloraceae have low germination rate which can be due to the existence of dormancy (Carvalho and Nakagawa, 2000; Pantano, 2007), which must be overcome for the continuation of the other germination processes.

In seeds, dormancy can be classified as primary or secondary and induced or natural. Primary dormancy is established in the seed maturation stage, developing in conjunction with the seed. Secondary dormancy begins after dispersion and does not always happen (Carvalho and Nakagawa, 2000).

Light is an extremely important factor for seed germination. However, there is disagreement in the response from different species to the absence or presence of this condition. For some species, the influence of light is favorable for germination, and for others it is negative or indifferent (Borges and Rena, 1993). This is because the light is responsible for the activation of phytochrome, a soluble chromoprotein which, in the inactive form (F_v), absorbs red (R) wavelength and is transformed into an active (F_{ve}) one (Taiz and Zeiger, 2013).

In passion fruit seeds, Passos et al. (2004) have studied the in vitro germination of *Passiflora nitida* and have not found a significant effect of light/dark on seed germination. As for Zucareli et al. (2009), they have studied the germination of *Passiflora cincinnata* seeds and observed an inhibitory effect of light on germination thereof.

Another environmental factor that also influences the seed germination process is temperature (Carvalho and Nakagawa, 2000; Marcos-Filho, 2005; Bewley et al., 2014). The influence can be in the germination percentage and rate, affecting water absorption and regulating the biochemical reactions that regulate the metabolism involved in the germination process (Bewley and Black, 1994; Marcos-Filho, 2005). Therefore, germination will occur within certain temperature limits and consequently the maximum germination efficiency will occur in a particular temperature range (Carvalho and Nakagawa, 2000; Bewley et al., 2014).

Santos et al. (1999) have studied the germination of *Passiflora edulis* f. *flavicarpa* seeds under constant temperature (25 °C) and alternating temperatures (20-30 °C) and have indicated the temperature alternation as the most appropriate for the species germination. Duarte Filho et al. (2000) have assessed the germination of *Passiflora gibertii* seeds at constant temperatures 20 °C, 25 °C and 30 °C and alternating temperatures 30-25 °C, 30-20 °C and 25-20 °C, all under constant white light and have found that temperature influences the percentage of emission of primary roots and normal and abnormal seedlings, and the alternating temperature (30-20 °C) has provided greater uniformity of germination and constant temperatures have provided lower averages for the variables germination percentage and normal seedlings percentage.

Zucareli et al. (2003) have assessed the germination of *Passiflora alata* seeds and concluded that, regardless of the treatment used, alternating temperatures between 20 °C and 30 °C has improved the germination. Osipi and Nakagawa (2005) have studied the germination of seeds of different plants of *Passiflora alata* under two temperature conditions (25 °C and 20-30 °C) and have observed that the alternating temperature 20-30 °C has allowed a higher percentage of germination, regardless of the plant, and have suggested that this may have favored the overcoming of seed dormancy.

In a study with seeds of *Passiflora cincinnata*, Zucareli et al. (2009) have concluded that the alternating temperature (20-30 °C) is more suitable for germination. In the Regras para Análise de Sementes (RAS; Rules for Seed Analysis) (Brasil, 2009) there is a recommendation for alternating temperature and absence of light for germination of *Passiflora edulis*. However, there are no recommendations for the remaining species of the genus.

Thus, this study aimed to assess the influence of light and temperature on the germination of *Passiflora incarnata* L. seeds.

Material and Methods

The work was conducted in the Laboratório de Botânica e Fisiologia Vegetal (Laboratory of Botany and Plant Physiology) of UEM (Universidade Estadual de Maringá), Regional Campus of Umuarama, PR, Brazil. The seeds used in the experiment were donated by the company Grupo Centroflora, Botucatu, SP, Brazil.

The experimental design was completely randomized in a 2 x 5 factorial arrangement (light versus temperature), totaling 10 treatments and 5 replicates containing 25 seeds per plot. The treatments consisted of five temperature conditions (25 °C, 30 °C, 35 °C, 20-30° C and 30-20 °C) and two light conditions (constant light and constant dark). For alternating temperature, the periods of 16 and 8 h were used and, to simulate the photoperiod, transparent acrylic boxes were used (constant light) and black coloring (constant darkness).

The *Passiflora incarnata* seeds were treated with fungicide Captan® (2 $g.kg^{-1}$). After treatment, the seeds were placed in acrylic boxes on two sheets of blotting paper previously moistened with distilled water. The boxes containing the seeds were kept in a germination chamber for 30 days.

