Revista Ciência Agronômica, v. 57, e202595324, 2026
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Physiological quality of chia seeds as a function of sowing time and phosphorus rate[1]

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ABSTRACT - Chia has attracted interest in Brazilian agriculture due to the high nutritional value of its seeds. However, there is still limited information on agronomic practices aimed at producing seeds with high physiological quality. Among the factors that influence this attribute, sowing time and phosphorus fertilization stand out, especially in the sandy and acidic soils of northwestern Paraná. Therefore, it is necessary to investigate practices that enhance chia seed vigor and germination. The experiment was conducted in the Seed Laboratory of the State University of Maringá, Umuarama Campus. The design was a randomized complete block design with a 4 × 4 factorial arrangement and 4 replications. Germination percentage, germination speed index, seedling shoot length, seedling root length, and electrical conductivity were determined. Sowing time and phosphorus rate significantly influenced the physiological quality of chia seeds. The sowing time for producing chia seeds with high physiological quality in the study area is at the end of March, combined with the application of approximately 60 kg ha^{-1} P_2O_5.

Keywords: Germination. Physiological potential. Seed analysis. Vigor. *Salvia hispanica*.

DOI: 10.5935/1806-6690.v57e202595324
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Received for publication 28/03/2025; approved on 12/08/2025
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INTRODUCTION

Salvia hispanica, commonly known as chia, is an annual herbaceous plant belonging to the family Lamiaceae. Native to southwestern Mexico and northern Guatemala, it has become one of the main crops in Mesoamerica due to its nutritional properties (Madaan *et al*., 2020). Chia cultivation has attracted growing interest from producers and researchers. Studies on the nutritional and medicinal properties of chia seeds showed *that* they are rich in linoleic and alpha-linolenic acids (De Falco; Amato; Lanzotti, 2017). These essential fatty acids are associated with a reduced risk of developing cardiovascular diseases, hypercholesterolemia, hypertriglyceridemia, and obesity, contribute to the regulation of intestinal function and support the immune system (Grancieri; Martino; Gonzalez de Mejia, 2019).

Chia is also noteworthy for its capacity to adapt to tropical and subtropical climates (Herman *et al*., 2016; Win *et al*., 2018). The main form of propagation is through seeds, which measure from 1 to 2 mm (Stefanello *et al*., 2016). As the crop has been recently introduced to Brazil, there is still limited agronomic and technological information on sowing times, fertilization practices, and seed germination parameters. Chia yield is strongly influenced by climatic conditions and sowing date, underscoring the importance of the growing environment. Brazil's climate is generally favorable to chia development. The crop is intolerant to frost and sensitive to low temperatures, which can impact grain yield (Bochicchio *et al*., 2015).

There is no fertilization guide available for chia cultivation in Brazil. One key nutrient that should be considered in fertilization strategies for chia crops is phosphorus. This element serves as a structural component of nucleic acids, coenzymes, phosphoproteins, and phospholipids (Moura *et al*., 2015). It is crucial to understand the effects of phosphorus fertilization on chia development. Research has demonstrated that soils with low phosphorus availability, mainly acidic soils in tropical and subtropical regions, can limit the production of numerous crops (Bucher *et al*., 2018). Additionally, there is a need for studies that go beyond the nutritional properties of chia seeds, particularly investigations that address physiological quality. By enhancing our understanding of seed production and germination parameters, it will be possible to improve field cultivation (Stefanello *et al*., 2015).

There are no standardized methods to assess the viability and vigor of chia seeds according to national seed analysis guidelines. Key parameters, such as germination rates at first and second counts, follow specific patterns for each crop (Brasil, 2009). Further studies are needed to standardize viability and vigor tests for chia seeds. Currently, Brazilian production is confined to small-scale systems that rely on imported seeds (Stefanello *et al*., 2015), which ultimately hinders our understanding of physiological quality.

