

## Pre-germination treatment of carrot seeds with bioactivator

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### ABSTRACT

Carrot is a vegetable with an important socio-economic role, both due to the employment of labor and high commercialization. To optimize the establishment of this crop in fields, seeds with rapid and uniform germination are essential. Therefore, various seed treatment techniques have been used to enable and enhance the germination percentage and optimize the vigor of the seeds. In continuation with this, the objective of this study was to evaluate the effects of thiamethoxam on the physiological performance of carrot seeds during germination and initial development of seedlings. The experimental design was a completely randomized design in a 5 × 4 factorial scheme, with five carrot cultivars and four doses of thiamethoxam (0, 0.4, 0.8, and 1.2 mL). Parameters such as germination, first germination count, seedling length, and dry mass were evaluated. In addition, biochemical analyses of total sugars, total amino acids, and proline content were performed. In general, the treatment of carrot seeds with thiamethoxam positively influenced the germination of the seeds of the cultivars studied. However, thiamethoxam doses of 0.4 and 0.8 mL had no positive effect on the seedling development of the Tropical cultivar. The thiamethoxam dose of 1.2 mL was beneficial to the development of seedlings and accumulation of metabolites in the Alvorada cultivar.

**Keywords:** *Daucus carota*, Germination, Vegetables, Thiamethoxam.

### Tratamentos pré-germinativos em sementes de cenoura com bioativador

#### RESUMO

A cenoura é uma hortaliça com papel socioeconômico importante, tanto pelo elevado emprego de mão-de-obra, quanto por sua alta comercialização. Para otimizar o estabelecimento dessa cultura em campo se torna imprescindível sementes com germinação rápida e uniforme. Por isso, tem-se utilizado o tratamento de sementes como técnica para viabilizar o desempenho germinativo e otimizar a expressão de vigor. Desse modo, objetivou-se avaliar os efeitos do tiametoxam no desempenho fisiológico de sementes de cenoura durante a germinação e desenvolvimento inicial de plântulas. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 5 x 4, sendo cinco cultivares de cenoura e quatro dosagens de tiametoxam (0; 0,4; 0,8 e 1,2 mL). Avaliaram-se a germinação, primeira contagem de germinação, comprimento e massa seca de plântula, além de análises bioquímicas do conteúdo de açúcares totais, aminoácidos totais e prolina. O tratamento de sementes de cenoura com tiametoxam, no geral, influenciou positivamente a germinação das sementes das cultivares estudadas. No entanto, nas dosagens de 0,4 e 0,8 mL de tiametoxam para cultivar Tropical não houve influência positiva para o desenvolvimento de plântulas. A dose de 1,2 mL do bioativador, para a cultivar Alvorada, foi benéfico ao desenvolvimento de plântulas e acúmulo de metabólitos.

**Palavras-chave:** *Daucus carota*, Germinação, Hortaliças, Tiametoxam.

## 1. Introduction

The seeds of carrot (*Daucus carota* L.) usually present uneven germination, which is a limiting factor for its cultivation (Kist et al., 2021). One of the reasons for the low seed quality of this species can be attributed to the difference between primary and secondary umbels in the same plant during maturation period (Panngom et al., 2018). Thus, commercial seed lots may present different maturation stages, which affect the performance and consequently the establishment of stands in the field and the profitability of the crop per area (Silva et al., 2016). Hence, carrot production requires large quantities of seeds during sowing and subsequent thinning, making the process costly, because the acquisition of this input corresponds to 4% of the production costs (Almeida et al., 2020; Kist et al., 2021).

Some techniques increase the germination and establishment of carrot seedlings in the field. One of these is the use of insecticides with bioactivating function, which has shown to positively affect germination, emergence, and initial development of the seedlings (Nisha et al., 2012). Further, thiamethoxam, which is an insecticide with broad-spectrum activity and used as a bioactivator in rice and wheat for seed treatment, is reported to improve seed performance in the field (Annamalai et al., 2018; Tsaganou et al., 2021).

The effects of bioactivators on seed physiology ensures faster germination and induction of embryonic axis development at certain doses (Lanka et al., 2014). In sorghum, thiamethoxam (7 mL kg<sup>-1</sup> of seeds) increased germination and maintained values above 96% (Perales-Rosas et al., 2019); its use in lettuce seeds resulted in physiological stimulation of 5–7% compared with that by the use of zero dose during germination, and the maximum technical efficiency was reached at doses of 0.4–0.6 mL 1000 seeds<sup>-1</sup> (Deuner et al., 2014).

