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EFFECTS OF TEMPERATURE, SUBSTRATE AND LUMINOSITY CONDITIONS ON CHIA SEED GERMINATION[1]

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ABSTRACT – The aim of the present study was to evaluate the effects of temperature, substrate, and luminosity on chia seeds in order to determine adequate conditions for a germination test. The experiment consisted of a completely randomized design, in a 4 × 5 × 2 factorial scheme (four substrates × five temperatures × two luminosity conditions), with four replications. The following variables were analyzed: first germination count, germination, germination speed index, and mean germination time. Germination was best at 15, 20, 25, and 30 °C for the on-paper, paper roll, and sand substrates. However, in the presence of luminosity and at 25 °C, the on-paper substrate had the highest mean. For all substrates, the lowest germination was achieved at 35 °C, proving that high temperatures reduce chia seed germination. Germination speed index was highest at 25 and 30 °C for on-paper, paper roll, and sand substrates, in both the presence and absence of luminosity. Chia seeds are considered to be neutral photoblasts. Germination tests should be performed with the on-paper substrate, at 25 °C, and with a duration of seven days. The first count should be carried out four days after establishment of the test.

Keywords: Lamiaceae. *Salvia hispanica*. Germinative potential. Photoblastism.

TEMPERATURA, SUBSTRATO E CONDIÇÃO DE LUMINOSIDADE NA GERMINAÇÃO DE SEMENTES DA CHIA

RESUMO - Este trabalho teve como objetivo avaliar a temperatura, o substrato e a luminosidade na germinação de sementes de chia, a fim de determinar procedimentos adequados para um teste de germinação. O experimento consistiu de um delineamento inteiramente casualizado, em esquema fatorial 4 x 5 x 2 (quatro substratos x cinco temperaturas x duas condições de luminosidade), com quatro repetições. As seguintes variáveis foram analisadas: primeira contagem de germinação; germinação; índice de velocidade de germinação e tempo médio de germinação. A germinação apresentou as maiores porcentagem de germinação a 15, 20, 25 e 30 °C para os substratos em papel, rolo de papel e areia; no entanto, na presença de luminosidade, o substrato em papel, a 25 °C, diferiu de todos os demais, resultando na maior média. Para todos os substratos, a menor germinação foi obtida a 35 °C, comprovando que o uso de altas temperaturas reduz a germinação das sementes de chia. O índice de velocidade de germinação apresentou os maiores valores a 25 e 30 °C para substratos em papel, rolo de papel e areia, tanto na presença quanto na ausência de luminosidade. Sementes de chia são consideradas como fotoblasticas neutras. O teste de germinação deve ser realizado com o substrato em papel, a 25 °C, com duração de sete dias, sendo a primeira contagem realizada aos quatro dias após a instalação do teste.

Palavras-chave: Lamiaceae. *Salvia hispanica*. Potencial germinativo. Fotoblastismo.

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INTRODUCTION

Salvia hispanica L. (Lamiaceae), commonly known as chia, is an herbaceous and oleaginous plant native to midwestern Mexico and northern Guatemala (CAPITANNI et al., 2012). In recent years, the seeds of this plant have gained attention due to the benefits they provide to human health (SANDOVAL-OLIVEROS; PAREDES-LÓPEZ, 2013), which include reducing cardiovascular diseases, cholesterol, triglycerides, and obesity, in addition to bowel regulation and prevention of type II diabetes and some cancers (VÁZQUEZ-OVANDO et al., 2010; IXTAINA et al., 2011; JIN et al., 2012).

Several studies on the nutritional and medicinal values of chia have been conducted (MARINELI et al., 2015; OLIVEIRA-ALVES et al., 2017; ALCÂNTARA et al., 2019), however, information on the agronomic aspects of the crop is still scarce. In Brazil, the regions of Western Paraná and Northwestern Rio Grande do Sul have successfully invested in chia cultivation in recent harvests, despite the lack of technical information on the crop. The emergence of new commercial cultivation areas has led to research on the cultivation priorities for the crop, such as the use of high-quality seeds, aiming at rapid emergence, and establishment of plants, all of which simplify crop management and lower the risks to the capital invested (HOFS et al., 2004).