Despite these limitations, the growing interest in chia cultivation is promising due to its nutritional properties. The use of high-quality seeds is crucial for the success of propagation and commercialization efforts. According to national seed analysis guidelines, the physiological potential of seeds should be evaluated in the laboratory by the germination test, which provides information on the number of normal seedlings produced from a seed batch (Brasil, 2009). For a comprehensive evaluation of physiological quality and a more accurate prediction of field results, it is necessary to complement the germination test with vigor tests. Seed vigor encompasses all properties that determine seed activity and performance during germination and development under different climatic conditions (Krzyzanowski *et al*., 2020)

In view of the relevance of this plant species and the lack of information on seed technology, this study aimed to assess the physiological quality of chia seeds as a function of sowing times and phosphorus rates in Umuarama, Paraná, Brazil.

MATERIAL AND METHODS

The experiment was conducted at the Seed Laboratory of the State University of Maringá (UEM), Umuarama Campus, Paraná, Brazil (23°47′28.4″S 53°15′24″W, 379 m a.s.l.). The soil is a typical Dystrophic Red Latosol with sandy texture (Embrapa, 2018). Soil chemical properties are summarized in Table 1.

The climate of the region is of the Cfa type, characterized as subtropical, with average temperatures below 18 °C in the coldest month and above 22 °C in the hottest month. Summers are hot, frosts are rare, and rainfall tends to concentrate in the summer. There is no well-defined dry season.

Seeds of chia were produced in a field experiment carried out at the Experimental Farm of the State University of Maringá, Umuarama campus. Crops received different amounts of water from sowing to harvest depending on the sowing date, as follows: batch 1 (sown on March 21), 467.7 mm; batch 2 (sown on April 4), 452.1 mm; batch 3 (sown on April 18), 356.4 mm; and batch 4 (May 2), 317.8 mm. The last harvest date was July 20. Rainfall, temperature, and relative humidity data for the seed production period are presented in Table 2.

The experiment followed a randomized complete block design with a 4 × 4 factorial arrangement and 4 replications. The first factor was sowing time (T): T1, March 21, 2017; T2, April 4, 2017; T3, April 18, 2017; and T4, May 2, 2017. The second factor was phosphorus rate (0, 40, 80, and 120 kg ha^{-1} P_2O_5).

The physiological potential of seeds was assessed by germination and vigor tests. For the germination test, two evaluations were performed: the first count on the fifth day, which was considered as an indicator of seed vigor, and the final count on the eighth day, corresponding to the total germination percentage. As there is no official protocol established in the literature for chia seeds regarding the duration of germination tests, the time intervals adopted followed the recommendations established for soybean, common bean, peanut, and cowpea (Brasil, 2009).

The germination speed index (GSI) was calculated during the germination test. For this, germinated seeds were counted at the same time daily from the second day after sowing onward. Seeds were considered germinated when radicle length reached about 2 mm. Counting was stopped when seedling emergence plateaued (8 days after sowing). GSI was calculated as follows (Eq. 1) (Maguire, 1962):

$$GSI = G_1 / N_1 + G_2 / N_2 + \dots G_n / N_n \quad (1)$$

Where GSI is the germination speed index; G_1, G_2, and G_n are the number of germinated seeds recorded in the first, second, and last counts; and N_1, N_2, and N_n are the number of days from the date of sowing to the first, second, and last counts.

Seedling length was determined according to a previously described method (Walkley; Black, 1934). Briefly, four replications of 10 seeds were sown along a line drawn longitudinally in the upper third of moistened germination paper. The papers were rolled into cylinders and stored vertically in a germinator at 25 °C under a 12 h photoperiod. The lengths of the primary root and aboveground part of seedlings were measured on the eighth day after sowing by using a millimeter ruler. Root and shoot lengths (cm) are expressed as mean values, calculated by summing the measurement of each replicate and dividing by the number of normal seedlings.