Borges et al. (2015) reported an increase of 150% in the seedling performance of bean seeds when 200 mL of thiamethoxam per 100 kg of seeds was used compared with that in the control (zero). The application of thiamethoxam in cowpea seeds at a dose of 0.14 g.i.a L<sup>-1</sup> resulted in a 50% increase in winter cultivar germination compared with that in the control (Costa et al., 2017). In soybean seeds, after storage for 30 days, the use of thiamethoxam resulted in a satisfactory performance with a germination percentage above 86% (Camilo; Lazaretti, 2020). However, in wheat seeds, thiamethoxam resulted in a negative effect on the initial development of seedlings (Solariski et al., 2021).

Thus, it can be seen that thiamethoxam has the characteristics of enhancing germination, depending on the dose applied and the species used. Therefore, in addition to the use of seeds of high genetic and physiological quality, thiamethoxam can improve the

initial development of plants (Seraguzi et al., 2018). Thus, the objective of this study was to evaluate the effects of thiamethoxam on the physiological performance of carrot seeds during germination and initial development of seedlings.

## 2. Material and Methods

The study was conducted at the Universidade Federal Rural do Semi-Arido (UFERSA), Mossoró, Rio Grande do Norte, Brazil. The experimental design was completely randomized in a 5 × 4 factorial scheme, with five carrot cultivars and four doses of thiamethoxam (0, 0.4, 0.8, and 1.2 mL 1000 seeds<sup>-1</sup>), which were prepared using Cruiser 350® (Syngenta, 2020) and 8 mL of distilled water. From this syrup, 1 mL was used to treat the seeds used in the experiment.

The carrot cultivars were spring-summer cultivars (adapted to warmer climate) and a hybrid of autumn-winter climate: Alvorada (C1), Brasília (C2), BRS Esplanada (C3), Tellus\* (C4), and Tropical (C5) cultivar seeds arranged in transparent acrylic boxes (11.0 × 11.0 × 3.5 cm), with four replicates of 50 seeds. For the treatment of carrot seeds, thiamethoxam was applied as a solution to form a syrup and then placed on the seeds in petri dishes to ensure the greatest contact of the product with their surface. Thereafter, the plates were placed on the bench and dried under a natural environmental condition for 10 min.

After the seed treatment, a germination test was set-up for physiological and biochemical evaluations. Physiological analyses were: a) Germination: Four replicates of 50 seeds were sown on blotting paper moistened with distilled water at 2.5 times the dry weight of the substrate. The transparent acrylic boxes were placed in a germination chamber (Mangelsdorf) at 20 °C with a 12 h photoperiod, and the normal seedlings were quantified at seven (first count) and fourteen days after the test started, and the values were expressed in percentage (Brazil, 2009); b) Seedling length: At the end of the germination test, ten normal seedlings were randomly selected from each replicate, their length was determined by measuring from the cap to the apex of the seedling using a ruler graduated in millimeters, and the results were expressed in centimeters; c) Seedling dry mass: After measuring the lengths, the seedlings were placed in Kraft paper bags and placed in a forced air circulation oven at 65 °C for 72 h. They were then weighed on precision analytical scales (0.0001), and the results were expressed as mg seedling<sup>-1</sup> (Carvalho; Nakagawa, 2012).

A fresh mass of 0.2 g of normal seedlings (from the germination test) was weighed and placed in hermetically sealed tubes, and 3 mL of 60% alcohol was subsequently added. The material was macerated and

centrifuged at 10000 rpm at 4 °C for 8 min. The supernatant was obtained for quantification of the following components: a) Total soluble sugars (TSS): The TSS were determined using the anthrone method (Yemm; Willis, 1954) with glucose as the standard substance of the curve. The results were expressed in  $\mu\text{g}$  of AST  $\text{g}^{-1}$  of fresh mass; b) Total amino acids (TA): The acid ninhydrin method (Yemm; Cocking, 1955) was applied, with glycine as the standard substance of the curve to measure the TA. The results were expressed in  $\mu\text{mol}$  TA  $\text{g}^{-1}$  of fresh mass; c) Proline: The proline content was measured using the method proposed by Bates et al. (1973), and the results were expressed in  $\mu\text{mol}$  proline  $\text{g}^{-1}$  of fresh mass.

