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Methodology for germination tests accomplishment in chia seeds

Metodologia para condução do teste de germinação em sementes de chia

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Abstract: Chia seeds are known for their high nutritional content and for being an excellent source of energy. Chia has gained notoriety since the 1990s with the growing demand for functional and healthy foods. With the significant increase in demand for this product, the production of high-quality seeds is essential to achieve high yields. In addition, there are few studies on methodologies for seed analysis of this species, making this study relevant. Thus, the objective was to determine the methodology regarding temperature, substrate and photoperiod for the germination test in chia seeds. The experiment was carried out in a completely randomized design with triple factorial treatments 7 x 3 x 2 (temperatures x substrates x photoperiod), with four replications. Mass tests of 1000 seeds, moisture content and germination test with temperature variations of 10, 15, 20, 25, 30, 35 and 40 ºC were performed on the substrates on paper, between paper and between sand, with presence or absence of light. Results were expressed as germination percentage, speed index, average time and average germination speed. The water absorption curve was also obtained at three temperatures (20, 25 and 30 ºC). Under the conditions tested, it was concluded that chia seeds can be germinated on paper and sand substrates, with or without light, at temperatures of 15 to 35 ºC, thus achieving high germination percentage rates.

Keywords: Photoperiod, *Salvia hispanica*, substrate, temperature

Resumo: As sementes de chia são conhecidas por seus elevados teores nutricionais e por ser uma excelente fonte de energia. A chia ganhou notoriedade a partir da década de 90 com a crescente demanda por alimentos funcionais e saudáveis. Com o aumento expressivo da demanda por este produto, a produção de sementes de alta qualidade é imprescindível para alcançar elevados índices de produtividade. Além disso, existem poucos estudos sobre metodologias para análise de sementes dessa espécie, tornando este estudo relevante. Dessa forma, objetivou-se determinar a metodologia quanto à temperatura, substrato e fotoperíodo para o teste de germinação em sementes de chia. O experimento foi instalado em delineamento inteiramente casualizado, com tratamentos em esquema fatorial triplo 7 x 3 x 2 (temperaturas x substratos x fotoperíodo), com quatro repetições. Foram realizados os testes de massa de 1000 sementes, teor de água e teste de germinação com variações de temperaturas de 10, 15, 20, 25, 30, 35 e 40 ºC, nos substratos sobre papel, entre papel e entre areia, com presença ou ausência de luz. Os resultados foram expressos em porcentagem de germinação, índice de velocidade, tempo médio e velocidade média de germinação. Também foi obtida a curva de absorção de água em três temperaturas (20, 25 e 30 ºC). Nas condições testadas, concluiu-se que sementes de chia podem ser germinadas em substratos de papel e areia, com presença ou ausência de luz, nas temperaturas de 15 a 35 ºC, conseguindo assim, obter elevadas taxas de porcentagem de germinação.

Palavras-chave: Fotoperíodo, *Salvia hispanica*, substrato, temperatura

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Introduction

Chia is an annual herbaceous plant and was one of the basic foods of the Central America civilizations, from Southern Mexico and Northern Guatemala. It was one of the most produced crops by ancient Mesoamerican civilizations, for thousands of years (Capitani et al., 2012). For centuries, the chia had its cultivation extinguished, being resumed in the early 90s by Argentine researchers (Ayerza, et al., 2005). From this decade, research has been resumed, including in Brazil, where the cultivation is still recent. It is currently cultivated in many countries, including Australia, Mexico, Argentina, Ecuador, Bolivia, Peru, Paraguay, Guatemala, Colombia, and Southeast Asia, among others (De Kartzow, 2013).

Chia can be consumed in whole grain form or as commercial derivatives in flour and oil (Spada et al., 2014). The consumption has increased every year, because it is important for human health, due to its high content of omega 3 and fatty acids. Consequently, it has shown several beneficial effects to health (Ayerza and Coates, 2011). The contents of alpha-linolenic and linoleic acids are responsible for chia seeds importance (Mohd et al., 2012), due to the fact that these compounds help immune functions, inhibit proliferation of lymphocytes and pro-inflammatory cytokinin, keep integrity of cell membranes and neurotransmitters and act out on cardiovascular diseases prevention (Ludwig et al., 2013).