The counting of germinated seeds was done daily from the day of the experiment deployment and the seeds that had at least 2 mm of primary root were considered germinated (Hadas, 1976).

At the end of the experiment were calculated: the percentage of germination (Brasil, 2009), the germination speed index (GSI) (Maguire, 1962) and the germination rate (GR) (Edmond and Drapala, 1958). GSI was calculated by the formula: $GSI = E_1/N_1 + E_2/N_2 + ... + En/N_n$, where: E_1, E_2, E_n = number of germinated seeds, computed in the first, second, ..., last count; N_1, N_2, N_n = number of days after the test deployment. The germination rate was obtained from the formula: $GR = ((N_1 \times E_1) + (N_2 \times E_2) + ... + (N_n \times E_n)) / (E_1 + E_2 + ... + E_n)$, where: E_1, E_2, E_n, N_1, N_2, N_n = with the same meanings of the above formula.

The data were subjected to analysis of variance and means compared by Tukey test at 5% probability. The relative frequency of germination was calculated according to Labouriau and Agudo (1987) and represented as graphs.

Results and Discussion

In Figure 1, it is possible to see that the germination started on the fifth day after the experiment deployment for the seeds subjected to temperatures of 30 °C, 35 °C, 30/20 °C and 20/30 °C and that the seeds subjected to temperature 25 °C germination only started from the seventh day. It is also observed that the highest percentage of germination occurred in the treatment using the constant temperature of 35 °C, in which case the germination stabilization occurred on the 13th day after the experiment deployment.

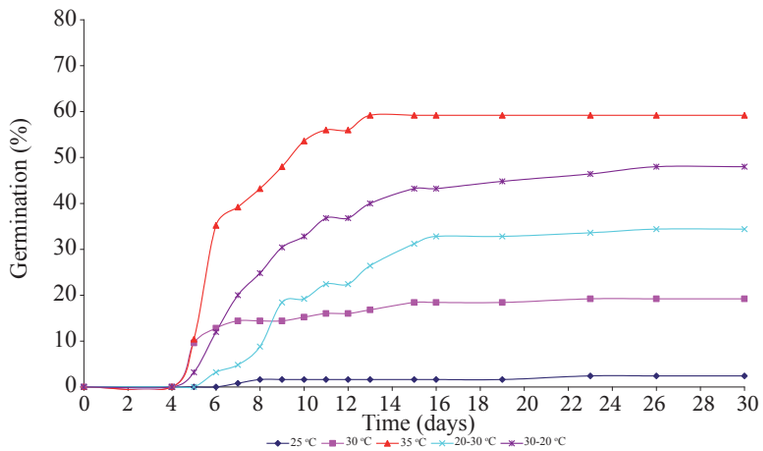

Figure 1. Germination (%) of *Passiflora incarnata* L. seeds subjected to different temperatures and constant darkness.

By analyzing Figure 1 it is possible to establish that for germination tests the first count and final assessment can be performed on the 10th and 15th days, respectively, for already on the tenth day the maximum cumulative average germination was obtained and after that period the accumulated average germination was constant. However, a period of five days was stipulated to verify nonoccurrence of germination. These results differ from the recommended ones for the species *P. edulis* in the Regras para Análise de Sementes (RAS; Rules for Seed Analysis) (Brasil, 2009), which advocates 7 and 28 days for the first count and final assessment, respectively, showing the existence of a differentiated behavior for the species of the genus.

Table 1 shows the averages for germination, germination speed (GS) and Germination Speed Index (GSI) obtained from *P. incarnata* seeds undergoing germination under different temperatures and two light conditions. In this one it is possible to see that the seeds subjected to the light absence condition had the highest averages for the variable germination percentage, regardless of the temperature used. This result corroborates that obtained by Zucareli et al. (2009), who reported higher germination values for *P. cincinnata* when the seeds remained in constant darkness. The are also in agreement with the Regras para Análise de Sementes (RAS; Rules for Seed Analysis) (Brasil, 2009), which recommend conducting germination tests in the dark for *P. edulis*.

Regarding temperatures, the lowest percentage of germination was observed in seeds that remained under the constant temperature of 25 °C, significantly differing from the others. The treatment that had higher germination values was the one of 35 °C (59%), which did not differ from the treatment using alternating temperatures of 30/20 °C (48%).