Understanding such an important seed for human health is essential, not only for the preservation of the plant species, but also to obtain seeds of higher physiological quality, which is only possible when the optimal conditions for germination and development of the crop are made available. The germination test is the primary parameter used to evaluate the physiological quality of seeds, since it predicts the germination potential of a set of seeds under favorable conditions (CARVALHO; NAKAGAWA, 2012). Thus, the test should follow a standard procedure recommended by the Rules for Seed Analysis (BRASIL, 2009), which is an official publication that regulates seed analysis, so that germination occurs under the optimal conditions of each species. No standard methods have been developed for chia seed analysis, making it difficult to evaluate seed quality for this species.

Seeds perform differently according to the temperature and substrate used, which are basic components of the germination test. These components influence both germination percentage and speed, interfering with water absorption, pathogen infestation, and biochemical reactions (CARVALHO; NAKAGAWA, 2012). Therefore, knowing the influence of these components on the germination of a species is fundamental (MONDO et al., 2008).

The germination process can also be affected by internal or external factors (LUZ et al., 2014). Seed sensitivity to luminosity is quite variable depending on the species. Some seeds (positive and negative photoblasts) are positively or negatively influenced by luminosity, whilst others are not influenced at all (neutral photoblasts) (GUEDES; ALVES, 2011; CARVALHO; NAKAGAWA, 2012; POSSENTI et al., 2016; PAIVA et al., 2016).

The initial success of a crop is dependent on the use of high-quality seeds. Therefore, the aim of the present study was to evaluate the effects of temperature, substrate, and luminosity on the physiological potential of chia seeds, in order to determine adequate procedures for a germination test.

MATERIAL AND METHODS

The experiment was carried out using chia seeds purchased from producers from Chapadão do Sul, Mato Grosso do Sul (MS), Brazil (18° 47′ 39″ S; 52° 37′ 22″ W). The experiment consisted of a completely randomized design in a 4 × 5 × 2 factorial scheme, with four replications. Treatments comprised four substrates (on-paper, paper roll, on-sand, and between-sand), five temperatures (15, 20, 25, 30, and 35°C), and two luminosity conditions (presence and absence of luminosity). For the paper roll substrate, four replicates of 50 seeds were distributed over two sheets of germitest paper, moistened with a volume of distilled water equivalent to 2.5 times the mass of the non-hydrated paper, and were covered with another sheet of the same paper. Afterward, the papers were rolled and stored in plastic bags to avoid dehydration and kept in a B.O.D chamber under the previously described conditions.

For the paper substrate, four replicated of 50 seeds were placed in plastic boxes (gerbox type) with two sheets of germitest paper moistened with a volume of distilled water equivalent to 2.5 times the mass of the non-hydrated paper. Germination on-sand and between-sand were carried out with four replicated of 50 seeds in plastic boxes (gerbox type), using 200 g of sand that had previously been sieved, washed, autoclaved, and moistened with 40 mL of distilled water (based on 60% water retention capacity) (BRASIL, 2009). For the between-sand substrate, seeds were placed at 5.0 mm depth.

All replicates for all treatments were stored in a B.O.D (Biological Organism Development) chamber, at the temperatures described above, either in the presence of luminosity (four 20 W daylight fluorescent lamps with radiant flux density of 15 mmol m^{-2} s^{-1} at the height of the plastic boxes) (STEFANELLO et al., 2015) or in the absence of luminosity.

The effect of the treatments on seeds was evaluated using germination and vigor variables (first germination count, germination speed index, and mean germination time). For the germination test (G), seeds were counted daily, with root protrusion (greater than 2.0 mm in length) evaluated categorically (presence/absence). The duration of the test was determined as the number of days from sowing to germination stabilization. The first germination count (FGC) was performed four days after test installation (STEFANELLO et al., 2015). The germination speed index (GSI) was calculated according to the formula proposed by Maguire (1962), and the mean germination time (MGT) was obtained using the formula suggested by Labouriau (1983). Data were subjected to analysis of variance, and the means of treatments were compared using the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The interaction between temperature, substrate, and luminosity was significant for all variables analyzed in this study. For first germination count (Table 1), the temperatures of 15, 20, 25, and 30°C and the on-paper, paper roll, and on-sand substrates were the most suitable both in the presence and absence of luminosity. The temperature of 35°C was harmful regardless of substrate, and in both the presence and absence of luminosity, and germination count was lower than 25% at this temperature for the between-sand substrate.

