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Physiological quality of chia seeds as a function of coat color and fungicide treatment[1]

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ABSTRACT - Chia (*Salvia hispanica* L.) seeds have attracted great economic interest for their high levels of linoleic and α-linolenic acids. In Brazil, there is a lack of studies on the agronomic characteristics and physiological quality of chia, necessitating research on germination processes and strategies to enhance yield under field conditions. There are no studies assessing the development of seeds with different coat colors in the presence of fungicides. This study aimed to assess the physiological quality of chia seeds as a function of coat color and fungicide treatment. The experiment followed a completely randomized design with a 2 × 2 factorial arrangement and six replications. For this, seeds were divided into two groups according to coat color (black and white) and subjected or not to treatment with carboxin + thiram. Germination speed index, germination rate, and shoot length were evaluated in plants sown at 0, 90, and 180 days after seed treatment. Germination rate and speed index differed significantly between coat colors, with white seeds having higher vigor than black seeds. Shoot length, however, differed only for seeds sown on the day of treatment, indicating that treated seeds were more vigorous.

Key words: *Salvia hispanica* L. Seed treatment. Vigor.

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INTRODUCTION

Salvia hispanica, commonly known as chia, belongs to the family Lamiaceae. This annual herbaceous plant originated in southwestern Mexico and northern Guatemala (Busilacchi *et al*., 2013) and, because of its important nutritional properties, became one of the major crops of Mesoamerica (USDA, 2016). This plant species shows great adaptability to tropical climates, such as those found in Brazil (Silva *et al*., 2020), and can be easily propagated from its small seeds, which measure between 1 and 2 mm (Bordin-Rodrigues *et al*., 2021).

Chia production has attracted great economic and scientific interest owing to the nutritional and medicinal properties of seeds, particularly their high contents of linoleic and α-linolenic acids (Dal'Maso *et al*., 2013). Chia seeds are a natural source of omega-3 fatty acids, fibers, and proteins, as well as other important nutritional components, such as antioxidants (Coelho; Salas-Mellado, 2014) and secondary metabolites with beneficial effects on immune function (Ludwig *et al*., 2013).

As chia crops have been recently introduced to Brazil, there is scarce information regarding their agronomic and technological properties (e.g., sowing time, fertilization strategies, and germination rate). Further studies assessing the physiological quality of chia seeds are needed to deepen our knowledge of germination processes and support the production of high-quality seeds with improved yield under field conditions (Stefanello *et al*., 2015).

Currently, no standardized protocols are available in the national Rules for Seed Testing to assess the viability and vigor of chia seeds during the first and second days of germination (Brasil, 2009). Specific guidelines are needed because different species follow different germination patterns. In Brazil, chia crops are mainly grown on a small scale from imported seeds (Stefanello *et al*., 2015). Therefore, standardized viability and vigor tests for this species are crucial for obtaining accurate information on physiological quality, thereby minimizing economic losses. The production of high-quality seeds is of great importance not only for propagation but also for commercialization, as chia seeds are widely used in cooking.

There are no studies investigating the relationship between coat color and physiological quality in chia seeds. Chia seeds can vary in color from black to white. At harvest, light-colored seeds tend to be larger and denser than darker seeds. Larger and denser seeds can be expected to contain higher levels of reserves, suggesting increased vigor in the field. Such an association was reported by Bispo *et al*. (2017), who concluded that seed size directly influences seedling growth and can be used as an indicator of vigor in angico seeds.

Seed treatment is recommended for most crop species grown in Brazil. In assessing the effectiveness of seed treatment for soybean, Soares *et al*. (2019) observed that treated seeds had higher emergence and yield under field conditions because they were less susceptible to the negative effects of pests and diseases. Seed treatment was found to improve germination percentage in some crops (Lorenzo-Barrera *et al*., 2024). However, not all plant species respond positively to seed treatment. In the study of Mattei *et al*. (2013), fungicide treatment of crambe seeds negatively affected initial plant development, resulting in delayed growth, seedling abnormalities, and uneven emergence.

