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Light regime and temperature on seed germination in *Salvia hispanica* L.

Emanoela Pereira de Paiva[1], Salvador Barros Torres[1*], Francisco Vanies da Silva Sá[1], Narjara Walessa Nogueira[1], Rômulo Magno Oliveira de Freitas[2] and Moadir de Sousa Leite[1]

*[1]Universidade Federal Rural do Semi-Árido, Av. Francisco Mota, 572, 59625-900, Mossoró, Rio Grande do Norte, Brazil. [2]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Valença, Bahia, Brazil. *Author for correspondence. E-mail: sbtorres@ufersa.edu.br*

ABSTRACT. This study evaluated the effects of different light regimes and temperatures on the germination of *S. hispanica* seeds and used a factorial scheme in a completely randomized experimental design, in which three light conditions were combined with six temperatures (constant 20, 25, 30 and 35°C and alternating 20-30 and 25-30°C), totaling 18 treatments with four 50-seed replications. The seeds were sown on blotting paper in transparent plastic boxes and then allowed to germinate in Biochemical Oxygen Demand germinators. The seeds were evaluated over a period of eight days with respect to the following variables: germination percentage, average germination time, shoot and root lengths, shoot and root dry weights and root-shoot ratio. The data were submitted to Tukey's test ($p \leq 0.05\%$). The *S. hispanica* seed (beige colored) germination test can be carried out at constant (25°C) or alternating (25-30°C) temperatures. The germination of the seeds was indifferent to the light conditions evaluated; however, there was increased seedling growth and dry matter accumulation in the presence of light. The duration of the *S. hispanica* seed germination test can be five days, with the first count on the second day after sowing.

Keywords: chia, seeds analysis, germination test.

Regime de luz e temperatura na germinação de sementes de *Salvia hispanica* L.

RESUMO. A pesquisa teve como objetivo avaliar os efeitos de diferentes regimes de luz e temperaturas sob a germinação de sementes de *S. hispanica*. Adotou-se delineamento experimental inteiramente casualizado em esquema fatorial constituído pela combinação de três condições de luminosidade (luz constante, escuro constante e alternância luz/escuro com 8h de luz e 16h de escuro) e seis temperaturas (quatro temperaturas constantes 20, 25, 30 e 35°C, e duas alternadas 20-30 e 25-30°C), perfazendo 18 tratamentos em quatro repetições de 50 sementes. As sementes foram semeadas em caixas plásticas transparentes, tendo como substrato papel mata-borrão e colocadas para germinar em Biochemical Oxigen Demand. As sementes foram avaliadas durante oito dias através das seguintes variáveis: percentagem de germinação, tempo médio de germinação, comprimento da parte aérea e da radícula, massa seca da parte aérea e da radícula e relação raiz/parte aérea. Os dados foram submetidos ao teste de Tukey ($p \leq 0,05\%$). O teste de germinação de sementes de *S. hispanica* (coloração bege) pode ser conduzido em temperaturas constante de 25°C e alternada de 25-30°C. A germinação das sementes mostrou-se indiferente à luminosidade, no entanto, verificou-se maior crescimento das plântulas e acúmulo de massa seca na presença de luz. A duração do teste de germinação de sementes *S. hispanica* pode ser de cinco dias, com a primeira contagem realizada no segundo dia após a semeadura.

Palavras-chave: chia, análise de sementes, teste de germinação.

Introduction

Salvia hispanica L. (Lamiaceae), commonly known as chia, is an oleaginous native to the region extending from west-central Mexico to northern Guatemala and stands out for its adaptation to regions of tropical and subtropical climates (Capitanni, Sportorno, Nolasco, & Tomás, 2012). In recent years, *S. hispanica* seed has become important in human nutrition for providing health benefits, such as bowel regulation; reduction of cardiovascular diseases, obesity, cholesterol and triglycerides; and prevention of type II diabetes (Jin et al., 2012; Poudyal, Panchal, Waanders, Ward, & Brown, 2012). Seeds of *S. hispanica* have also been under investigation because of their high levels of protein, antioxidants and dietary fiber, as well as the quality of their oil (30 g oil 100 g^{-1} seed weight), which presents high concentrations of alpha-linolenic acid (50-57%) and linoleic acid (17-26%) (Ixtaina et al., 2011).

S. hispanica has been relatively well studied with respect to its nutritional and medicinal values; however, regarding its agronomic aspects, experimental data are still very scarce, especially in the scope of seed technology. In Brazil, the cultivation of this species is recent; therefore, this species does not yet have recommended cultivars. The lack of standardized methods of analyses, as *S. hispanica* is a species not yet covered by the Rules for Testing Seeds (Brasil, 2009; ISTA, 2013), makes it difficult to assess the quality of its seeds.