Regardless of the luminosity condition, a negative effect of using the between-sand substrate was noted for all temperatures evaluated in this study. Moreover, no seeds germinated at four days after test installation in the absence of luminosity at 15°C, using the between-sand substrate (Table 1). This could be due to the difficulty of maintaining moisture in sand due to its medium texture, and uneven retention and distribution, which drains it excessively and leaves the top layers dry (ALVES et al., 2015). According to Figliolia et al. (1993), the water retention capacity and the amount of luminosity that the substrate provides to seeds can result in different responses even at the same temperature, as observed in this work.

Table 1. First germination count (%) and chia seed germination (%) as a function of different substrates, temperatures, and luminosity conditions.

Temp.	Presence of luminosity				Absence of luminosity			
	Substrates							
	OP	PR	OS	BS	OP	PR	OS	BS
	First germination count (%)							
15	70 Aa1	66 Aa1	70 Aa1	19 Bb1	64 Aa1	64 Aa1	61 Ab1	0 Bc2
20	77 Aa1	73 Aa1	68 Aa1	52 Ba1	71 Aa1	68 Aa1	75 Aa1	53 Ba1
25	77 Aa1	63 Aa2	70 Aa1	49 Ba1	74 Aa1	77 Aa1	72 Aa1	43 Bb1
30	72 Aa1	62 Aa2	72 Aa1	45 Ba2	73 Aa1	74 Aa1	70 Aa1	57 Ba1
35	58 Ab1	51 Ab1	55 Ab1	23 Bb1	67 Aa1	50 Bb1	63 Ab1	9 Cc2
	Germination (%)							
15	73 Aa1	71 Aa1	73 Aa1	66 Aa1	75 Aa1	78 Aa1	68 Ab1	68 Aa1
20	79 Aa1	77 Aa1	71 Aa1	70 Aa1	75 Aa1	72 Aa1	78 Aa1	65 Aa1
25	81 Aa1	71 Ba1	72 Ba1	64 Ba1	77 Aa1	80 Aa1	76 Aa1	65 Ba1
30	72 Aa1	64 Bb2	75 Aa1	55 Bb2	73 Aa1	75 Aa1	72 Aa1	66 Aa1
35	62 Ab1	54 Ab1	57 Ab1	35 Bc1	69 Aa1	52 Bb1	64 Ab1	20 Cb2

*Means followed by the same uppercase letter in the row, lowercase letter in the column, and same number (luminosity) do not differ significantly from each other according to a Scott-Knott test at 5% probability. OP - on-paper, PR – paper roll, OS - on-sand, BS – between-sand.

The highest percentages of germination were found at 15, 20, 25, and 30°C for the on-paper, paper roll, and on-sand substrates; however, in the presence of luminosity, the on-paper substrate differed from all other substrates at 25°C, resulting in the highest overall mean (Table 1). For all substrates, the lowest germination percentage was achieved at 35°C, proving that high temperatures reduce chia seed germination.

In most species, temperature influences germination speed and percentage because it alters the water absorption speed and the metabolic reactions of the reserves necessary for seedling survival (BEWLEY; BLACK, 1994). As observed in the first count, the between-sand substrate produced inferior germination values compared to the other substrates.

Chia seeds had maximum germination potential at 25°C using an on-paper substrate, which agrees with the results of several studies. For instance, Possenti et al. (2016) reported the same temperature as the most adequate for germination tests carried out on *S. hispanica* seeds; and Paiva et al. (2016) stated that chia seed germination tests could be carried out at a constant temperature of 25°C, or at alternating temperatures (between 25 and 30°C).

In general, the least suitable temperatures for chia germination tests were found to be 15 and 35°C. This might be because high temperatures alter membrane permeability and denature proteins necessary for germination, whereas low temperatures delay metabolic activities, leading to a reduced germination percentage and a germination delay (BEWLEY; BLACK, 1994). In addition, higher temperatures tend to stimulate germination; however, beyond a certain threshold temperature the effect is reversed, and germination decreases until the maximum temperature is reached, at which point no seeds germinate (CARVALHO; NAKAGAWA, 2012). Therefore, excessively high or low temperatures can be detrimental to germination (OLIVEIRA et al., 2014), as observed in the present study on chia seeds.