Several studies have already been carried out on nutritional and medicinal values of chia seeds. However, in relation to the agronomic aspects and in terms of seed technology, studies are still scarce. There are no standard methods of analysis to verify seed quality, since methodologies for this species have not yet been covered by the Rules of Seed Analyses (ISTA, 2013). In addition, research on the evaluation of seed physiological potential is important to ensure crop success, to understand crop establishment and to estimate seed performance in the field (Martins et al., 2014).

The seed germination process should consider factors that affect its process, which requires ideal internal and external conditions, so that seed has an optimal germinative index. Water has considerable influence during the germination process, because its absorption reflects on tissue rehydration, so, respiration increases and influences on metabolic activities, providing energy that allows embryonic axis to grow. Water absorption causes volume to increase during the process, which causes seed coat to rupture, providing satisfactory germination (Carvalho and Nakagawa, 2012).

Temperature takes part of the environmental factors that can significantly affect germination. It also affects both germination speed and percentage, mainly influencing water absorption by seeds and in biochemical reactions and physiological processes (Carvalho and Nakagawa, 2012). There is an optimum temperature range for each species, and this allows greater efficiency in the germination process associated to higher germination speed (Marcos Filho, 2015).

Substrate is a complex factor that can influence in different ways the germinative and post-germinative processes. In order to choose the material used as substrate, seed size, moisture and light requirement, water absorption and retention capacity, aeration and drainage, absence of pests, diseases and toxic substances should be taken into account. Besides, when those factors are considered, they help on seedlings evaluation. Based on the information above, the present trial aimed to evaluate the best temperature, substrate and photoperiod for germinating chia seeds.

Material and Methods

The experiment was carried out at the Seed and Plant Evaluation Laboratory of Western Paraná State University, Campus of Cascavel - PR, in 2015. Untreated chia seeds, harvested in July 2014, were packed in transparent plastic boxes and stored at room temperature during the experiment. In order to determine seed physical quality, mass of 1000 seeds and moisture content were evaluated (Brasil, 2009).

The treatments for germination evaluation consisted of three substrates (seeds on paper, seeds between paper and sand), seven temperatures (10, 15, 20, 25, 30, 35 and 40 ºC) and two photoperiods (12-hour light and 24-hour dark).

For the paper substrates, two filter sheets were, moistened with distilled water 2.5 times the paper mass and 50 chia seeds were manually sowed in each box, totaling four replications per treatment. For the substrate between paper, the same process described above was carried out, and after sowing, a sheet of moistened paper covered the seeds. The boxes in the third treatment, that contained sieved and washed sand as substrate, were prepared with 50 seeds per replication. B.O.D. cameras were used in order to control the studied temperatures and photoperiod.

Countings were daily recorded and starting on the second day after sowing and ending on the fifth. The results were expressed in percentage, calculated by the following formula: $G = (N/100) \times 100$, where: N = number of seeds germinated at the end of the test. Based on these values, the germination speed index - GSI was determined by this formula: $GSI = \sum (ni/ti)$, where: ni = number of seeds germinated at time 'i'; Ti = time after test installation; $I = 1 \rightarrow 5$ days, with dimensionless unit (Maguire, 1962); average germination period - AGP calculated by the formula $AGP = (\sum niti)/\sum ni$, where ni = number of seeds germinated per day; Ti = incubation time; $I= 1 \rightarrow 5$ days, with unit in days (Labouriau and Agudo, 1987); and the average germination speed – AGS calculated by the formula: AGS 1/t where: t = average germination time, with unit in $days^{-1}$ (Edmond and Drapala, 1958).