The data were subjected to normality analysis using the Shapiro–Wilk test and analysis of variance using the F test ( $p \leq 0.05$ ). Significantly different data were subjected to Tukey test and regression analysis using the statistical program System for Analysis of Variance - Sisvar<sup>®</sup> (Ferreira, 2019). The data were also subjected to multivariate analysis of principal components using the Past 4 program (Hammer, 2001).

### 3. Results and Discussion

All the variables analyzed had significant interaction between cultivars and doses (Table 1). The application of 0.8 mL of the bioactivator to the Brasília and Tellus cultivars did not produce plant material for biochemical analyses but generated them only for physiological evaluations. Thiamethoxam dose had different germination effects on the various cultivars (Figure 1A).

In the Alvorada and Brasília cultivars, the control (0 mL) and 1.2 mL doses were superior to the 0.4 and 0.8 mL doses with germination values close to 70%, as established by the legislation for the marketing of seeds of these species (Brazil, 2019). However, in the Tropical cultivar, the 0.4 and 0.8 mL doses presented higher values in the trend curve, with germination values of approximately 77%. In the Tellus cultivar, the adjustment of the values presented cubic equations;

the 0.4 and 1.2 mL doses resulted in 80% germination, followed by a decrease of 78% in the 0.8 mL dosage, and then a resumption of higher germination values (80%) in the 1.2 mL dosage. In the BRS Esplanada cultivar, germination remained below 60% in the doses 0.4 and 0.8 mL of thiamethoxam, and the same occurred with the other cultivars subjected to the 0.8 mL dose, excluding the Tropical cultivar.

The results of the first count fitted into a quadratic equation (Figure 1B). In the Alvorada, Brasília, and BRS Esplanada cultivars, the highest scores were found in the 0 (42, 62, and 47%) and 1.2 mL (70, 70, and 59%) doses, respectively. In contrast, the doses 0.4 and 0.8 mL produced the highest averages in the Tropical cultivar, that is, 63 and 51%, respectively. Similar results were observed in rice seed germination, where the beneficial effects of thiamethoxam were evident in seed lots with low vigor (Castro et al., 2007).

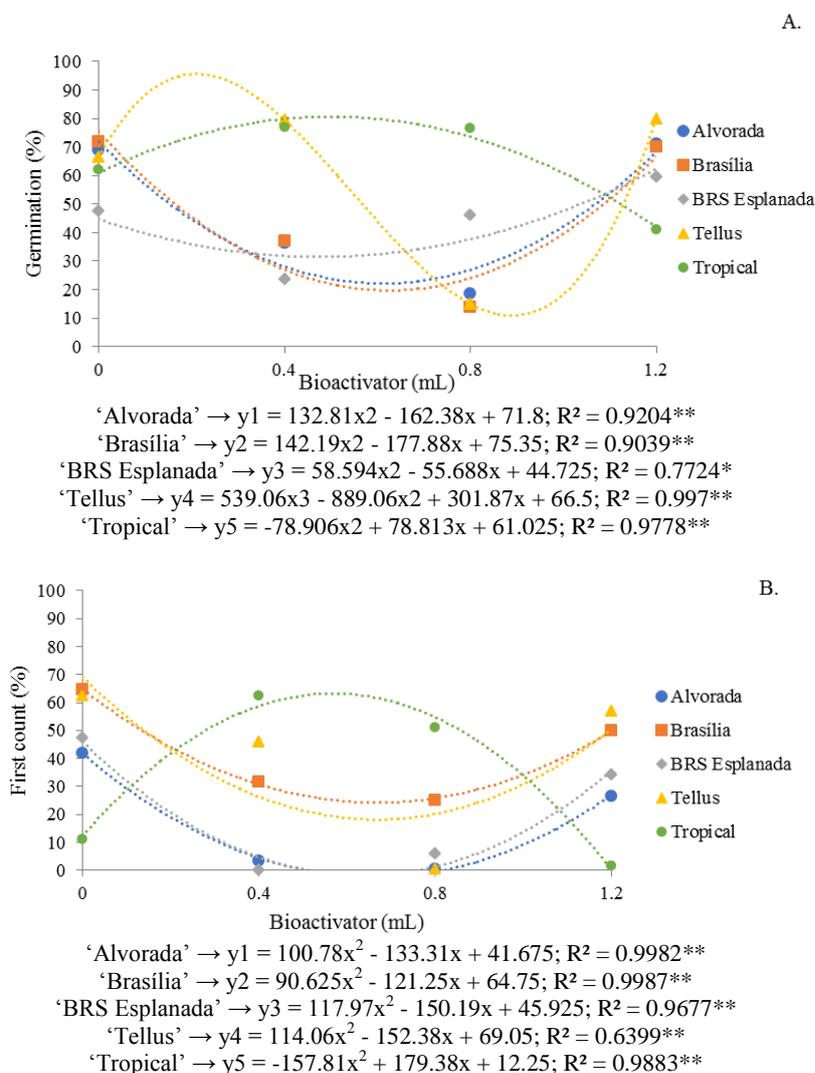
The use of thiamethoxam (0.4 and 0.6 mL  $\text{kg seeds}^{-1}$ ) in pumpkin promoted germination (above 93%) even in less vigorous plots, with a 6% increase compared with that in the control (Lemes et al., 2015). In sorghum seeds, treatment with 7 mL of Cruiser<sup>®</sup> resulted in excellent germination with 99% seedling emergence (Perales-Rosas et al., 2019). The same neonicotinoid is considered crucial in sunflower treatment at a product concentration of 350  $\text{g L}^{-1}$  due to its benefits during early development (Gvozdenac et al., 2019). The beneficial effects of thiamethoxam may also be related to the water balance of seeds, absorption of water during the mobilization of reserves, and activation of proteins involved in the defense mechanism (Almeida et al., 2014).

