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# Heat stress mitigation on the germination and initial development of carrot seedlings using plant growth regulators<sup>1</sup>

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## ABSTRACT

The adoption of technologies that allow the mitigation of environmental stresses, such as the use of regulators in seed treatment, is an alternative for crops in high temperatures regions. This study aimed to assess the heat stress mitigation on the germination and initial development of carrot seedlings using plant growth regulators. The study was divided into three experiments performed at the temperatures of 25, 30 and 35 °C. A 5 × 3 factorial arrangement was used, with five carrot cultivars (Alvorada, Brasília, BRS Esplanada, Tellus and Tropical) and two products (Stimulate® and thiamethoxam), in addition to the control (untreated seeds). The following variables were evaluated: germination, first germination count, seedling length and dry mass. When the cultivars had their seeds treated with the plant growth regulators at 25 °C, the germination for the Tellus cultivar was 28 % higher than for the control, while, at 30 °C, the seed germination for BRS Esplanada and Tellus was twice as high as for the control, and showed a higher vigor than that of Alvorada, Brasília and Tropical. Overall, the thiamethoxam promoted increments at the initial development of carrot seedlings at 25 and 30 °C. However, the germination of the carrot cultivars was disrupted at 35 °C.

KEYWORDS: *Daucus carota* L., biostimulant, thiamethoxam.

## RESUMO

Mitigação do estresse térmico na germinação e desenvolvimento inicial de plântulas de cenoura por meio de reguladores de crescimento vegetal

A adoção de tecnologias que permitam a atenuação de estresses ambientais, como o uso de reguladores no tratamento de sementes, é uma alternativa para cultivos em regiões com temperaturas elevadas. Objetivou-se avaliar a mitigação do estresse térmico na germinação e desenvolvimento inicial de plântulas de cenoura, por meio de reguladores de crescimento vegetal. O estudo foi dividido em três ensaios, utilizando-se temperaturas de 25, 30 e 35 °C. Empregou-se esquema fatorial 5 x 3, sendo cinco cultivares de cenoura (Alvorada, Brasília, BRS Esplanada, Tellus e Tropical) e dois produtos (Stimulate® e tiametoxam), além do controle (sementes não tratadas). Avaliaram-se as seguintes variáveis: germinação, primeira contagem de germinação, comprimento e massa seca de plântula. Quando as cultivares tiveram as sementes tratadas com os reguladores de crescimento vegetal a 25 °C, Tellus apresentou germinação 28 % superior ao controle, enquanto, a 30 °C, BRS Esplanada e Tellus apresentaram germinação duas vezes maior que o controle e expressaram melhor vigor, em detrimento da Alvorada, Brasília e Tropical. De modo geral, o tiametoxam promoveu incrementos no desenvolvimento inicial de plântulas de cenoura aos 25 e 30 °C. No entanto, a germinação das cultivares de cenoura foi interrompida a 35 °C.

PALAVRAS-CHAVE: *Daucus carota* L., bioestimulante, tiametoxam.

## INTRODUCTION

Climate change has transformed agricultural productivity in general, resulting in severe socioeconomic implications. In the agricultural sector, the United Nations recommend that countries

adopt measures to mitigate the consequences of climate change in order to maintain the sustainability of production systems (Santos & Alves 2020).

This requires an interdisciplinary response and approach (Gaertner 2020). High temperatures and water restriction or excess, enhanced by climate

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change, are abiotic stress factors that impede the growth and development of plants, putting food production at risk (Kul et al. 2020).

Carrot is a root vegetable of great nutritional importance, and its cultivation is socioeconomically important, as it offers significant employment and income generation. In 2014, the cultivation of this species generated 150,000 jobs and, in 2019, its production reached 760,000 t year<sup>-1</sup>; however, there was a decline in yield (-5.5 %) in the summer and winter, due to climatic variations (Kist et al. 2021). This oscillation renders the carrot production system uncertain for farmers. This leads to farmers being reluctant to invest in new areas, thereby causing discontinuity of supply.

Moreover, the cultivation of carrots requires vigorous seeds that germinate quickly and uniformly. However, the cost of this input is high and germination, in most cases, is heterogeneous. Seed treatment was conceived to be a possible remedy to this issue. Moreover, plant growth regulators have been used in rice (Grohz et al. 2016) and wheat (González-Guzmán et al. 2021) seeds, with the aim of obtaining a more efficient germination and vigorous seedlings. Other products, such as systemic insecticides, are also widely used to promote germination, stand and vigor (Morzelle et al. 2017, Solarski et al. 2021, Ferreira et al. 2022).