During seed germination there is a physiological sequence of events that is influenced by intrinsic (dormancy, physiological immaturity and genotype) and extrinsic (light, temperature, water availability and substrate) factors (Kleczewski, Herms, & Bonello, 2010). As each factor has a specific influence on the process, acting alone or in combination with other factors, the sensitivity of each species must be considered (Bewley & Black, 1994).

Temperature is one of the main factors affecting the seed germination rate and speed because it acts directly on seed water absorption and the biochemical reactions that regulate the metabolism involved in the germination process (Marcos Filho, 2015). In addition, the period of germination may be altered completely in response to temperature (Bewley & Black, 1994).

Similar to temperature, light acts directly on the germination of the seed, which is influenced by the quality, intensity and duration of light irradiation (Aud & Ferraz, 2012), as well as by the period of imbibition and the temperature during this period. Menezes, Franzin, Roversi, and Nunes (2004), working with a congeneric species (*Salvia splendens* Sellow), reported that its seeds are indifferent to light quality, although they germinate better under far-red light or dark conditions, but a temperature of 15°C retards their germination.

The germination test is one of the main methods used to evaluate the physiological quality of seeds. Using this test, it is possible to determine the sowing rate and compare different seed lots for storage and commercialization (Martins, Marini, Bandeira, Villela, & Moraes, 2014). The methodology of this test follows the standards outlined in the national and international rules for seed testing, but with regard to *S. hispanica* seeds, there is no information about light regime, temperature and suitable substrate to provide a secure germination test. Given the above information, this study was carried out to evaluate the effects of different light regimes and temperatures on the germination of *S. hispanica* seeds.

Material and methods

Seed materials

The experiment was carried out in the Seed Analysis Laboratory, Department of Plant Sciences, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte State, Brazil. A simple sample of *S. hispanica* seeds (90% beige, 5% white and 5% black) from the 2013/2014 crop was used, purchased from producers based in Santana do Livramento (30° 53' 27" S, 55° 31' 58" W and 208 m altitude), Rio Grande do Sul State, Brazil. The beige-colored seeds were manually separated, Homogenized, placed in plastic bags and stored in a cold room (10°C and 50% ambient relative humidity) until the date of installation of the experiment.

Conducting germination tests

The seeds were placed in transparent plastic boxes (11 × 11 × 3 cm) containing two sheets of blotting paper as a substrate that before sowing had been moistened with distilled water in an amount equal to 2.5 times their dry weight and put into germination Biochemical Oxygen Demand (BOD) chamber.

The lighting conditions were constant light, constant dark and alternating light/dark, the latter equivalent to 8h of light plus 16h of dark; 20-W fluorescent lamps were used as a source of white light (Oliveira Bento, Torres, Oliveira, Paiva, & Bento, 2013). The absence of light was ensured using plastic boxes with aluminum foil; under these conditions, the evaluation was carried out under green light from a flashlight that was coated with two green cellophane sheets (Coelho, Sanches, & Azevedo, 2012).

The temperature conditions that were used were four constant (20, 25, 30, and 35°C) and two alternating (20-30 and 25-30°C for 8 and 16h, respectively, for higher and lower temperatures) temperatures. For the treatments with alternating light/dark conditions, the duration of each condition corresponded to periods of light and dark.

The germination test was evaluated for eight days after sowing. Seedlings with hypocotyl elongation and primary root protrusion were considered normal. The results were expressed as percentages.

Mean germination time

Daily counts were performed at the same time during the evaluation period of the germination test, and seeds were considered germinated if they matched the same normality standards as defined for the germination test. The average germination times were

calculated according to Labouriau and Valadares (1976) using $t = \sum_{i=1}^{i} n_i t_i / \sum_{i=1}^{i} n_i$, where t = average germination time, n_i = number of seeds germinated on the i^{th} day, and t_i = time for germination (days).

Seedling length and dry matter

At the end of the germination test, the lengths of the shoot (from the collar to the apex) and primary root (from the collar to the cap) in normal seedlings of each experimental unit were measured. After these measurements, each seedling had its shoot separated from the root, and these parts were then conditioned in paper bags and put to dry in an air-forced circulation stove at 65°C for 72h. The shoot and root dry weights were obtained using an analytical scale (0.001 g), and calculations were made to determine the seedling root-shoot ratio reflecting the biomass accumulation in the root system in relation to the shoot.