In relation to seedling length, the Tropical cultivar showed similar growth behavior to the Tellus cultivar with the dose of up to 0.4 mL of thiamethoxam; however, higher doses of up to 0.8 mL negatively influenced growth behavior, with a decrease of 83% compared with that of the Tropical cultivar (Figure 2A). The thiamethoxam dose of 1.2 mL promoted better seedling growth than the other doses of the Alvorada cultivar (55% greater than the control).

**Table 1.** Summary of analysis of variance for the following variables: germination (G), first count (FC), seedling length (SL), seedling dry mass (SDM), total soluble sugars (TSS), total amino acids (TAA), and proline (PRO) of carrot cultivars subjected to seed treatment with bioactivator.

	G	FC	SL	SDM	TSS	TAA	PRO
Cultivar (C)	1182.7**	1989.9**	40.16**	2.71**	3.78**	411.8*	0.025**
LEVEL (L)	4014.0**	2871.0**	31.58**	0.253 <sup>ns</sup>	4.85**	1284.6**	0.070**
C x L	1892.8**	2185.6**	13.54**	0.264*	3.19**	806.0**	0.020**
CV%	32%	55%	23.9%	37.7%	29.80%	31.90%	21.70%

\*\* significant at 1%; \* significant at 5%; <sup>ns</sup> not significant.



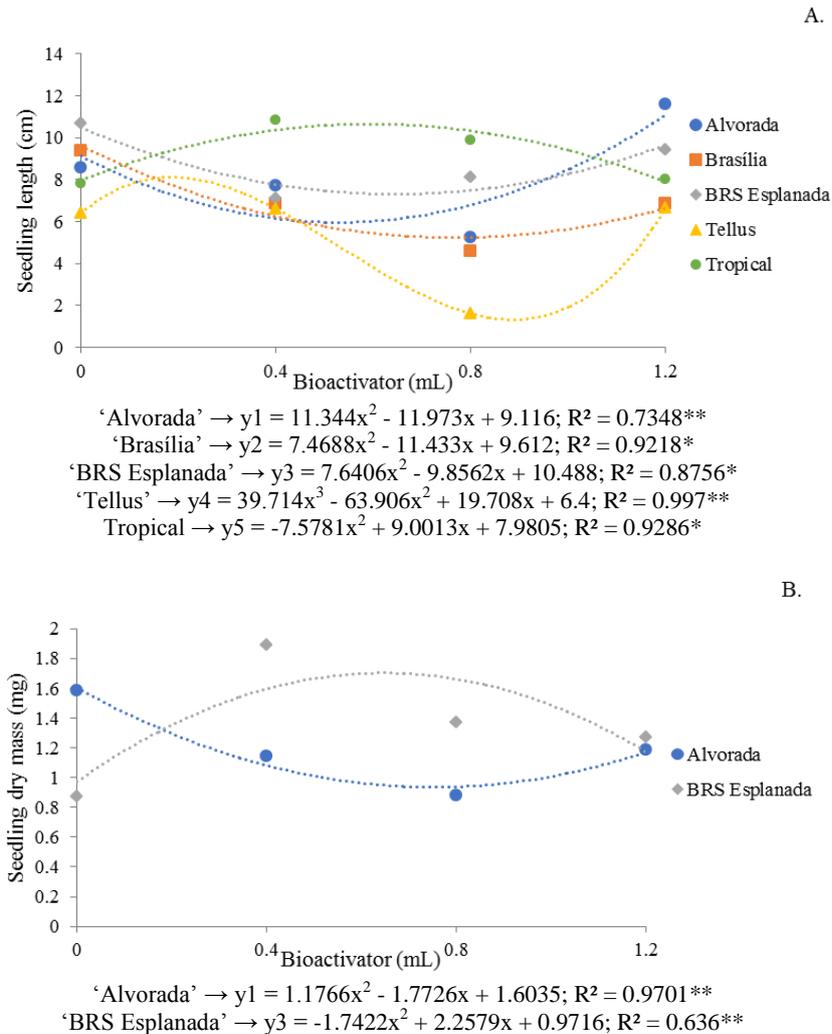
**Figure 1.** Characteristics of the initial development (A. Germination; B. First count) of seedlings of five carrot cultivars (Alvorada, Brasília, BRS Esplanada, Tellus, and Tropical cultivars), treated with bioactivator at doses of 0, 0.4, 0.8, and 1.2 mL. **\*\*** significant at 1%; **\*** significant at 5%; **ns** not significant.