Although studies have demonstrated the efficacy of these products in seeds, some results are contradictory. Therefore, further data are required to verify the adequacy of the used methods to the species and cultivars and the timing of application of these products. Thus, this study aimed to assess the heat stress mitigation on germination and initial development of carrot seedlings using plant growth regulators.

## MATERIAL AND METHODS

The study was performed at the Universidade Estadual do Piauí (Corrente, Piauí State, Brazil), from February to March 2020. A randomized design, in a 5 × 3 factorial arrangement, with four replicates of 50 seeds, was employed. The study was sectioned into three trials performed at the temperatures of 25, 30 and 35 °C. The treatments consisted of a combination of five carrot cultivars (Alvorada, Brasília, BRS Esplanada, Tellus and Tropical) and two products [Stimulate® (plant growth regulator) and

thiamethoxam (systemic insecticide with bioactivator function)], in addition to the control (untreated seeds). The concentrations of the two products used in the trials were determined in preliminary tests.

Stimulate® was the biostimulant used, composed of 0.005 % gibberellic acid, 0.005 % indolebutyric acid and 0.009 % kinetin, at a concentration of 10 mL of commercial product per liter of water. The carrot seeds were placed on blotting paper (substrate) moistened with the solution 2.5 times the dry weight. They were then placed in transparent acrylic boxes (11.0 cm × 11.0 cm × 3.5 cm) for the germination test assembly, as they were watered to prevent dehydration of the substrate after the first count.

To prepare the bioactivator solution (thiamethoxam), 8 mL of distilled water were added to 1.2 mL of the commercial product Cruiser 350®. Subsequently, 1 mL of this mixture was used in the treatment of 1,000 seeds. The seeds were then placed in Petri dishes and swirled to achieve a greater contact with the surface product. Then, the plates were left to dry on the laboratory bench for 10 min, at room temperature (26 °C), to improve the homogenization and fixation of the product. Moreover, the seeds were sown in acrylic boxes with blotting paper as substrate moistened with distilled water 2.5 times the weight of the paper.

After treating the seeds with a biostimulant and a bioactivator, the experiments were conducted at 25, 30 and 35 °C. The boxes were placed in a biochemical oxygen demand (BOD) germinator, with a 12 h photoperiod, for 14 days (Brasil 2009), until they were subjected to the following physiological analyses: a) germination: the evaluation of normal seedlings was performed after 7 (first count) and 14 days, with values expressed as percentage of normal seedlings (Brasil 2009); b) seedling length: at the end of the germination test, ten normal seedlings of each replicate were randomly selected and the length (expressed in cm) from the root cap to the apex of the seedling was determined using a ruler in mm; c) seedling dry mass: after the length was measured, the seedlings were placed in Kraft paper bags, and then in an oven with forced air circulation at 65 °C, for 72 h. Then, the seeds were weighed on an analytical scale (0.0001) and the results expressed in mg seedling<sup>-1</sup>. The Shapiro-Wilk test was used to test for normality of distribution, and analysis of variance was performed using the F-test ( $p \leq 0.05$ ). In case of significance, the Scott-Knott test was used. The

analyses were performed using the Sisvar<sup>®</sup> statistical software (Ferreira 2019). To ease interpretation, the data were also submitted to multivariate analysis of principal components using the Past 4 software (Hammer et al. 2001).

## RESULTS AND DISCUSSION

A significant interaction was detected between the cultivars and products for all variables at 25 and 30 °C (Table 1). At 35 °C, there was no germination of carrot seeds, even after treatment.

The carrot seeds of the Alvorada and Tropical cultivars treated with the biostimulant experienced a 23 and 15 % reduction in their germination, respectively, relatively to the untreated seeds. At 25 °C, Alvorada showed the best germination (Figure 1A) without the use of products (biostimulant and bioactivator). Tellus and Tropical showed a higher germination when treated with the bioactivator than when treated with the biostimulant, although the Tropical variety did not differ from the control in this treatment. The Tellus seeds treated with the bioactivator showed significant differences, such as a germination rate 28 % higher than that of the control (untreated) and 22 % higher than those treated with the biostimulant. However, the germination rate of the Tropical seeds treated with the biostimulant was 21 % lower than that obtained in the control treatment, at the same temperature.

The values obtained for the first count of carrot seedlings differed among the cultivars (Figure 1B). At 25 °C, the seed treatment with the bioactivator led to a 49 % increase in germination for Brasília and a 58 % increase for Tropical, relatively to that of the control. Under the treatment with the biostimulant, Brasília was 35 % more responsive, if compared to the control. The plant growth regulators did not have a positive effect on the other cultivars.