The luminosity condition was not a determinant for most of the treatments. For total germination, the presence of luminosity was favorable in the between-sand substrate at 35°C; conversely, the absence of luminosity was favorable in the paper roll and between-sand substrates at 30°C (Table 1). Thus, chia seeds probably have phytochromes in the form of phyA controlling germination through Very Low Fluence Responses (TAKAKI, 2005). Chia seeds can be classified as neutral photoblastic (indifferent to luminosity) since they germinate regardless of the luminosity condition (GUEDES; ALVES, 2011; CARVALHO; NAKAGAWA, 2012; ORZARI et al., 2013). These results corroborate those of Paiva et al. (2016), which reported that chia seeds were indifferent to the luminosity condition; however, the authors observed higher seedling growth and dry matter accumulation in the presence of luminosity. Similar results were recorded by Menezes et al. (2004) for sage (*Salvia splendens* Sellow) seeds and by Pacheco Junior et al. (2013) for long-pepper (*Piper hispidinervum*) seeds, both of which were indifferent to the luminosity condition, despite better germination being observed under extreme red light in addition to under white and red lights.

Germination speed index (GSI) (Table 2) was highest at 25 and 30 °C for on-paper, paper roll, and sand substrates, both in the presence and absence of luminosity. Nevertheless, higher values were reported for the on-paper substrate. The lowest GSI values were observed at 15°C for all substrates and both luminosity conditions. For the sand substrate, all temperatures resulted in lower values compared to the other substrates (Table 2). This fact can be explained by the fact that low temperatures usually reduce germination speed, while high temperatures increase germination speed, up to a certain threshold (NASCIMENTO et al., 2011).

Regardless of the luminosity condition, the lowest mean germination times (MGT) (Table 2) were obtained at 25, 30, and 35 °C for on-paper, paper roll, and on-sand substrates. Of all the treatments evaluated in this study, the between-sand substrate produced the highest MGT values at all temperatures. Moreover, the least suitable temperature was 15 °C (Table 2). According to Carvalho and Nakagawa (2012), the optimum temperature for total germination may be different from the optimum temperature for germination speed. This is because water absorption speed and the speed of chemical reactions increase at higher temperatures, justifying the results of this study, in which seeds germinated slowly at lower temperatures, and rapidly at higher temperatures.

Table 2. Germination speed index (GSI) and mean germination time (days) of chia seeds as a function of different substrates, temperatures, and luminosity conditions.

Temp. (°C)	Presence of luminosity				Absence of luminosity			
	Substrates							
	OP	PR	OS	BS	OP	PR	OS	BS
	---Germination speed index---							
15	30.3Ac^{1}	29.4Ac^{1}	28.5Ac^{1}	13.6Ba^{1}	23.7Ac^{1}	24.4Ad^{1}	20.5Ad^{2}	11.3Bb^{1}
20	63.8Aa^{1}	37.4Cb^{1}	45.0Bb^{1}	17.5Da^{1}	43.3Ab^{2}	41.2Ac^{1}	36.8Ac^{2}	17.8Ba^{1}
25	65.4Aa^{1}	49.0Ba^{2}	58.1Aa^{1}	17.3Ca^{1}	65.8Aa^{1}	61.2Ab^{1}	62.5Aa^{1}	16.8Ba^{1}
30	64.1Aa^{1}	45.1Ba^{2}	62.1Aa^{1}	19.4Ca^{1}	65.3Aa^{1}	69.2Aa^{1}	65.5Aa^{1}	22.8Ba^{1}
35	52.6Ab^{2}	40.2Bb^{1}	45.9Bb^{2}	10.0Ca^{1}	62.8Aa^{1}	45.7Cc^{1}	55.1Bb^{1}	4.4Db^{1}
	---Mean germination time (days)---							
15	1.95Ab^{1}	1.94Ab^{1}	2.04Ab^{1}	3.30Bc^{1}	2.69Ac^{2}	2.79Ac^{2}	2.39Ac^{1}	4.12Bd^{2}
20	1.20Aa^{1}	1.75Bb^{1}	1.40Aa^{1}	2.95Cb^{2}	1.63Ab^{2}	1.56Ab^{1}	1.78Ab^{1}	2.52Bc^{1}
25	1.33Aa^{1}	1.44Aa^{1}	1.11Aa^{1}	2.62Bb^{1}	1.12Aa^{1}	1.29Ab^{1}	1.27Aa^{1}	2.69Bc^{1}
30	0.91Aa^{1}	1.15Aa^{1}	1.18Aa^{1}	1.87Ba^{1}	0.93Aa^{1}	0.94Aa^{1}	0.94Aa^{1}	2.18Bb^{1}
35	0.96Aa^{1}	0.98Aa^{1}	0.92Aa^{1}	1.49Ba^{2}	0.92Aa^{1}	0.79Aa^{1}	0.90Aa^{1}	1.01Aa^{1}