Four subsamples of 10 seeds were used at each temperature to obtain the absorption curve. Seeds were placed in petri dishes on two sheets of filter paper moistened with 6 mL of distilled water. These seeds remained in growth chamber (B.O.D.) at 20, 25 and 30 °C during imbibition. The seeds were then removed from the petri dish and laid over dry paper to remove excess of water. They were weighted every 15 minutes during the first hour, then at each 30 minutes in the second hour, then, this procedure was carried out at every hour during the next four hours. Finally, every three hours until 12 hours to determine the mass gain of each seed, that is, the amount of absorbed water. Those results were expressed in grams and the data submitted to the polynomial regression analysis to obtain water absorption equation.

The experiment was carried out in a completely randomized design, with treatments distributed as a 7 x 3 x 2 triple factorial scheme (temperatures x substrates x photoperiod), with four replications. The averages obtained were compared by Tukey test, at 5% probability, after being submitted to analysis of variance.

Results and Discussion

The tests performed before the others for the physical characterization allowed to determine average data of weight of one thousand seeds that was 1.3 grams and moisture content of 8.4%.

The values obtained from the analysis of variance and interaction among temperature, photoperiod and substrate factors for germination data, speed germination index (SGI), average germination period (AGP) and average germination speed (AGS) in chia seeds (Table 1).

The analysis of variance shows that there was a triple interaction between photoperiod and substrate factors for germination percentage, SGI and AGS in chia seeds. The coefficients of variation for all parameters were superior to 40%, showing high dispersion among the data, and that they are heterogeneous (Gomes, 2000).

The average values of germination percentage (G) and speed index (SGI) in chia seeds submitted to the variation of photoperiod, substrate and temperature were shown in Table 2. The germination percentage was statistically affected by triple interaction, in the presence of light, only in sand substrate at 35 ºC (78%). For temperatures such as 10, 15, 20, 25 and 30ºC, it was observed that the averages were statistically the same. The germination percentage in light absence and substrate interaction was not significant at temperatures of 15, 20, 25, 30, 35 and 40 ºC, and did not differ statistically.

Table 1. Analysis of variance of germination percentage (G), germination speed index (GSI), average germination period (AGP) and average germination speed (AGS) for chia seeds under seven temperatures, two photoperiods and three substrates

	p-valor

Source of	DF	G (%)	GSI	AGP (days)	AGS (days^{-1})
Temp	6	673.2703**	535.6208**	702.9918 **	638.2941 **
Photo	1	0.3155 ns	124.0702**	314.8569 **	169.8783 **
Subs	2	2.1237**	19.0156**	22.4501 **	26.1501 **
TempxPhoto	6	957.1356**	261.2130**	930.6334 **	330.4401 **
TempxSubs	12	2.8455**	4.0080**	6.1144 **	7.5946 **
PhotoxSubs	2	3.5214*	2.0038 ns	7.2787 **	1.7976 ns
TempxPhotoxSubs	12	2.5464**	2.1349*	1.4932 ns	2.5048 **
Residue	126				
CV (%)		41.30	54.96	57.25	53.71

G: germination, Temp: temperature, Photo: photoperiod, Subs: substrate, CV: coefficient of variation, * significant at 5%, ** significant at 1%.

Table 2. Percentage (G), speed index (GSI), of germination for chia seeds according to photoperiod x substrate interaction, under seven temperatures

Photo X Subs		Temperature (°C)						
		10	15	20	25	30	35	40
L/OP	G (%)	98aA	98aA	97aA	98aA	97aA	99aA	0bB
L/BP		95aA	97aA	92aA	95aA	99aA	94aA	0bB
L/BS		94aA	98aA	98aA	98aA	99aA	78bB	0cB
WL/OP		0bB	97aA	92aA	96aA	98aA	92aA	96Aa
WL/BP		0bB	95aA	98aA	98aA	98aA	98aA	99aA
WL/BS		0bB	94aA	100aA	93aA	97aA	93aA	96aA
L/OP	GSI	16.1dA	24.2cA	23.8cA	37.4bBC	46.8aA	47.9aA	0eC
L/BP		15.4dA	23.9cA	21.9cA	39.2bABC	47.6aA	41.8abB	0eC
L/BS		16.2dA	23.5cA	24.4cA	33.5bCD	44.4aA	34.3bC	0eC
WL/OP		0dB	23.1cA	22.3cA	41.8bAB	48.0aA	44.7abAB	47.6abAB
WL/BP		0cB	22.6bA	24.1bA	44.6aA	47.4aA	46.6aAB	48.2aA
WL/BS		0dB	19.5cA	24.9cA	33.0bD	44.9aA	42.3aAB	41.8aB