It is necessary to evaluate the influence of coat color and fungicide treatment on chia seed germination. This information is not available in the literature, and there are few studies on seed treatment strategies for this important crop. Thus, this study aimed to assess the physiological quality of chia seeds as a function of coat color and fungicide treatment.

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.). Chia seeds were purchased at a local store in Londrina, Paraná, Brazil.

Tests were performed to evaluate the physiological potential of chia seeds. Seeds were separated according to coat color (black or white) and subjected or not to treatment with a commercial product based on carboxin + thiram. Assessments were performed at 0, 90, and 180 days after seed treatment. Seeds were stored in paper bags at room temperature until analysis. The experiment followed a completely randomized design with a 2 × 2 factorial arrangement and six replications.

The germination test was performed with four replications of 50 seeds per treatment. Seeds were spread on two sheets of germination paper, covered with a third sheet, and moistened with a volume of distilled water 2.5 times the weight of dry paper. Sheets were rolled and placed in a biochemical oxygen demand (BOD) germinator at 25 °C under a photoperiod of 12 h light. Seed vigor was assessed on day 5 of germination and seed viability on day 8.

The germination speed index (GSI) was determined by counting the number of germinated seeds daily, at the same time of day, from the second day after sowing onward. Seeds were considered germinated when the radicle measured about 2 mm long. Counting was interrupted when seedling emergence stabilized (day 8), and the index was calculated according to Maguire (1962), by using the formula GSI = $G_1/N_1 + G_2/N_2 + ... + G_n/N_n$, where G_1, G_2, and G_n represent the number of germinated seeds in the first, second, and last counts, respectively, and N_1, N_2, and N_n represent the number of days from sowing to the first, second, and last counts, respectively.

Seeding length was determined according to the method of Nakagawa (1999). Briefly, four replications of 10 seeds each were sown on a line longitudinal to the upper third of moistened substrate paper. The substrate paper was then rolled and placed vertically in a BOD chamber at 25 °C under a photoperiod of 12 h light. Assessment of the length of the primary root and aerial part of seedlings was performed 8 days after sowing by using a millimeter ruler. The mean shoot and root lengths of seedlings were obtained by adding the lengths of each replication and dividing by the number of normal seedlings germinated in each replication; results are expressed as centimeter per seedling.

Data were subjected to analysis of variance (*F*-test), and means were compared by Tukey's test using SISVAR software.

RESULTS AND DISCUSSION

The results of the germination test were used to determine the germination percentage, which is indicative of commercialization potential. At 8 days after sowing (Table 1), there was a higher number of normal seedlings grown from white seeds than from black seeds. Such a difference was observed in the three evaluation periods. Fungicide treatment (carboxin + thiram) did not influence germination percentage.

Significant differences in germination rate were observed between chia seed colors; the germination percentage of white seeds was 65.4% – 69.5% and that of black seeds was 53.2% – 55.0%. White seeds showed better germination capacity. According to Brazilian legislation (Brasil, 2009), seed lots must reach a minimum germination percentage to be commercialized, but this threshold has not yet been determined for chia seeds.

It is believed that differences in seed color stem from changes to the integument. Santos *et al*. (2007) found that yellow soybean seeds had lower lignin content in the integument than brown seeds and that yellow seeds exhibited higher imbibition rates. Thus, white chia seeds might have lower integument lignin contents, having therefore a greater imbibition capacity, which influences germination.

Seed vigor in the first germination count is described in Table 2. Seed color had a significant effect on this parameter. White seeds had higher vigor than black seeds, resulting in a higher proportion of normal seedlings at 5 days after sowing.

The vigor of white seeds ranged from 62.0% to 68.0% and that of black seeds from 51.2% to 56.2%, demonstrating that white seeds had higher vigor. Seed viability and vigor directly influence emergence rate and total emergence.