The electrical conductivity of the imbibing solution was determined on four replications of 75 seeds. Seeds were selected from a physically pure sample and weighed into plastic cups containing 50 mL of deionized water using a precision scale (0.01 g precision). Then, seeds were incubated in a germinator at 25 °C. After 24 h, electrical conductivity was measured using a benchtop digital microprocessor-based conductivity meter (Tecnal TEC-4MP). The results are expressed as µS cm^{-1} g^{-1} seed (Vieira; Krzyzanowski, 1999).

Experimental data were subjected to analysis of variance (*F*-test) and means were compared by Tukey's test.

Table 1 - Soil chemical properties in the 0–20 cm layer at the experimental local

pH	P	OM	Ca	K	Mg	Al	CEC	BS
$CaCl_2$	mg dm^{-3}	g dm^{-3}	------------------------------ cmol$_c$ dm^{-3} ------------------------------					%
4.35	1.0	15.04	0.72	0.1	0.21	1.35	3.88	26.55

OM, organic matter; CEC, cation-exchange capacity; BS, base saturation. P and K were extracted with resin. Organic matter was extracted by the Walkley–Black method (1934). Ca, Mg, and Al were extracted with 1 cmol L^{-1} KCl

Table 2 - Climate data for the seed production period

Month	Rainfall (mm)	Minimum temperature (°C)	Maximum temperature (°C)	Relative humidity (%)
March*	15.6	18.9	30.2	71.2
April	134.3	19.8	31.1	73.5
May	247.7	18.0	25.2	82.9
June	69.1	14.8	24.2	72.9
July**	1.0	12.1	24.8	54.2

Chia seeds were sown on March 21 (batch 1), April 4 (batch 2), April 18 (batch 3), and May 2 (batch 4). Climate data refer to the period between March 21 (*, sowing of the first batch) and July 20 (**, harvest)

RESULTS AND DISCUSSION

The performance of chia seedlings was assessed in terms of root and shoot lengths (Table 3). There was a significant effect of sowing time on all variables analyzed. However, the interaction between sowing time and phosphorus rate was not significant.

Root and shoot lengths did not differ between T1, T2, and T3 but were significantly lower in T4. Crop development was impaired in T4 because of the low temperatures and occurrence of frost. This finding demonstrated that chia seeds are sensitive to thermal stress due to low temperatures (Jojoa *et al*., 2021). In the laboratory test, seedlings from seeds with earlier sowing dates (T1 and T2) were more vigorous, as indicated by their greater shoot and root lengths (Nakagawa, 1999). Seedling length is commonly used as a measure of vigor, suggesting that vigorous seeds produce seedlings with enhanced growth rates (Krzyzanowski *et al*., 2020), given the greater capacity for nutrient transfer from reserve tissues to the developing embryonic axis (Vieira; Krzyzanowski, 1999). As plants were sown at different times, they developed under different temperature conditions. Plants sown at later periods were subject to lower temperatures and photoperiods, which affected their growth, yield, and seed quality (Jojoa *et al*., 2021).

Phosphorus rate did not influence shoot or root length, possibly because seeds contained sufficient reserves for initial seedling development. According to Grant *et al*. (2001), at the beginning of development, plants utilize seed reserves to grow, and thus external inputs have a small influence on early plant growth. Plants in early development may have different mineral needs, depending on the balance between internal and external nutrient supply and their specific nutritional requirements.

GSI was influenced by the interaction effect of sowing time and phosphorus rate. The effects of sowing times within phosphorus rates and vice versa are shown in Table 4. Within phosphorus rate treatments, T1 and T2 did not differ from each other in GSI, being higher than T3 and T4 at all rates. Seeds from T4 plants, in addition to having a low germination rate, had a low germination percentage. These results can be attributed to the low temperatures observed in the last two sowing times in the field. As such, these plants produced less vigorous seeds with low reserves, resulting in low GSI in the laboratory. Optimal plant development is influenced by temperature variations, which affect seed water absorption and the biochemical reactions that control germination and growth processes, including speed, percentage, and uniformity (Krzyzanowski *et al*., 2020).