Photo: photoperiod, Subs: substrate, L: Light, WL: without light, OP: over paper, BP: between paper, BS: between sand. Averages followed by the lowercase letters in row and the uppercase letters in column differ from each other by Tukey test at 5% probability.

Chia seeds are indifferent to photoperiod, and germination may occur in the presence and absence of light. The occurrence of germination under different light conditions may be due to the active phytochrome-form present in these seeds in adequate quantity to induce the germinative process (Marcos Filho, 2015). Seeds submitted to 40 ºC with light and seeds submitted to light absence at the lowest temperature (10 ºC) did not germinate, regardless substrate. This indicates that, for chia seeds, temperatures of 15 to 35 ºC are the optimum range for germination.

According to Labouriau and Agudo (1987), chia seeds present different responses regarding temperature and light. The extreme temperature limits, 3.3 ± 0.4 ° C and 39.8 ± 0.4 ° C, demonstrated that low temperatures have limited seed germination, even though it is near the lowest limits of some cold tolerant tropical plants. High temperatures have also limited germination, as they were higher than the values of plants from average temperature (20-25 ºC). Thus, it can be concluded that chia is classified between tropical plants and plants from average temperature. This may be directly related to the geographical origin of the species.

Photoperiod and substrate factors from 25 to 40 ºC affected germination speed index. The highest values were verified when the seeds germinated in the light absence, on paper, at 30 ºC (48.0) and in light absence, between paper, at 40 ºC (48.2). Previously, it was observed the interaction between light absence and paper substrates at 40 °C to show high SGI values. However, under temperatures as low as 10 °C, without light and high temperatures (40 °C) with light, seeds did not germinate. As it was observed in this study, high temperatures may therefore have detrimental effects on seed germination (Oliveira et al., 2014).

Regarding average germination period, the triple interaction was not significant (Table 3). Different results were found out by Paiva et al. (2016), who observed that the highest average germination period in chia seeds were obtained under light absence conditions, despite temperature, ranging from 3.33-4.41 days for germination, on the other hand, under light, the lowest germination period was 30 °C (2.59 days).

Similar statistical results were observed when seeds were germinated at 30 and 35 ºC temperature, in the presence and absence of light for the average germination speed. While, in light absence at 40 ºC, it was faster, and statistically differed from the others. No significant difference was observed for the germination percentage at 15, 20, 25 and 30 ºC when they were associated to the photoperiod and substrate. The germination speed indices, at 10, 15 and 25 ºC, were similar, but different from one another statistically, despite photoperiod and substrate factors. In the triple interaction, it was observed significant difference for the average germination speed among the studied treatments.

The average values of germination percentage, germination speed index, average germination period and average germination speed, in chia seeds submitted to temperature, photoperiod and substrate variation, are presented on Table 4.

Table 3. Average period (AGP) and average speed (AGS) of germination for chia seeds according to photoperiod x substrate interaction, under seven temperatures