Table 1 - Germination rate of chia seeds as a function of coat color and carboxin + thiram treatment at 0, 90, and 180 days after treatment (DAT). Umuarama, PR, Brazil, 2021

Item	Germination rate (%)		
	0 DAT	90 DAT	180 DAT
Coat color			
White	65.7 a	65.4 a	69.5 a
Black	55.0 b	54.2 b	53.2 b
Treatment			
Untreated	61.7 a	66.0 a	59.0 a
Treated	59.0 a	61.2 a	63.7 a
CV (%)	7.0	8.6	10.3
F-test			
Coat color (C)	**	*	*
Treatment (T)	ns	ns	ns
C × T	ns	ns	ns

CV, coefficient of variation; ns, not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$. Means within columns followed by the same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 2 - Vigor (first germination percentage) of chia seeds as a function of coat color and carboxin + thiram treatment at 0, 90, and 180 days after treatment (DAT). Umuarama, PR, Brazil, 2021

Item	Seed vigor (%)		
	0 DAT	90 DAT	180 DAT
Coat color			
White	62.0 a	62.5 a	68.0 a
Black	52.2 b	56.2 b	51.2 b
	Treatment		
Untreated	59.0 a	59.0 a	57.0 a
Treated	55.2 a	56.2 a	62.2 a
CV (%)	8.7	7.6	13.2
	F-test		
Coat color (C)	**	*	*
Treatment (T)	ns	ns	ns
C × T	ns	ns	ns

CV, coefficient of variation; ns, not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$. Means within columns followed by the same letter are not significantly different at $p < 0.05$ by Tukey's test

Fungicide treatment did not significantly influence seed vigor (Table 2); that is, seeds treated with fungicides did not differ from untreated seeds. In some cases, treatment of seeds with pesticides can influence physiological potential. For instance, Rubert *et al*. (2023) found that treatment with fungicides and insecticides considerably improved the physiological properties of lentil seeds. By contrast, Mattei *et al*. (2013) reported that fungicide treatment of crambe seeds negatively affected initial plant development, resulting in delayed growth, seedling abnormalities, and uneven emergence.

The GSI is an indicator of physiological vigor and can be used to determine the specific physiological activity of seeds. GSI values differed significantly between coat colors, indicating that white seeds germinated faster than black seeds. Such a difference was observed in all evaluation periods (Table 3). The GSI of white seeds ranged from 16.19 to 17.37; and that of black seeds, from 12.63 to 14.48.

Seed size is another trait possibly influencing physiological quality, as previously reported (Coelho *et al*., 2019). According to Carvalho and Nakagawa (2000), larger seeds have well-formed embryos with higher levels of reserves. As a result, they are more likely to be vigorous, increasing the probability of successful seedling establishment. This fact further corroborates that chia seeds with white integument have higher vigor because of their greater size compared with seeds with black integument. Coelho *et al*. (2019), in evaluating the size and storage time of soybean seeds, found that 6 mm seeds had higher germination rates than 5 mm seeds. The authors observed that seed grading has a direct influence on germination rate and vigor. Storage time was also found to influence germination, and seed size influenced vigor. Overall, the results suggest that white chia seeds may provide a better crop stand under unfavorable sowing conditions. This finding does not imply that black seeds do not provide good results; rather, it suggests that black seeds may necessitate more favorable environmental conditions for optimal development.

Seed treatment did not significantly influence GSI, not affecting vigor. Treatment influenced seedling length only for seeds sown on the day of treatment; fungicide-treated seeds exhibited larger root lengths. However, this difference was not observed on the other evaluation days, as shown in Table 4. Coat color did not influence root or shoot length.

No differences in root length were observed between black and white chia seeds. Therefore, although white seeds had greater vigor (as evidenced by germination rate and GSI), after emergence, white and black seeds developed similarly. These findings suggest that the difference in vigor between seeds is not evident at more advanced stages of development.