Table 3 - Shoot and root lengths (cm) of chia seedlings as a function of sowing time and phosphorus rate. Umuarama, Paraná, Brazil

Treatment	Shoot length (cm)	Root length (cm)
Sowing time		
T1	2.74 a	3.34 a
T2	2.75 a	3.43 a
T3	2.72 a	3.66 a
T4	1.86 b	1.90 b
CV (%)	35.6	37.2
Phosphorus rate (kg ha^{-1})		
0	2.19	2.65
40	3.00	2.91
80	2.58	3.59
120	2.30	3.16
Analysis of variance (F-test)		
Sowing time (T)	*	*
Phosphorus rate (P)	Ns	Ns
T × P	Ns	Ns
Linear regression	Ns	Ns
Quadratic regression	Ns	Ns

T1, March 21; T2, April 4; T3, April 18; T4, May 2; CV, coefficient of variation; * significant at $p < 0.05$; ns, not significant. For sowing time, means in a column followed by the same letter are not significantly different from each other by Tukey's test at $p < 0.05$

Phosphorus rate significantly influenced GSI at all sowing times. The relationship between phosphorus rate and GSI was modeled using quadratic regression, with the rates of maximum technical efficiency being 65.06, 57.37, 67, and 33.6 kg ha^{-1} P_2O_5 for T1, T2, T3, and T4, respectively. It can be concluded that T4 plants were negatively affected by low temperatures, demonstrating that this sowing time is not suitable for planting chia in the region. These plants were unable to use all available phosphorus. This nutrient is closely linked to the availability of energy for metabolism, stored in the form of ADP and ATP (Bucher *et al*., 2018).

Germination speed index is widely used in the evaluation of seed vigor, as it reflects both the speed and uniformity of the germination process. However, it is important to emphasize that GSI should not be interpreted in isolation. Seeds that germinate rapidly do not necessarily exhibit superior performance under field conditions, especially when subjected to environmental stresses. Solely relying on this index may overestimate the physiological potential of seed lots that, despite germinating quickly, may lack sufficient reserves or adequate cellular integrity to ensure proper seedling establishment (Krzyzanowski *et al*., 2020; Marcos-Filho, 2015). Therefore, the interpretation of GSI should be complemented by other vigor tests and physiological parameters, such as electrical conductivity, seedling length, and field emergence, to provide a more robust assessment of seed quality.

The first germination count (5 days after sowing) was influenced by the interaction effects of Sowing time × Phosphorus rate (Table 5). Within phosphorus rate treatments, T1 and T2 seeds did not differ from each other and had a higher germination count than T3 and T4 seeds. Seeds of T1 and T2 plants germinated faster in the first count, being considered more vigorous (Krzyzanowski *et al*., 2020). Seeds of T3 and T4 plants were less vigorous, attributed to the low temperatures and their effects on the rate of biochemical reactions, in addition to physiological processes influencing germination (Carvalho; Nakagawa, 2012). First count may be indicative of germination speed (Nakagawa, 1999), as occurred in this study.

Table 4 - Germination speed index (GSI) of chia seeds as a function of sowing time and phosphorus rate. Umuarama, Paraná State, Brazil

Sowing time	P_2O_5 rate (kg ha^{-1})				Regression equation
	0	40	80	120	
T1	59.45 a	61.18 a	63.11 a	59.61 a	$Y = -0.0008x^2 + 0.1041x + 59.169$, $R^2 = 0.81$
T2	65.03 a	51.84 a	58.27 a	69.85 a	$Y = 0.0032x^2 - 0.3672x + 64.107$, $R^2 = 0.86$
T3	22.58 b	34.74 b	35.18 b	28.20 b	$Y = -0.003x^2 + 0.4021x + 22.795$, $R^2 = 0.99$
T4	0.86 c	3.26 c	0.06 c	10.58 c	$Y = 0.0013x^2 - 0.0874x + 1.826$, $R^2 = 0.72$
CV (%)				16.1	

T1, March 21; T2, April 4; T3, April 18; T4, May 2; CV, coefficient of variation. Means in a column followed by the same letter do not differ from each other by Tukey's test at $p < 0.05$