In the case of the biostimulant, the positive results obtained for some cultivars regarding germination (Tellus) and first count (Brasília) are associated with the mode of action and the balance between the regulators. They are composed of gibberellin (which acts directly on the germination process and cell elongation), gibberellic acid (which increases the production of alpha-amylase at the level of gene transcription) and alpha-amylase and beta-amylase (which are enzymes responsible for the initial degradation of starch) (Taiz et al. 2017). The use of biostimulants also boosted the germination of rice and maize seeds under heat stress (Grohs et al. 2016, Carmo et al. 2021).

For Alvorada treated with plant regulators (biostimulant and bioactivator) at the temperature of 25 °C, there was a reduction in seedling length of approximately 28 %, relatively to that of the control (Figure 1C). At the same temperature, the seedling length was significantly affected by these regulators, except for BRS Esplanada treated with the bioactivator. This treatment resulted in seedlings that were 1.72 cm longer than those treated with the biostimulant.

In general, the treatments of carrot seeds with the biostimulant and bioactivator did not have positive effects at 25 °C (Figure 1D). However, the effect for the treatment of the Tropical cultivar with the bioactivator was similar to that of controls under the same treatment, though a 0.365 mg increase in mass was observed, when compared to those treated with the biostimulant. These indicated that seedlings under this treatment (bioactivator) had a superiority of 174 % (Figure 1D).

The plant growth regulators present in the biostimulant did not contribute to the heat stress mitigation in any of the carrot cultivars in this study. Negative results are expected when the temperature is increased during the germination of species,

Table 1. Summary of analysis of variance for the variables germination (G), first count (FC), seedling length (SL) and seedling dry mass (SDM) of carrots submitted to seed treatment with biostimulant and bioactivator under temperatures of 25 and 30 °C.

Source of variation	25 °C					30 °C				
	Degrees of freedom	Pr > Fc				Degrees of freedom	Pr > Fc			
		G	FC	SL	SDM		G	FC	SL	SDM
Cultivars (C)	4	161.44**	87.89**	17.45**	8.09 e-7**	4	241.0**	179.0**	2.85**	8 e-6 <sup>ns</sup>
Products (P)	2	33.65 <sup>ns</sup>	91.81**	9.86**	2.91 e-7 <sup>ns</sup>	2	82.8*	230.0**	0.63 <sup>ns</sup>	4 e-6 <sup>ns</sup>
C x P	8	90.12**	182.12**	2.21*	1.31 e-7**	8	266.0**	21.1 <sup>ns</sup>	2.01**	8 e-6 <sup>ns</sup>
CV (%)		11.79	8.52	13.24	61.6		21.6	11.61	18.40	73.4