In Brasília cultivar, the doses of the insecticide caused reductions of 27% (0.4 and 1.2 mL) and 51% (0.8 mL) compared with that in the control. In the BRS Esplanada cultivar, the doses of 0.0 and 1.2 mL resulted in the highest averages of 10.7 and 9.45 cm, respectively. In contrast, the intermediate doses of 0.4 and 0.8 mL had positive effects on the cultivar Tropical with averages of 10.82 and 9.88 cm, respectively, and were superior to the other doses, as they resulted in a length that is six times greater than that of the control. In the Tellus cultivar, the doses of 0, 0.4, and 1.2 mL gave the highest values of 6.39, 6.59 and 6.64 cm, respectively. Using 3.5 mL kg seed<sup>-1</sup>, thiamethoxam had no negative effect on the initial development of wheat plants (SolarSKI et al., 2021). However, the use of 0.21 g i.a. L<sup>-1</sup> of thiamethoxam in the treatment of cowpea seeds resulted in better initial seedling establishment performance (Costa et al., 2017).

The 0.4 and 0.8 mL doses significantly promoted the behavior of the Alvorada and BRS Esplanada cultivars,

but with the minimum points of seedling dry mass accumulation for the first cultivar (Figure 2B). In the Alvorada cultivar, the control treatment resulted in seedlings with dry mass of 1.58 mg, which is higher than that of the doses with the regulator by 44%, if we consider the dose of 0.8 mL. For the BRS Esplanada cultivar, maximum dry mass production of 1.89 mg was observed in the 0.4 mL dose, that is, an increase of approximately 100% compared with that in the control (0.87 mg).

The effects of thiamethoxam dose (175 g 100 kg seeds<sup>-1</sup>) on the seeds of the soybean cultivar M-SOY 6101 varied. Thiamethoxam reduced dry mass but maintained vigor above 80% for up to 45 days of storage (Dan et al., 2010). Moreover, thiamethoxam (0.21 g i.a. of the product L<sup>-1</sup>) stimulated the physiological performance of cowpea seeds, exhibiting positive effects on growth and dry matter assimilation by the roots (approximately 12% increase compared with that in the control (Costa et al., 2017)).



**Figure 2.** Seedling length (A) and seedling dry mass (B) of five carrot cultivars (Alvorada, Brasília, BRS Esplanada, Tellus, and Tropical cultivars), treated with bioactivator at doses of 0, 0.4, 0.8, and 1.2 mL. **\*\*** significant at 1%; **\*** significant at 5%; **ns** not significant.

For the Alvorada cultivar, there was no significant difference in the levels of total soluble sugars (Figure 3A). In the Brasília cultivar, there was a linear trend as the dose of thiamethoxam increased, and the 1.2 mL dose increased the levels of total soluble sugars by 63% compared with that in the control. However, with the control dose, the Tropical cultivar accumulated up to 46% more sugars than the other cultivars. The doses of 0.4 and 0.8 mL resulted in a reduction of total soluble sugars, which was 42% on an average. Finally, the dose of 1.2 mL promoted a soluble sugar content of  $3.0 \mu\text{g g}^{-1}$  FM, with a reduction of 27% compared with that in the control.