According to Salomão and Sousa-Silva (2003), the germination of species at high temperatures is not common, and the optimal temperature for the non-dormant seed germination is between 25 ºC and 30 ºC. As for the benefit of

alternating temperature in the passion fruit seeds germination, this was also reported by Santos et al. (1999) in seeds of *P. edulis*, by Osipi and Nakagawa (2005) in seeds of *P. alata* and by Zucareli et al. (2009) in seeds of *P. cincinnata*.

Table 1. Germination percentage, germination rate (GR) and germination speed index (GSI) obtained from *Passiflora incarnata* L. seeds subjected to germination under different temperatures and two light conditions.

Treatments	Germination (%)		GR		GSI	
	Light	Dark	Light	Dark	Light	Dark
25 ºC	0.0 Ba	2.4 Ad	0.00 Ba	6.40 Aa	0.000 Aa	0.072 Ad
30 ºC	0.0 Ba	20 Ac	0.00 Ba	7.68 Aa	0.000 Ba	0.934 Ac
35 ºC	0.8 Ba	59 Aa	4.40 Ba	6.28 Aa	0.058 Ba	2.630 Aa
20/30 ºC	2.4 Ba	35 Abc	6.20 Ba	10.46 Aa	0.034 Ba	1.050 Abc
30/20 ºC	1.6 Ba	48 Aab	4.60 Ba	9.06 Aa	0.008 Ba	1.678 Ab
Average	0.96	32.88	3.04	7.97	0.02	1.2728
F value						
Light	299**		27**		339**	
Temperature	24**		1.86^{ns}		24**	
Light x temperature	14**		0.49^{ns}		16**	
CV (%)	13.36		58		17	

Means followed by the same letter (uppercase in the row and lowercase in the column) do not differ by Tukey test at 5% probability. CV = Coefficient of variation; n.s.: not significant by Tukey test at 5% probability; *: significant by Tukey test at 5% probability and **: significant by Tukey test at 1% probability.

For the germination rate (GR) variable, it can be observed (Table 1) that there was a difference only for light and dark. It is also observed that despite the seeds being subjected to alternating temperatures 20-30 °C having a higher average germination rate, there was no difference between treatments, regardless of the lighting conditions. However, it should be noted that for germination speed the lower the value obtained, the higher the seed vigor, because it estimates the germination rate by the average days spent for germination (França-Neto et al., 1999).

For the germination speed index (GSI) there was a significant difference between treatments remaining in constant darkness. The higher the GSI obtained, the higher the germination rate implied and thus there is greater vigor of the lot, because the index calculated estimates the average number of normal seedlings per day (França-Neto et al., 1999).

It was found that seeds subject to constant temperature of 35 °C had higher GSI, differing from the other treatments. As for the lowest index, it was obtained in seeds subjected to low temperature (25 ºC). For Bewley et al. (2014), the germination rates (for example, GSI) are very sensitive to temperature, generally increasing with temperature until the optimal temperature and then decreasing sharply above the optimal temperatures. Although the total percentage of germination present a broad spectrum for maximum temperature, the germination rates can be used to more precisely identify the optimal temperature for germination.

Unlike the results obtained in this study, Zucareli et al. (2009) have observed that in the germination of *P. cincinnata* seeds the treatments with alternating temperatures of 20-30 °C and 30-20 °C showed the highest germination speed index when compared to constant temperatures, including 35 ºC.

Figure 2 depicts the germination frequencies for the seeds subjected to different temperatures and dark, whereas in the light germination was significantly lower. In seeds subjected to a temperature of 35 °C there was a single peak of germination on the 5th day after the start of the experiment. At the other temperatures, germination occurred over time with a higher number of peaks, except with the temperature of 25 °C, where it is possible to observe only two germination peaks, but they occurred in a greater time interval (days 6 and 19) than the other treatments. It is noteworthy that the temperature of 35 °C was the one that provided the highest germination rate concentrated in a shorter period of time, which results in a more uniform emergence and less exposure of the seeds to pathogens, as mentioned by Carvalho and Nakagawa (2000). According to Alexandre et al. (2004) the uniformity of the germination in passion fruit seeds can more efficiently provide seedling production on a commercial scale.

The higher germination rate obtained under higher temperatures can be explained by the need of these conditions to overcome the germination inhibitor system, as reported by Carvalho and Nakagawa (2000) and by Marcos-Filho (2005). Relatively high temperatures are essential in overcoming the seed dormancy of some species and it was also reported by Pereira et al. (2013) in *Peltophorum dubium* seeds, who

recommended the temperature of 35 °C for the seed germination of the species.