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

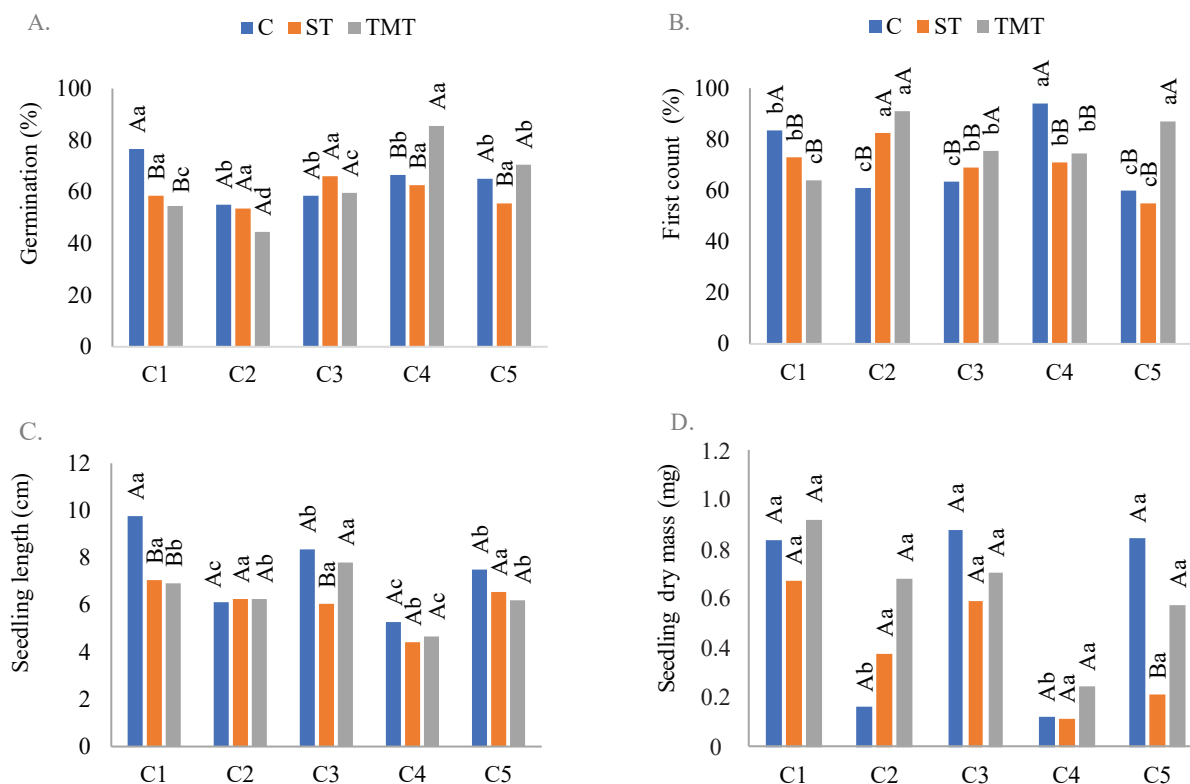


Figure 1. Germination, first count, seedling length and seedling dry mass of five cultivars [Alvorada (C1), Brasília (C2), BRS Esplanada (C3), Tellus (C4) and Tropical (C5)] treated with biostimulant (Stimulate®) and bioactivator (thiamethoxam), under temperature of 25 °C. C: control; ST: Stimulate®; TMT: thiamethoxam. Means followed by the same lowercase letter do not differ for the division of the cultivar factor within the plant regulator by the Scott-Knott test ( $p \leq 0.05$ ). Means followed by the same capital letter do not differ in the product factor within the same cultivar by the Scott-Knott test ( $p \leq 0.05$ ).

because high temperatures (30-35 °C) accelerate the absorption of water by the seeds and affect the germination process, thereby causing necrosis of the radicle (Hilgert et al. 2021). However, the Brasília cultivar showed no difference in germination at 20-32 °C (Pereira et al. 2007). Nevertheless, losses in the germination of carrot seeds were observed in the present study.

The germination of BRS Esplanada and Tellus at 30 °C was boosted by the treatment with the regulators (Figure 2A). Brasília germinated more without the use of the products, reaching values 33 and 62 % higher than when treated with the biostimulant and bioactivator, respectively. The germination for BRS Esplanada and Tellus did not differ from that of the other cultivars when treated only with the products (Stimulate® and thiamethoxam). However, the treatment with the biostimulant resulted in germination rates 2.7 and 2.0 times higher than that in the untreated seeds,

respectively. The beneficial effect of the bioactivator was also observed on the germination of seeds of BRS Esplanada (50 %) and Tellus (55 %), manifested by a 2.8 and 2.6-times higher germination rate than the control, respectively.

The use of the biostimulant decreased the first count of carrot seedlings for Alvorada by 36 % (Figure 2B). The cultivars responded differently to the plant growth regulators. The treatment with the bioactivator showed a higher sensitivity of action under stress conditions at 30 °C. This means that, under this condition, Alvorada, BRS Esplanada and Tellus were similar to the controls when submitted to the biostimulant, but the first count germination decreased by 35, 18 and 20 %, respectively, if compared to the treatment with the bioactivator.

The negative effects of high temperatures during germination were also observed for carrot (Pereira et al. 2007) and pepper (Diel et al. 2019) cultivars, which showed a lower germination rate