Table 5 - First germination count (5 days after sowing) of chia seeds as a function of sowing time and phosphorus rate. Umuarama, Paraná State, Brazil

Sowing time	P_2O_5 rate (kg ha^{-1})				Regression equation
	0	40	80	120	
T1	69.50 a	74.00 a	73.00 a	71.00 a	$Y = -0.001x^2 + 0.1306x + 69.725$, $R^2 = 0.92$
T2	76.50 a	60.00 a	67.50 a	75.50 a	$Y = 0.0038x^2 - 0.4481x + 75.325$, R2 = 0.84
T3	26.50 b	41.50 b	42.00 b	34.50 b	$Y = -0.0035x^2 + 0.4831x + 26.825$, $R^2 = 0.99$
T4	1.00 c	3.50 c	0.00 c	12.00 c	$Y = 0.0015x^2 - 0.1044x + 2.075$, $R^2 = 0.74$
CV (%)				16.4	

T1, March 21; T2, April 4; T3, April 18; T4, May 2; CV, coefficient of variation. Means in a column followed by the same letter do not differ from each other by Tukey's test at $p < 0.05$

First count showed a quadratic response to phosphorus rates within each sowing time. For this parameter, the maximum technical efficiency was estimated to be achieved with 65.3, 59, 69, and 35 kg ha^{-1} P_2O_5 for T1, T2, T3, and T4, respectively. Seed vigor is influenced by several factors, such as climatic conditions during maturation, nutrition of parent plants, seed size, and seed treatment, among others (Carvalho; Nakagawa, 2012). Phosphate fertilization is essential for the development of crops, as this nutrient composes cell membranes, nucleic acids, and ATP, which are vital to plant metabolism (Taiz; Zeiger, 2017). Adequate management of this nutrient can lead to more vigorous plants. T4 plants were not able to fully utilize phosphorus, in view of their stunted growth due to low temperatures.

Germination test was influenced by the interaction effects of factors (Table 6). The parameter was higher in T1 than in the other sowing times within the 40 kg ha^{-1} P_2O_5 treatment. In general, second count was higher in T1 and T2 in all phosphorus treatments. As previously mentioned, seeds from T3 and T4 were likely impacted by the low temperatures occurring during the crop cycle. The laboratory germination test confirmed that these conditions resulted in chia seeds with low viability and vigor. The germination capacity of plant species has a defined limit, with temperature exerting a direct influence (Krzyzanowski *et al*., 2020).

Germination test showed a quadratic response to phosphorus rates. The maximum technical efficiency was estimated at 60, 55.68, 70.56, and 33.96 kg ha^{-1} P_2O_5 for T1, T2, T3, and T4, respectively. Good germination conditions are crucial for the successful establishment of crops in the field (Krzyzanowski *et al*., 2020). Phosphorus influences several vital processes of plants; its adequate supply from germination onward may contribute to rapid root growth (Bucher *et al*., 2018). Nutrient availability is needed for the formation of embryos and reserve organs, affecting seed chemical composition and quality (Carvalho; Nakagawa, 2012).

Electrical conductivity was found to be influenced by the interaction effect of sowing time and phosphorus rate (Table 7). In general, electrical conductivity was lower in T1 and T2, confirming that seeds from these plants were more vigorous. During decay, cellular constituents are leached as seeds are soaked in water, resulting from the loss of membrane integrity. A low conductivity is indicative of high seed quality; the higher the conductivity value, the greater the leaching of cellular constituents, resulting in reduced seed vigor (Krzyzanowski *et al*., 2020). In the control treatment, electrical conductivity did not differ between T1 and T2, and T1 did not differ from T3; however, T4 had the lowest electrical conductivity. For the 40 and 80 kg ha^{-1} P_2O_5 treatments, electrical conductivity did not differ between T1 and T2 groups, which were lower than T3 and T4. Within the 120 kg ha^{-1} P_2O_5 treatment, T1 and T2 did not differ from each other, and T2 did not differ from T3, but all groups had lower electrical conductivities than T4.