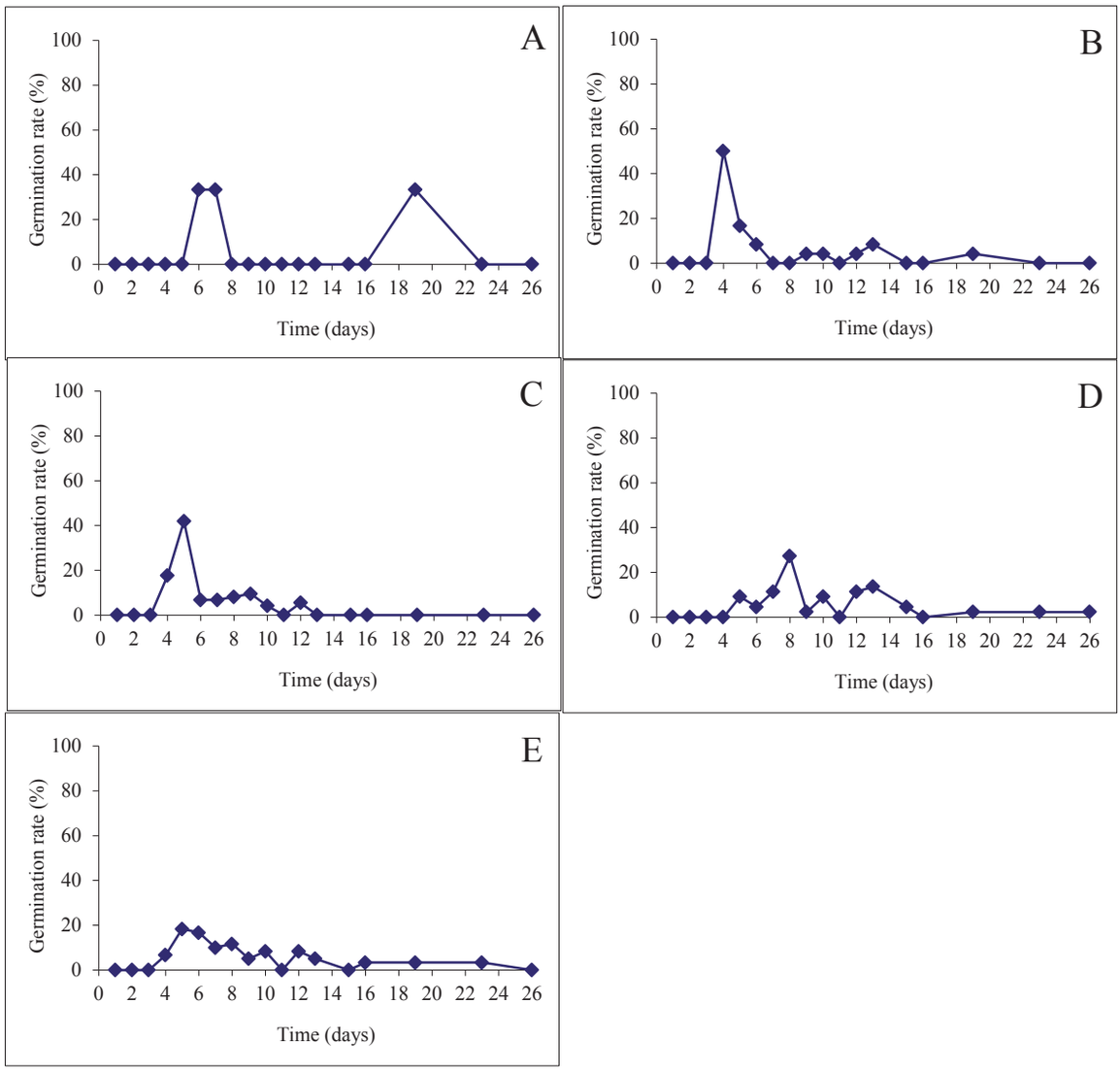

Figure 2. Germination rate (%) of *Passiflora incarnata* L. seeds subjected to different temperatures: 25 ºC (A); 30 ºC (B); 35 ºC (C); 30-20 ºC (D) and 20-30 ºC (E) and constant darkness.

Conclusions

From the results obtained in this study it can be concluded that light has an inhibitory effect on the germination of *Passiflora incarnata* seeds and that the temperature influences their germination, and the constant temperature of 35 °C and the alternating temperatures of 30/20 °C promoted the highest germination rates.

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