*Means followed by the same uppercase letter in the row, lowercase letter in the column, and same number (luminosity) do not differ significantly from each other according to a Scott-Knott test at 5% probability. OP - on-paper, PR – paper roll, OS - on-sand, BS - between-sand.

In the presence (Figure 1) and absence of luminosity (Figure 2), germination began from one day after installation at temperatures of 20, 25, 30, and 35°C for the on-paper (Figure 1A and 2A) and paper roll (Figure 1B and 2B) substrates. For the on-sand substrate, germination started from one day after installation at 20, 25, 30, and 35ºC in the presence of luminosity (Figure 1C); and at 25, 30, and 35ºC in the absence of luminosity (Figure 2C). At 15°C, germination started from three days after installation for the on-paper substrate in the presence of luminosity (Figure 1A), and from two days after installation in the absence of luminosity (Figure 2A). At the same temperature, for the paper roll (presence and absence of luminosity) (Figures 1B and 2B) and on-sand substrates (presence and absence of luminosity), germination began from two days after installation (Figures 1C and 2C). For the between-sand substrate, germination started from two days after installation at 30 and 35ºC (presence of luminosity; Figure 1D) and at 30ºC (absence of luminosity; Figure 2D); from three days after installation at 20 and 25ºC (presence of luminosity) and 20, 25, and 35ºC (absence of luminosity); and from four days after installation at 15ºC (presence and absence of luminosity).

For the on-paper substrate (Figure 1A), germination stabilization occurred seven days after installation at 20 and 25°C, four days after installation at 30°C, and eight days after installation at 15 and 35°C. For the paper roll substrate (Figure 1B), germination stabilization occurred eight days after installation at 15, 25, and 35°C, and seven days after installation at 20 and 30°C. For the on-sand substrate (Figure 1C), germination stabilization occurred seven days after installation at 15, 25, and 35°C, eight days after installation at 30°C, and nine days after installation at 20°C. For the between-sand substrate (Figure 1D), germination was impaired at all temperatures, and consequently germination stabilization occurred nine days after installation at 15ºC. Stabilization did not occur until ten days after installation at 20 and 25°C, and until nine days after installation at 30 and 35°C.