Statistical analysis

A factorial scheme in a completely randomized design was used in which three light conditions were combined with six temperatures, totaling 18 treatments with four 50-seed replications.

The data were submitted to an ANOVA (F test) and, in case of significance, the means were compared by Tukey's test ($p \leq 0.05$) using the software SISVAR (Ferreira, 2011).

Results and discussion

Data regarding seed germination and average germination time in *S. hispanica* were the result of significant interactions between light conditions and temperatures. The germination data that were obtained under conditions of constant temperatures (20, 25 and 30°C) were statistically similar to those obtained under alternating temperatures (20-30 and 25-30°C), independent of the light regime (Table 1). There was also a reduced germination percentage under conditions of constant dark and alternating light/dark combined with the constant temperatures of 30 and 35°C, as well as under conditions of constant light plus a temperature of 35°C (Table 1).

Table 1. Germination (%) of *Salvia hispanica* L. seeds under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	93 aA*	95 aA	95 aA
25	97 aA	80 abA	99 aA
30	95 aA	55 cB	69 bB
35	33 bB	56 bcA	66 bA
20-30	94 aA	85 aA	97 aA
25-30	92 aA	82 aA	94 aA
CV = 14.59%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

The seeds of *S. hispanica* were indifferent to the light regime, with germination occurring in the presence and absence of light. The occurrence of germination under different light conditions may be due to the active-form phytochrome present in the seed in a quantity sufficient to induce the germination process (Marcos Filho, 2015). Similar to the results observed in this study, seeds of several species are indifferent to light regime, including *Clitoria fairchildiana* R. A. Howard (Alves et al., 2012) and *Dalbergia cearensis* Ducke (Nogueira, Gallão, Bezerra, & Medeiros Filho, 2014), which are capable of germinating in the both presence and absence of light.

Concerning the various temperatures evaluated, there was an inhibition of *S. hispanica* seed germination, a stress that is less harmful when under constant light. Under this lighting condition, stimuli occurred during the respiratory activity in the seeds, resulting in thermal balance up to a temperature of 30°C; beyond this temperature, respiratory activity was insufficient to regulate seed temperature, causing thermal stress that likely affected enzymatic activity and resulted in germination reduction. However, the ability of seeds to germinate under both temperature conditions reflects the adaptability of the species to environmental thermal variations. According to Bita and Gerats (2013), species that are capable of establishing under most temperature conditions have greater resilience potential to adverse environmental conditions. It seems that a similar potential feature is present in *S. hispanica* as its seeds germinated satisfactorily under conditions of varying temperatures (20-30°C).

The highest average times of germination were obtained under conditions of constant darkness independent of temperature, ranging from 3.33-4.41 days for germination (Table 2). Under conditions of constant light and alternating light/dark, the lowest mean germination time was obtained under constant (25 and 30°C) and alternating (25-30°C) temperatures.

Table 2. Average seed germination time (days) in *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	3.14 bcA*	3.80 abB	3.29 aA
25	2.84 abcA	3.33 aB	2.83 aA
30	2.59 aA	3.50 aB	3.18 aB
35	4.04 dB	4.31 cB	3.15 aA
20-30	3.18 cA	3.98 bcB	3.28 aA
25-30	2.68 abA	3.66 abB	3.06 aA
CV = 6.81%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

The germination test in *S. hispanica* seeds can be carried out between the second (first count) and fifth (last count) days after sowing, given that the conditions that favor the highest germination percentage and shortest average germination time are constant temperature (25°C) and alternating temperature (25-30°C), either under conditions of constant light or alternating light/dark periods (8h light 16h dark^{-1}). The results that were obtained in this study were similar to those observed by Oliveira Bento et al. (2013) in *Calotropis procera* Aiton seeds that were submitted to different temperatures and light regimes, with the light regimes decreasing the average germination time when the germination test was carried out under temperatures of 20-30°C (alternating), 25-30°C (alternating) and 30°C (constant).

Compared with this study, diverging results were reported by Nogueira et al. (2014), who evaluated the effect of temperature and light on the germination of seeds of *Dalbergia cearensis* Ducke. These authors found that the lowest average germination times were obtained under a temperature of 30°C, 3.54 and 3.81, respectively, to light and dark conditions, and that the greatest average germination times were obtained under a temperature of 20°C under dark (5.86 days) and light (6.31 days) conditions. However, *D. cearensis* occurs in the Caatinga biome and is adapted to high temperatures, whereas *S. hispanica* is a subtropical adapted to mild temperatures. This fact confirms a postulate by Marcos Filho (2015), in which the influence of a factor in the germination process of the seed depends on the sensibility of each species.