Photo X Subs		Temperature (°C)						
		10	15	20	25	30	35	40
L/OP	AGP (days)	3.1	2.0	2.0	1.5	1.1	1.1	0
L/BP		3.1	2.0	2.2	1.4	1.1	1.2	0
L/BS		2.9	2.1	2.0	1.7	1.2	1.3	0
WL/OP		0	2.1	2.1	1.3	1.0	1.1	1.0
WL/BP		0	2.1	2.0	1.2	1.1	1.1	1.1
WL/BS		0	2.6	2.0	1.6	1.2	1.2	1.3
L/OP	AGS ($days^{1}$)	0.3dA	0.5cA	0.5cB	0.7bBC	0.9aAB	0.9aA	0eC
L/BP		0.3dA	0.5cAB	0.5cB	0.7bB	0.9aAB	0.8abABC	0eC
L/BS		0.3dA	0.5cAB	0.5cB	0.6bC	0.8aC	0.8aC	0eC
WL/OP		0dB	0.5cAB	0.5bAB	0.8bAB	0.9aA	0.9aA	0.9aA
WL/BP		0cB	0.5bAB	0.5bB	0.9aA	0.9aAB	0.9aAB	0.9aA
WL/BS		0dB	0.4cB	0.6bA	0.6bC	0.8aBC	0.8aBC	0.8aB

Photo: photoperiod, Subs: substrate, L: Light, WL: without light, OP: over paper, BP: between paper, BS: between sand. Averages followed by the lowercase letters in row and the uppercase letters in column differ from each other by Tukey test at 5% probability.

Table 4. Percentage (G), speed index (GSI), average period (AGP) and average speed (AGS) of germination for chia seeds according to temperature x photoperiod, under three substrates

Temp x Photo	G (%)			GSI			AGP (days)			AGS ($days^{-1}$)		
	Substrate											
	OP	BP	BS	OP	BP	BS	OP	BP	BS	OP	BP	BS
10xL	98Aa	95aA	94aA	16.1dA	15.4dA	16.2dA	3.1	3.1	2.9	0.3dA	0.3dA	0.3eA
10xWL	0bA	0bA	0cA	0eA	0eA	0eA	0	0	0	0eA	0eA	0.5dA
15xL	98aA	97aA	98aA	24.2cA	23.9cA	23.5cA	2.0	2.0	2.1	0.5cA	0.5cA	0.5dA
15xWL	97aA	95aA	94aA	23.1cdA	22.7cA	19.5cdA	2.2	2.1	2.6	0.5cA	0.5cA	0.4deA
20xL	97aA	92aA	98aA	23.8cA	21.9cdA	24.4cA	2.1	2.2	2.0	0.5cA	0.5cA	0.5cdA
20xWL	92aB	98aA	100aA	22.3cdA	24.1cA	24.9cA	2.1	2.0	2.0	0.5cB	0.5cB	0.6bcA
25xL	97aA	95aA	98aA	37.4bAB	39.2cA	33.5bB	1.5	1.4	1.7	0.7bA	0.8bA	0.6bcB
25xWL	96aA	98aA	93aA	41.8abA	44.6abA	33.0bB	1.3	1.2	1.6	0.8bA	0.9abA	0.6bB
30xL	97aA	99aA	99aA	47.8aA	47.6aA	44.4aA	1.1	1.1	1.2	0.9aA	0.9aA	0.8aB
30xWL	98aA	98aA	97aA	48.0aA	47.4aA	44.9aA	1.0	1.1	1.2	0.9aA	0.9aA	0.8aB
35xL	99aA	94aA	78bB	47.9aA	41.8abB	34.3bC	1.1	1.2	1.3	0.9aA	0.8abB	0.8aB
35xWL	92aA	98aA	93aA	44.7aA	46.6aA	42.3aA	1.1	1.1	1.2	0.9aA	0.9aAB	0.8aB

40xL	0bA	0bA	0cA	0eA	0eA	0eA	0	0	0	0eA	0eA	0fA
40Xwl	96aA	99aA	96aA	47.6aA	48.3aA	41.8aB	1.0	1.1	1.3	0.9aA	0.9aA	0.8aB

Photo: photoperiod, Subs: substrate, L: Light, WL: without light, OP: over paper, BP: between paper, BS: between sand. Averages followed by the lowercase letters in row and the uppercase letters in column differ from each other by Tukey test at 5% probability.

The triple interaction was not significant for average germination period. Germination percentages, GSI and AGS presented significant difference when they were associated to temperature, photoperiod and substrate. In all parameters, the association between temperature at 10 ºC and light absence, despite substrates, seeds did not germinate. Likewise, at 40 ºC, in light absence and in all substrates, germination was not observed.