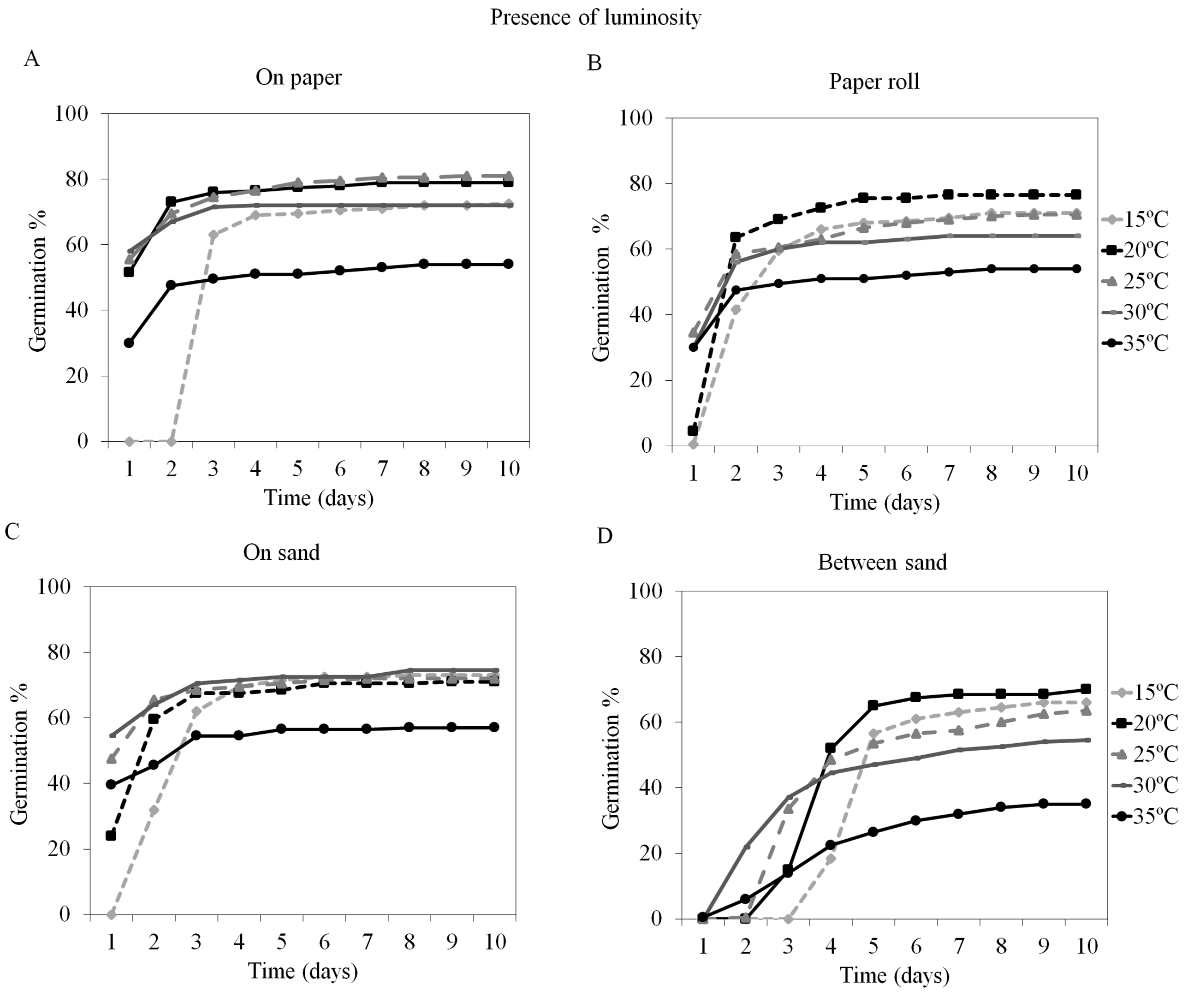

Figure 1. Chia (*Salvia hispanica* L.) seed germination in the presence of luminosity, using on-paper (A), paper roll (B), on-sand (C), and between-sand (D) substrates, as a function of different temperatures.

In the absence of luminosity (Figure 2), for the on-paper substrate (Figure 2A), germination stabilization did not occur until ten days after installation at 15ºC. It occurred eight days after installation at 20 and 25°C, and seven and five days after installation at 30 and 35°C, respectively. For the paper roll substrate (Figure 2B), germination stabilization occurred eight days after installation at 15°C, seven days after installation at 20 and 25°C, six days after installation at 30°C, and eight days after installation at 35°C. For the sand substrate (Figure 2C), germination stabilization occurred eight days after installation at 15 and 25°C, and seven days after installation at 20, 30, and 35°C. For the between-sand substrate (Figure 2D), germination stabilization occurred eight days after installation at 20, 25, 30, and 35°C, and nine days after installation at 15°C.

Faster germination is crucial to reduce the exposure of seeds and seedlings to unfavorable conditions, to prevent failures in initial seedling development, and to ensure seedling uniformity, all of which can significantly hinder production (NASCIMENTO et al., 2011).

Germination tests are performed with the aim of maximizing seed germination potential and producing rapid results. Therefore, the on-paper, paper roll, and sand substrates meet these goals. However, considering the fastest and easiest way of performing the test, and taking into account seed size, the most suitable substrate is on-paper, and the optimum temperature is 25°C. Furthermore, seeds can be counted at four and seven days after installation of the test, as this was the period in which germination stabilized for most of the conditions tested, including at 25°C for the on-paper substrate.