Seed treatment led to a significant difference in root length for seeds sown on the day of treatment. Treated seeds had a root length of 3.69 cm, and untreated seeds had a root length of 2.83 cm, demonstrating that treated seeds had more vigor. No differences were observed between treated and untreated seeds stored for 90 or 180 days. Thus, fungicide treatment did not affect seed quality, showing that seeds may be treated and stored without negative effects.

Table 3 - Germination speed index of chia seeds as a function of coat color and carboxin + thiram treatment at 0, 90, and 180 days after treatment (DAT). Umuarama, PR, Brazil, 2021

Item	Germination speed index		
	0 DAT	90 DAT	180 DAT
	Coat color		
White	17.37 a	16.19 a	16.73 a
Black	12.86 b	14.48 b	12.63 b
	Treatment		
Untreated	15.44 a	14.56 a	14.28 a
Treated	14.79 a	16.11 a	15.11 a
CV (%)	8.4	9.3	10.9
F-test			
Coat color (C)	**	**	**
Treatment (T)	ns	ns	ns
C × T	ns	ns	ns

CV, coefficient of variation; ns, not significant; ** significant at $p < 0.01$. Means within columns followed by the same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 4 - Root length as a function of seed coat color and carboxin + thiram treatment of chia plants sown at 0, 90, and 180 days after treatment (DAT). Umuarama, PR, Brazil, 2021

Item	Root length (cm)		
	0 DAT	90 DAT	180 DAT
	Coat color		
White	3.06 a	4.61 a	6.47 a
Black	3.47 a	4.29 a	6.58 a
Treatment			
Untreated	2.83 b	4.63 a	6.28 a
Treated	3.69 a	4.27 a	6.76 a
CV (%)	16.9	13.8	11.5
	F-test		
Coat color (C)	ns	ns	ns
Treatment (T)	**	ns	ns
C × T	ns	ns	ns

CV, coefficient of variation; ns, not significant; ** significant at $p < 0.01$. Means within columns followed by the same letter are not significantly different at $p < 0.05$ by Tukey's test

A significant influence of treatment on shoot length was observed only for seeds sown on the day of treatment (Table 5). Seeds treated with fungicide obtained higher values (2.64 cm) than untreated seeds (1.98 cm). Such a difference was not observed between seeds sown at 90 or 180 days after treatment.

The findings show that seeds treated at the time of sowing exhibit improved vigor and performance, in agreement with results obtained for soybean seeds (Brzezinski *et al*., 2015). No differences were observed between treated and untreated seeds sown 90 or 180 days after treatment. Coat color did not influence shoot length, indicating that both types of seed had similar vigor.

Table 5 - Shoot length as a function of seed coat color and carboxin + thiram treatment of chia plants sown at 0, 90, and 180 days after treatment (DAT). Umuarama, PR, Brazil, 2021

Item	Shoot length (cm)		
	0 DAT	90 DAT	180 DAT
		Coat color	
White	2.05 a	2.52 a	3.21 a
Black	2.47 a	2.37 a	3.47 a
Treatment			
Untreated	1.98 b	2.44 a	3.38 a
Treated	2.64 a	2.44 a	3.30 a
CV (%)	13.2	13.1	11.7
		F-test	
Coat color (C)	ns	ns	ns
Treatment (T)	**	ns	ns
C × T	ns	ns	ns

CV, coefficient of variation; ns, not significant; ** significant at $p < 0.01$. Means within columns followed by the same letter are not significantly different at $p < 0.05$ by Tukey's test

It should be noted that the results obtained here refer to only one seed lot. Future studies should investigate the effects of treatment and seed color using different lots. Given that there are no registered varieties of chia seeds nor literature data on their agronomic characteristics, further studies are needed to advance our knowledge about chia seed production.

CONCLUSION

White chia seeds showed higher vigor in the germination test, first germination count, and GSI test. Fungicide treatment of chia seeds did not compromise physiological quality, demonstrating that it is possible to subject seeds to storage after treatment.

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