The highest germination percentage (100%) was when the seeds were submitted to 20 ºC, in light absence, in sand. The nearest values (99%) were observed when seeds germinated at 30 ºC at light, both in sand and between paper. At 35 ºC, at light on paper, and at 40 ºC, in light absence between paper, showed the same germination percentage (99%). The observed results, in this study, allowed the following conclusion: chia seeds are both indifferent photoblasts and have easily germinated in sand and on paper in a large temperature range. However, Paiva et al. (2016) verified that the ideal temperature for chia seeds germination is 25 ºC or 25-30 ºC in alternate form. And, although they are indifferent to photoperiod, chia seedlings grow better and accumulate higher amount of dry matter in light presence. Different results were observed by Stefanello et al. (2015), who verified that photoperiod (presence or absence of light) and temperatures (20, 25 and 30 ºC) did not influence germination rate of *Salvia hispanica* seeds.

The highest germination speed indexes were at 30 and 35 ºC, both in light presence and absence, in the three substrates evaluated, as well as at 40 ºC in light absence despite the substrate (Table 5). The highest values of average speed were verified at 30 and 35ºC in paper and sand substrates, in both photoperiods. They were statistically similar, as well as at 40ºC in light absence, in the three substrates. The percentage, speed index and average germination speed were negatively influenced by the association of temperature at 10 °C with different substrates and light absence. The same parameters were similarly affected at 40 °C in light presence in the three substrates. According to these results, Labouriau and Agudo (1987) pointed out that chia seeds are considered physiologically heterogeneous, containing positive photoblastic subpopulations at 15 ºC and negative photoblastic subpopulations at 35 ºC. However, between 20 and 31 ºC, the germination was indifferent to light, which was confirmed in this trial.

Table 5: Percentage (G), speed index (GSI), average period (AGP) and average speed (AGS) of germination for chia seeds according to temperature x substrate interaction, under two photoperiods

Temp (°C) X Subs	G (%)		GSI		AGP (days)		AGS (days^{-1})	
	Photoperiod							
	Light	Without light	Light	Without light	Light	Without light	Light	Without light
10xOP	98aA	0Bb	16.1fgA	0dB	3.1	0	0.3hA	0gB
10xBP	95aA	0bB	15.4gA	0dB	3.1	0	0.3hA	0gB
10xBS	94aA	0bB	16.2fgA	0dB	2.9	0	0.3ghA	0gB
15xOP	98aA	97aA	24.2eA	23.1cA	2.0	2.1	0.5efA	0.5fA
15xBP	97aA	95aA	23.9eA	22.6cA	2.0	2.1	0.5efA	0.5fA
15xBS	98aA	94aA	23.5efA	19.5cA	2.1	2.6	0.5efA	0.4fB
20xOP	97aA	92aA	23.8eA	22.3cA	2.0	2.1	0.5efA	0.5fA
20xBP	92aA	98aA	21.9efgA	24.1cA	2.2	2.0	0.5fgA	0.5efA
20xBS	98aA	100aA	24.4eA	24.9cA	2.0	2.1	0.5dfB	0.6deA
25xOP	98aA	96aA	37.4bcdB	41.7aA	1.5	1.3	0.7cdB	0.8cA
25xBP	95aA	98aA	39.2bcdB	44.6aA	1.4	1.2	0.7bcB	08abcA
25xBS	98aA	93aA	33.5dA	33.00bA	1.7	1.6	0.6deA	0.6dA
30xOP	97aA	98aA	46.8aA	48.00aA	1.1	1.0	0.9aA	0.9abA
30xBP	99aA	98aA	47.6aA	47.4aA	1.1	1.1	0.9aA	0.9abA
30xBS	99aA	97aA	44.4abA	44.6aA	1.2	1.2	0.8abcA	0.8bcA