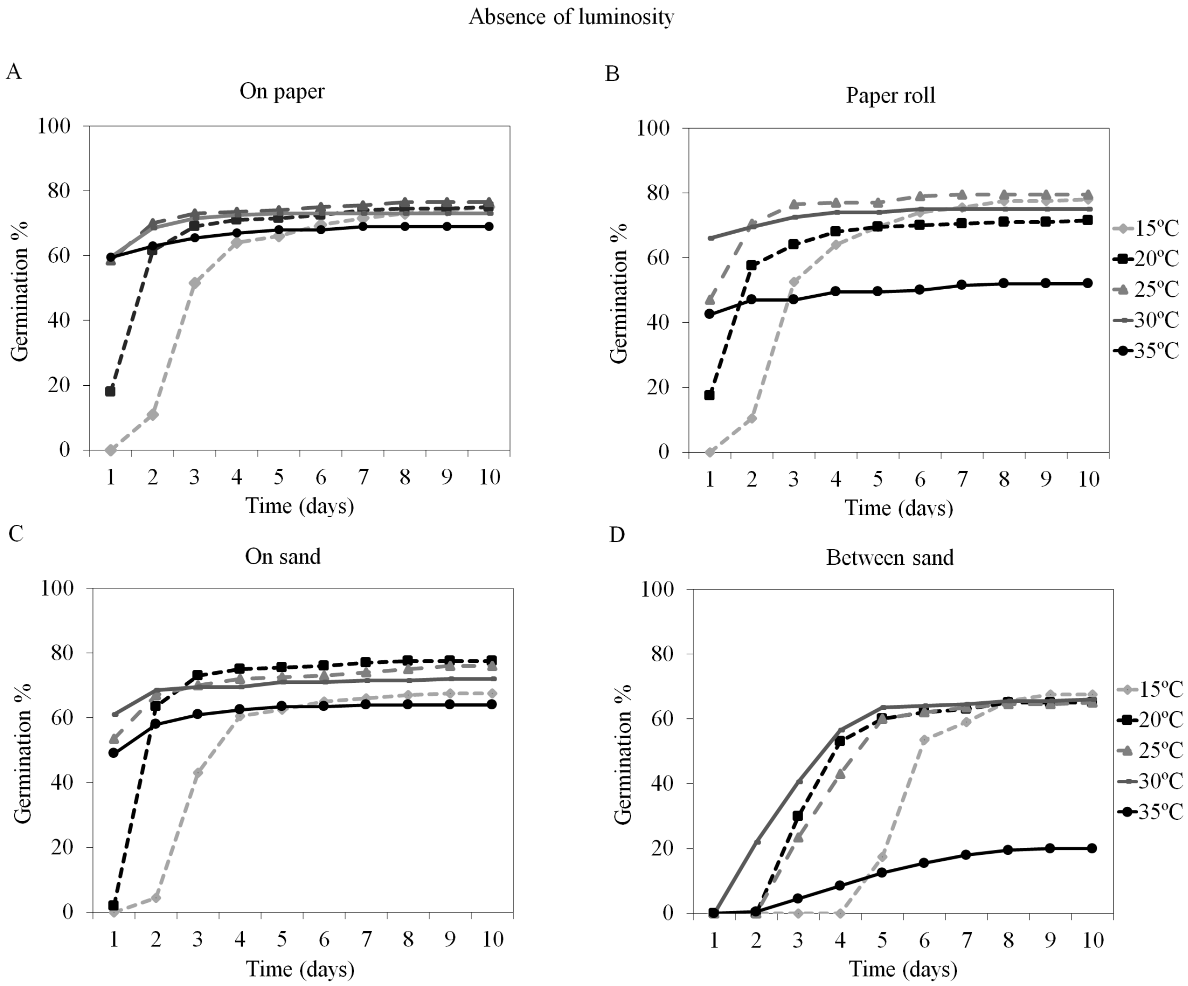

Figure 2. Chia (*Salvia hispanica* L.) seed germination in the absence of luminosity, using on-paper (A), paper roll (B), on-sand (C), and between-sand (D) substrates, as a function of different temperatures.

CONCLUSION

Chia seeds are considered as neutral photoblasts. Germination tests should be performed with an on-paper substrate, at 25°C. The duration of chia germination tests should be seven days, with the first count carried out four days after installation of the test.

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