Longer shoots were obtained in *S. hispanica* seedlings under constant darkness conditions at all of the evaluated temperatures. In addition, seedlings from seeds that germinated under constant light conditions had shorter shoots independent of temperature (Table 3).

Table 3. Average shoot length (cm) in seedlings of *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	2.32 bC*	4.38 eA	3.22 bcB
25	2.82 abC	6.68 cA	4.47 aB
30	2.98 aC	7.94 aA	4.26 aB
35	1.07 cC	4.67 eA	2.90 cB
20-30	2.28 bC	5.87 dA	3.50 bB
25-30	3.11 aC	7.27 bA	4.48 aB
CV = 6.40%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test $p \leq 0.05$); CV = coefficient of variation.

In the absence of light, the seedlings grew the longest shoots independent of temperature conditions (Table 3); thus, it is believed that dark conditions caused a hormonal imbalance, thus stimulating seedling etiolation in *S. hispanica* as etiolation is caused by an increase in gibberellin synthesis, which consequently promotes the excessive growth of the stem in search of light (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009), as seems to be the case in the present study. In contrast, seedlings that were germinated under constant light had the shortest shoots irrespective of temperature conditions. It is likely that the excess absorbed light triggered a stress condition known as photoinhibition, a process defined as the inhibition of photosynthesis caused by excess light. It is also believed that some enzymatic changes have occurred, including an increase in the synthesis of chlorophyllase, intensifying the degradation of chlorophyll in the cytochrome (Damatta & Ramalho, 2006). In general, the shortest shoots in seedlings of *S. hispanica* were found under high temperature conditions. This fact may have been due to the thermal stress causing decreases in the supply of free amino acids and protein synthesis (Santos, Sugahara, & Takaki, 2005).

The conditions of alternating light/dark at constant (25 and 30°C) and alternating (25-30°C) temperatures provided the longest shoots. However, at 35°C, the shortest seedling shoot (2.90 cm) was found, which was lower by 35, 32 and 35% compared with the seedlings under 25°C (4.47 cm), 30°C (4.26 cm) and alternating 25-30°C (4.48 cm), respectively (Table 3).

S. hispanica seedlings from seeds that were germinated under constant light conditions had the longest roots with regard to all of the evaluated temperature conditions, except in the treatments under 35°C temperature conditions (Table 4). Longer roots were also found in seedlings from seeds that germinated under alternating light/dark + alternating temperature (20-30 and 25-30°C) conditions, followed by those from seeds that germinated under constant temperature (25 and 30°C) conditions. Otherwise, under 20 and 30°C constant temperature conditions, the roots were shorter (Table 4).

Table 4. Average root length (cm) in seedlings of *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	4.91 aA*	3.66 aB	4.15 bB
25	5.13 aA	3.49 aB	5.03 abA
30	5.65 aA	3.20 aC	4.36 abB
35	1.82 bB	1.83 bB	3.12 cA
20-30	5.41 aA	3.78 aB	5.16 aA
25-30	5.19 aA	3.34 aB	4.52 abA
CV = 10.59%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

The root length increased under constant light conditions in all of the evaluated temperatures, except at 35°C (Table 4). In contrast, shoots in seedlings from seeds that were germinated under constant light conditions were the shortest. In this case, it is possible that the excess light absorbed decreased auxin synthesis, thus stimulating root growth, given that this hormone is responsible for balancing the growth of shoot and root (Farooq et al., 2009). Constant temperatures (20 and 35°C) caused the smallest root lengths in *S. hispanica*. This fact indicates that the seeds of this species, in addition to having sensitivity to thermal stress under high temperatures, are also sensitive to low temperatures, given that under this last condition, metabolic rates are reduced, and the growth process is affected from germination to seedling stage (Bita & Gerats, 2013). Thus, it is believed that the promising results that were obtained in the treatments under alternating temperature (20-30°C) conditions, are possibly related to the period of exposition (8h) to 30°C as the optimal temperature range for the development of *S. hispanica* seedlings in the experiment was 25-30°C.

For shoot dry mass, there was a difference between the light regimes only at constant temperatures of 20 and 35°C (Table 5). Also in this table, at a temperature of 20°C under constant darkness, there was a greater accumulation of dry matter in seedlings of *S. hispanica*, likely due to the greater shoot length caused by seedling etiolation (Table 3).