35xOP	99aA	92aB	47.9aA	44.7aA	1.1	1.1	0.9aA	0.9abA
35xBP	94aA	98aA	41.8abcB	46.6aA	1.2	1.1	0.8abA	0.9abcA
35xBS	78bB	94aA	34.3cdB	42.3aA	1.3	1.2	0.8bcA	0.8bcA
40xOP	0cB	96aA	0hB	47.6aA	0	1.0	0iB	1.0aA
40xBP	0cB	99aA	0hB	48.3aA	0	1.1	0iB	0.9abA
40xBS	0cB	96aA	0hB	41.8aA	0	1.3	0iB	0.8cA

Temp: temperature, Subs: substrate, OP: over paper, BP: between paper, BS: between sand. Averages followed by the lowercase letters in row and the uppercase letters in column differ from each other by Tukey test at 5% probability.

The substrates did not interfere significantly in the parameters evaluated when they were associated with 10 ºC in light presence, at 15 ºC in both photoperiods, and at 20 ºC in light presence, consequently their averages were statistically similar. Different results were found out by Pilau et al. (2012), which verified that the use of paper substrate under temperatures below 20 °C underestimates the germination potential of *Crambe abyssinica* Hochst seeds. The authors registered that substrates of paper and clay soil + sand are adequate for germination test, since, the correct temperature, ideal for seed germination, in this case *Crambe abyssinica* Hochst, was defined as 25 ºC.

The germination percentage was negatively affected when associated to 10 ºC in light absence, and 40 ºC in light, in the three substrates. Under this condition, values of germination percentage, germination speed index, average germination period and average germination speed were not observed. Seeds germinated at 30 ºC, on paper and between paper, and at 35 ºC on paper, presented the highest germination speed indexes (46.8, 47.6 and 47.9, respectively), showing greater vigor when in light presence. Similar results were observed by Stockman et al. (2007) in which temperature and substrate interfered in the germination of *Tabebuia roseo-alba* Ridl seeds. The most favorable condition for germination test of these seeds was 30 °C in paper substrate and at 8-hours daily photoperiod. Seeds germinated up from 25 ºC, despite the substrate, in light absence, also showed high seed germination indices, which were the highest at 30 ºC on paper (48.00) and at 40 ºC between paper (48.3).

The triple interaction was not significant for the average germination period. The average germination speed was positively influenced at 30 °C on paper substrates, at 35 °C on paper in light presence and at 40 °C on paper in light absence. These results were similar to those obtained by Santos Neto et al. (2008), which verified that *Mentha pulegium* L. seeds, exposed to higher temperatures, presented higher germination speed. The photoperiod did not affect significantly the parameters studied at 15 and 20 ºC, in paper substrates, at 25 ºC, in sand and at 30 ºC, despite the substrates. Likewise, Martins et al. (2007) found out that seeds of *Chenopodium ambrosioides* can be classified as insensitive to light because they germinate both in light absence and presence.

According to the Rules for Seed Analysis (Brasil, 2009), there are intervals of 20-30 ºC for *Salvia* spp seeds germination. Based on the established *Salvia* spp temperature intervals, in the present study, the observed results showed that the most adequate temperature ranges are in accordance with this recommendation. However, for each species, as evidenced by several authors cited here, there are more adequate conditions for seeds germination, and specific characteristics of each species, so this knowledge is essential.

In Figure 1, the water absorption curves by chia seeds at three temperatures during 720 minutes were observed.