Seeds from T1 and T2 treatments exhibited better electrical conductivity values because of the favorable environmental conditions in the field experiment. Rainfall levels and temperatures were adequate for chia growth. In the last sowing periods, temperatures were low and frost occurred, possibly contributing to the low vigor of seeds from these treatments.

The relationship between electrical conductivity and phosphorus rate was explained by a quadratic model. The rates of maximum technical efficiency were estimated to be 83.2, 25.9, 67.3, and 57 kg ha^{-1} P_2O_5 for T1, T2, T3, and T4, respectively. A lower conductivity indicates a lower amount of leached ions (Nunes, 2017), as the electrical conductivity indirectly assesses the intensity of the damage caused to cellular membranes resulting from the seed deterioration process (Silva Neta *et al*., 2024), the higher the value, the more ions are leached, indicating low vigor (Muraro *et al*., 2017). Phosphorus is essential for metabolic processes. Energy in the form of ATP is used in germination, nutrient absorption, physiological maturation, flowering, and seed formation (Taiz; Zeiger, 2017), factors that are crucial for crop development.

Table 6 - Germination test (%) of chia seeds as a function of sowing time and phosphorus rate. Umuarama, Paraná State, Brazil

Sowing time	P_2O_5 rate (kg ha^{-1})				Regression equation
	0	40	80	120	
T1	70.50 a	74.50 a	74.50 a	71.00 a	$Y = -0.0012x^2 + 0.1444x + 70.525$, $R^2 = 0.99$
T2	77.00 a	60.00 b	68.00 a	79.00 a	$Y = 0.0044x^2 - 0.49x + 75.9$, $R^2 = 0.89$
T3	28.50 b	43.00 c	42.50 b	37.00 b	$Y = -0.0031x^2 + 0.4375x + 29$, $R^2 = 0.96$
T4	1.00 c	4.00 d	0.00 c	12.50 c	$Y = 0.0015x^2 - 0.1019x + 2.175$, $R^2 = 0.71$
CV (%)				15.46	

T1, March 21; T2, April 4; T3, April 18; T4, May 2; CV, coefficient of variation. Means in a column followed by the same letter do not differ from each other by Tukey's test at $p < 0.05$

Table 7 - Electrical conductivity of chia seeds as a function of sowing time and phosphorus rate. Umuarama, Paraná State, Brazil

Sowing time	P_2O_5 rate (kg ha^{-1})				Regression equation
	0	40	80	120	
T1	442.61 ab	293.89 a	279.95 a	291.86 a	$Y = 0.0251x^2 - 4.1773x + 437.16$, $R^2 = 0.96$
T2	312.35 a	294.99 a	360.57 a	468.14 ab	$Y = 0.0195x^2 - 1.0101x + 310.3$, $R^2 = 0.99$
T3	549.95 b	677.97 b	668.86 b	610.07 b	$Y = 0.0292x^2 + 3.9308x + 554.32$, $R^2 = 0.96$
T4	1233.09 c	774.97 b	1176.21 c	1154.57 c	$Y = 0.0682x^2 - 7.7698x + 1169$, $R^2 = 0.37$
CV (%)				16.32	

T1, March 21; T2, April 4; T3, April 18; T4, May 2; CV, coefficient of variation. Means in a column followed by the same letter do not differ from each other by Tukey's test at $p < 0.05$

CONCLUSIONS

1. Vigor tests revealed that sowing time and phosphorus rate significantly influenced the physiological quality of chia seeds;
2. The best sowing time for chia production in the study area was at the end of March, combined with the application of approximately 60 kg ha^{-1} P_2O_5.

ACKNOWLEDGMENT

This study was financed in part by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES, Finance Code 001) and the Brazilian National Council for Scientific and Technological Development (CNPq).

DATA AVAILABILITY STATEMENT

Data-available: The research data are available from the corresponding author upon reasonable request.

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