The results of the sugar contents of cultivar seeds subjected to intermediate treatment (0.4 and 0.8 mL) of thiamethoxam were similar to that obtained for the physiological variables. Results showed that the 0.8 mL dose of the bioactivator had greater effect on the smaller seedlings and reduced seedling dry mass, plant development, growth promoters, and other metabolites (sugars, amino acids, and proline). Under stress conditions and low sugar content, sucrose-nonfermentation1-related protein kinase1 genes were activated, triggering a process

of reduced energy expenditure, which consequently reduced the metabolite processes associated with plant growth (Oliveira et al., 2021).

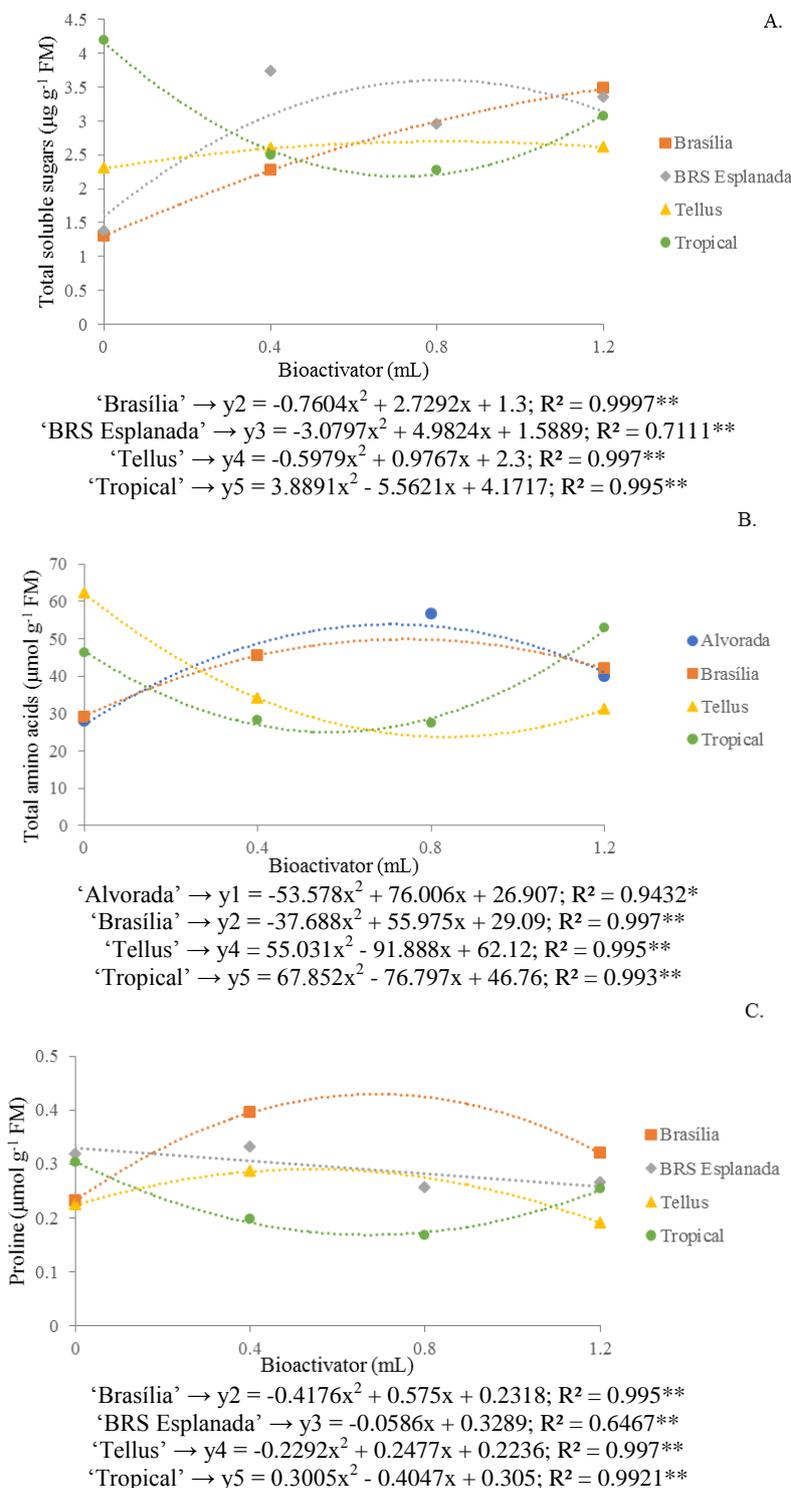
The doses of thiamethoxam showed significant differences in the production of total amino acids in the Alvorada, Brasília, Tellus, and Tropical cultivars (Figure 3B). At intermediate doses, Alvorada ( $45 \mu\text{mol g}^{-1}$  FM) and Brasília ( $56 \mu\text{mol g}^{-1}$  FM) cultivars accumulated more total amino acids than the other cultivars by approximately 24% at the 0.4 mL dose and 51% at the 0.8 mL dose. However, for the Tellus and Tropical cultivars, these same doses resulted in lower mean values of accumulation, which were  $31 \mu\text{mol g}^{-1}$  FM at the dose of 0.4 mL and  $27 \mu\text{mol g}^{-1}$  FM at the dose of 0.8 mL.