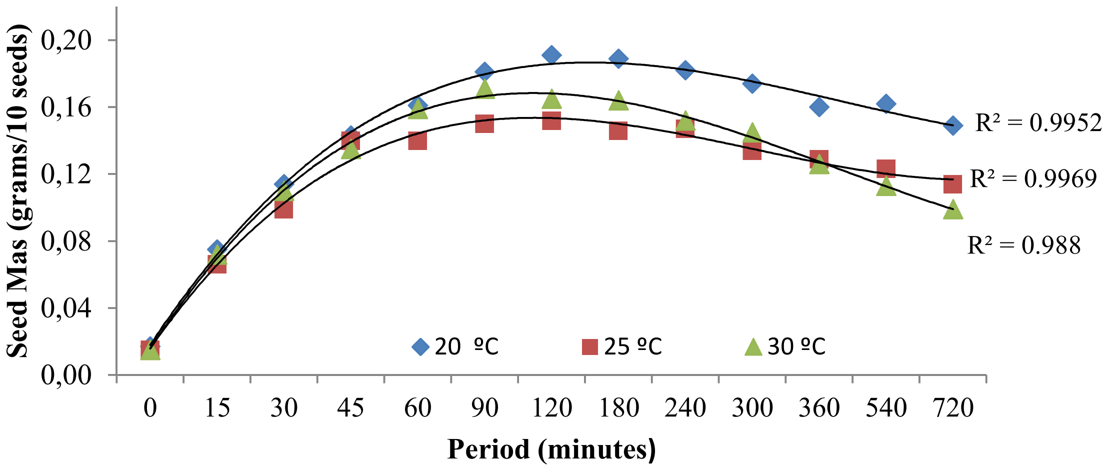

Figure 1. Water uptake of chia seeds during 720 minutes.

The water uptake of chia seeds at all temperatures was faster in the first 60 minutes, followed by slower absorption up to 180 minutes. There was a decrease in absorption up to 720 minutes. The obtained curve in this trial was not adjusted to triphasic pattern, which, according to Bewley and Black (1994), the seed imbibition process is characterized by an initial phase of fast water absorption, followed by a stationary phase, in which seeds almost do not absorb water and finally, a new increase in absorption rate, which coincides with the radicle protrusion and seedling growth.

In the first 15 minutes, it was observed that the water absorption of seeds was almost the same at all studied temperatures. The amount of water absorbed in this period was 33, 37 and 37% at 20, 25 and 30 °C, respectively. According to Bewley et al. (2013), when the seeds are in a dry state at maturity, water absorption is necessary for metabolism recovering that initially triggers cellular events, providing radicle emergence, by physical and structural changes associated with water imbibition. And during the next 15 minutes there is a slow differentiation of imbibition amount. The seeds that were submitted to 20 ºC showed a higher percentage of absorption when compared to seeds under other temperatures. The greatest water absorption peak was at 120 minutes with a mass of 0.191 g/10 seeds.

According to Carvalho and Nakagawa (2012), phase I is a short-lived process, whose, on average, seeds absorb water from two to four hours. When studied chia seeds were analyzed, it was observed that phase I was the fastest, around one hour. This probably happened due to their tiny size.

The seeds submitted to 30 and 25 ºC absorbed less water amount than those ones submitted to 20 ºC. The amount of water absorbed at 120 minutes was 0.15 and 0.96 g/10 seeds for seeds at 30 and 25 °C, respectively. However, there was an inversion on the water absorption curve at 360 minutes and at the last two temperatures, in which chia seeds submitted to 25ºC (83%) showed lower water loss than seeds at 30 ºC (71 %).

Chia seeds absorbed water rapidly in the initial period, and the highest absorption peak was observed at 120 minutes, while seeds kept at 20 ºC absorbed a greater amount of water. Differently from what was observed by Nunes et al. (2015), with *Phaseolus vulgaris* L, *Vigna unguiculata*, *Vigna angularis,* and *Vigna radiate*, whose seeds continued to absorb water after 48 hours of imbibition, even though it has been a slower process than the 12 first hours.

The amount of water absorbed by the seed is essential for the recovering of seeds metabolic activity (Marcos Filho, 2015). The data observed in this study, in which the average germination period was 1.1 to 2.6 days from 15 to 35 ºC, showed that, since chia seeds have fast absorption, they germinate easily in a short period of days, and in a wide temperature range. This behavior is probably due to the fact that the seed, in addition to the reduced size, has a mucilaginous layer, which hydrates fast addition to the reduced size, has a mucilaginous layer, which hydrates fast.

Conclusion

Germination of chia seeds can be evaluated on paper and sand substrates, both with and without light supply, at temperatures of 15 to 35 ºC.

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