Table 5. Shoot dry matter (mg) weight in seedlings of *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	9.0 aAB*	9.5 aA	8.0 abB
25	8.2 aA	9.0 abA	9.4 aA
30	8.5 aA	7.5 bA	7.5 bA
35	5.2 bB	4.7 cB	7.0 bA
20-30	7.5 aA	8.5 abA	7.5 bA
25-30	8.3 aA	8.7 abA	8.5 abA
CV = 10.56%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

Higher shoot dry matter weight values were obtained under conditions of 20°C and constant darkness (Table 5), likely due to seedling shoot etiolation (Table 3). In *Salvia splendens* seedlings, the highest dry mass contents were obtained also under conditions of 20°C, but only under a white light regime (Menezes, Franzin, Roversi, & Nunes, 2004), denoting that species of *Salvia* respond differently with regard to light regime. At 35°C, the highest dry matter accumulation occurred under the alternating light/dark regime, an accumulation in excess of 25 and 32% compared with that obtained under constant light and constant dark regimes, respectively. It is possible that the alternating regime (8h light/16h dark) was responsible for the lack of etiolation or light saturation in the seedlings, thus causing sufficient metabolic balance to stimulate greater biomass accumulation. It is noteworthy that the largest accumulation of shoot dry matter occurred at temperatures of 20 and 25°C. Pacheco Junior, Silva, Negreiros, Silva, and Farias (2013) reported similar results in seedlings of *Piper hispidinervum* C.DC. at 25°C.

The greatest accumulation of root dry mass occurred in seedlings that were germinated at 20°C in constant darkness, 20 and 25°C in alternating light/dark and under alternating temperatures of 20-30°C in constant light (Table 6).

Table 6. Root dry matter (mg) weight in seedlings of *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	3.7 abA	2.7 aB	3.7 aA
25	3.7 abA	2.0 abB	4.1 aA
30	3.2 abA	1.7 abB	2.2 bcB
35	1.7 cA	1.0 bA	1.5 cA
20-30	4.2 aA	1.7 abC	3.2 abB
25-30	3.0 bA	1.5 bB	3.2 abA
CV = 20.58%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

The dry matter accumulation in the roots of *S hispanica* seedlings was influenced by different temperatures and light regimes (Table 6). These results agree with those regarding the growth of the root system, which responded variously to the temperature under different light regimes, with the best results found in the seedlings at 25°C under alternating light conditions. An explanation for this higher dry matter content is the possibility that the treatment provided the necessary conditions for germination; that is, the seeds originate seedlings with higher growth rates as a result of the higher capacity in processing the reserve supplies stored in the tissues, followed by a higher incorporation rate of by-products in the embryo axis and radicle (Kleczewski et al., 2010).

The root-shoot ratio was higher in seedlings from seeds that germinated under constant temperatures of 20 and 25°C and alternating temperatures of 20-30 and 25-30°C under constant light and alternating light/dark regimes (Table 7). Under these light conditions, seedlings from seeds that were germinated at 30 and 35°C had the lowest root-shoot ratio.

Table 7. Root-shoot ratio in seedlings of *Salvia hispanica* L. under different light and temperature regimes.

Temperature (°C)	Light regimes		
	Constant light	Constant dark	Alternating light/dark
20	0.42 abA	0.29 aB	0.46 aA
25	0.45 abA	0.22 aB	0.44 abA
30	0.38 bA	0.23 aB	0.30 bcAB
35	0.33 bA	0.23 aA	0.22 cA
20-30	0.56 aA	0.20 aC	0.43 abB
25-30	0.36 bA	0.17 aB	0.38 abA
CV = 21.58%			

(*) Means followed by the same letter (lowercase in the column and uppercase in the row) are not significantly different (Tukey's test, $p \leq 0.05$); CV = coefficient of variation.

The root-shoot ratio reflects the balance of seedling growth, that is, root growth corresponds to shoot growth. According to this assumption, at constant temperatures of 20 and 25°C and alternating temperatures of 20-30 and 25-30°C, seedlings had the best root-shoot ratio under constant and alternating light/dark regimes (Table 7). It is noteworthy that, under these lighting conditions, *S. hispanica* seedlings from seeds that were germinated at 30 and 35°C had the least root-shoot ratios, which may reflect the inhibition of root growth likely caused by the increase in temperature. In contrast, seedlings that originated under constant light conditions had the lowest root-shoot ratio compared with those under the other light regimes. This fact may be due to seedling etiolation caused by the irregular growth of the shoot in relation to the root system.

Conclusion

The germination test in *S. hispanica* seeds (beige colored) can be carried out at constant (25°C) and alternating (25-30°C) temperatures. Although, its seeds are indifferent to the light regime, seedlings grow better and accumulate a greater dry matter content in the presence of light. In this species, the duration of the germination test can be five days, with the first count on the second day after sowing.

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