In the Alvorada cultivar, the AT contents were between 28.01 (0 mL) and  $56.74 \mu\text{mol g}^{-1}$  FM (0.8 mL). In the Brasília cultivar, an average of  $45.45 \mu\text{mol g}^{-1}$  FM (0.4 mL) was observed. For the BRS Esplanada cultivar, the AT values were between 29.92 (0.8 mL) and  $55.73 \mu\text{mol g}^{-1}$  FM (0.4 mL), with no significant difference. When the BRS Esplanada cultivar was treated with 0.4 and 1.2 mL of thiamethoxam, it showed an accumulation

of sugars and dry mass but with less growth, expression of vigor, and germination, indicating that the product had a greater influence on some reserve accumulation variables.

In general, for the Tellus cultivar, the doses 0.4 and 1.2 mL reduced the levels of AT by 45 and 50% compared with that in the control (62.12  $\mu\text{mol g}^{-1}$  FM). In the Tropical cultivar, the doses 0 and 1.2 mL were superior to the other treatment doses with means equal to 46.29 and

52.78  $\mu\text{mol g}^{-1}$  FM, respectively. These results indicate that the treatment of the cultivars resulted in lower vigor and germination, probably because thiamethoxam interfered with the accumulation of amino acids, whose functions are related to various biosynthesis pathways and secondary metabolism of seedlings (Dinkeloo et al., 2018). The proline contents in the cultivars were significantly affected by the doses of thiamethoxam, except in the Alvorada cultivar (Figure 3C).



**Figure 3.** Biochemical characteristics (A. Total sugars; B. Total amino acids; and C. Proline) of seedlings of five carrot cultivars (Alvorada, Brasília, BRS Esplanada, Tellus, and Tropical cultivars), treated with bioactivator at doses of 0, 0.4, 0.8, and 1.2 mL.

\*\* significant at 1%; \* significant at 5%; <sup>ns</sup> not significant.

The Brasília cultivar showed a quadratic trend with greater accumulation between doses of 0.4 and 0.8 of TMT, followed by BRS Esplanada cultivar, which showed a linear decreasing trend as the bioactivator dose increased. Proline production remained between 0.23 and 0.32  $\mu\text{mol g}^{-1}$  FM, 0.26 and 0.33  $\mu\text{mol g}^{-1}$  FM, and 0.19 and 0.28  $\mu\text{mol g}^{-1}$  FM in the Alvorada, BRS Esplanada, and Tellus cultivars, respectively. In the Brasília cultivar, the doses 0.4 and 1.2 mL increase its proline content by 69 and 39% compared with that by the control dose (0.23  $\mu\text{mol g}^{-1}$  FM).

In the Tropical cultivar, the highest levels of proline were found in the doses 0 and 1.2 mL with averages of 0.3 and 0.25  $\mu\text{mol g}^{-1}$  FM, respectively. The application of thiamethoxam increased the expression of seedling vigor of cotton seeds (Almeida et al., 2020) and wheat (Carvalho et al., 2011). These authors found that with the application of this insecticide, the synthesis of amino acids resulted in the production of new proteins.

The results of the proline content showed that the Tropical cultivar under 1.2 mL of thiamethoxam application did not have greater seedling length, similarly to the result obtained for Brasília cultivar using 0.4 mL of the bioactivator. Proline plays a role in osmotic adjustment, membrane protection and protein stabilization (Iqbal et al., 2014; Nelson; Cox, 2019). It is a signaling molecule that can increase antioxidant activity in response to different stresses (Rejeb et al., 2014).

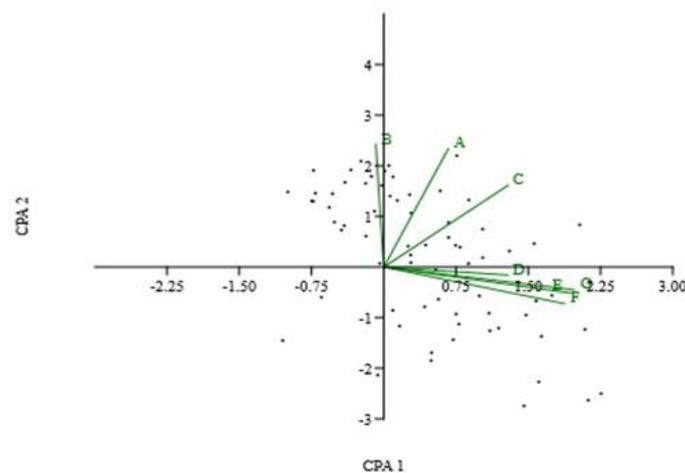
Proline is described in the literature as one of the main amino acids that acts against the negative effects of various stresses. A high synthesis of this amino acid may indicate phytotoxicity, owing to its role in glutamate and arginine metabolic routes, which are related to nitrogen metabolism. In stress situations, the plant prioritizes this nitrogen for the formation of proline, which performs osmotic adjustment, but it results in an imbalance of the nutrient in plants and consequently impairs development

(Ferreira et al., 2002; Hussain et al., 2022).

In the present study, the cultivars responded differently to the same concentrations of thiamethoxam. Generally, many species perform osmotic adjustment when there is an increase in proline, sugar, and amino acid contents under plant stress conditions. The seedling length of the Tropical cultivar reduced (1.2 mL of thiamethoxam), despite the accumulation of amino acids. The bioactivator possibly interfered in the metabolism of biomolecules and distinctly affect the development of seedlings and germination, depending on the cultivar.

For the Tropical cultivar, which showed lower germination performance and expression of vigor than the other cultivars, ionic stress in the seeds may have occurred. The metabolism was directed to overcome the abiotic stress promoted by the bioactivator treatment, resulting in greater energy expenditure. Some studies revealed that bioactivators are related to cell cycle retardation; for example, bioactivator had cytotoxic and genotoxic effects on sunflower root cell chromosomes, and it caused various abnormalities at higher doses of 4  $\text{g L}^{-1}$  of the product (Georgieva et al., 2021).

Based on the genetic material studied (cultivars), these results showed that thiamethoxam can promote germination and seedling development, because according to the principal components analysis, the physiological variables (germination and seedling growth) showed similar grouping of these components, that is, positive to the right (Figure 4). In addition, results of the biochemical characteristics and accumulation of dry mass of seedlings evaluated showed proximity of its components. In general, all the doses of thiamethoxam used in this study either inhibited the twinning of carrot cultivars or did not result in any difference from the control. However, its mode of action related to protein production routes may have influenced the development of carrot cultivars seedlings.



**Figure 4.** Principal component analysis of carrot cultivars (A. Germination, B. First count, C. Seedling length, D. Seedling dry mass, E Total soluble sugars, F. Total amino acids, and G. Proline).

The potential of thiamethoxam on seedling development was verified in this study. Thus, further studies on the appropriate doses of this bioactivator on cultivars, its mode of application, and time of exposure to the product are needed. Caution should be practised regarding the safe doses for seed treatment to avoid residual effects associated with phytotoxicity and other subsequent risks of contamination.

#### 4. Conclusions

Treatment of carrot seeds with thiamethoxam at 1.2 mL positively influences the germination of carrot cultivars, except Tropical cultivar. The intermediate doses of 0.4 and 0.8 mL provide better initial development for Tropical cultivar than the other doses. The carrot cultivars show different behavior of accumulation of biomolecules to different doses of bioactivator.

#### Authors' Contribution

Keylan Silva Guirra, José Eduardo Santos Barboza da Silva, Francisco Assis Nogueira Neto, Bruno Silva Guirra, Washington Aparecido da Luz Brito, contributed to the execution of the experiment, data collection, writing, interpretation and review of the manuscript. Salvador Barros Torres and Leomara Vieira de França Cardozo contributed to the writing, interpretation and revision of the manuscript.

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