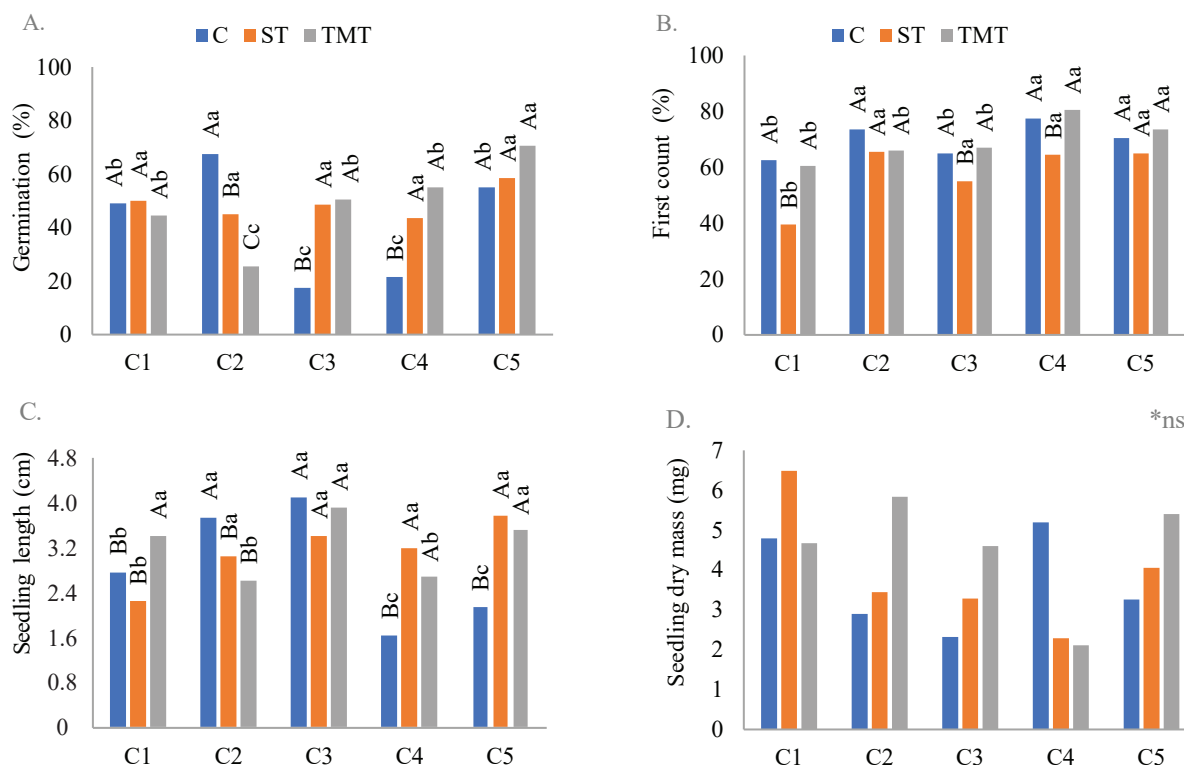


Figure 2. Germination, first count, seedling length and seedling dry mass of five cultivars [Alvorada (C1), Brasília (C2), BRS Esplanada (C3), Tellus (C4) and Tropical (C5)] treated with biostimulant (Stimulate®) and bioactivator (thiamethoxam), under temperature of 30 °C. C: control; ST: Stimulate®; TMT: thiamethoxam. Means followed by the same lowercase letter do not differ for the division of the cultivar factor within the plant regulator by the Scott-Knott test ( $p \leq 0.05$ ). Means followed by the same capital letter do not differ in the product factor within the same cultivar by the Scott-Knott test ( $p \leq 0.05$ ). ns: not significant.

when subjected to temperatures above standard ones for the species. In watermelon seeds, the temperature of 30 °C caused a reduction of 80 % in germination (Silva et al. 2018).

The first count of the germination test is one of the variables used to indicate vigor. In this study, the seeds of the cultivars treated with the bioactivator resulted in a faster establishment of seedlings, a very good characteristic for overcoming adverse environmental conditions. In the evaluation of germination at the end of 14 days, the results were similar to those of the cultivars treated with the biostimulant (Figure 2A). The bioactivator acts on the plant defense pathway, working as a plant tonic by attenuating or eliminating the heat stress effects (Almeida et al. 2014).

The seedling length for Alvorada (3.4 cm), Tellus (2.7 cm) and Tropical (3.5 cm), at 30 °C, were greater than that for Brasília under the treatment with the biostimulant (Figure 2C). The treatment with the biostimulant led to mean increases of 1.2 cm

(78 %) and 1.4 cm (69 %) for Tellus and Tropical, respectively, relatively to that of the control. The use of the same biostimulant (Stimulate®) in doses of 10 mL and 15 mL boosted the growth of lettuce (Soares 2012) and corn (Carmo et al. 2021) seedlings under high temperatures, respectively.

Opposite tendencies were observed for the seedling length and dry mass of carrot cultivars (Figures 2C and 2D). The length of the seedlings was greater at 25 °C than at 30 °C, and the dry mass of the seedlings was lower at 25 °C. Seedlings under heat stress probably underwent osmotic adjustments and direct the metabolism for the accumulation of matter in this condition. The accumulation of these metabolites is associated with the mode of action of gibberellin (present in the biostimulant) and with the production of enzymes (such as alpha and beta amylase) responsible for the breakdown of reserves stored in the endosperm (Taiz et al. 2017).

Thiamethoxam, a systemic insecticide, activates various physiological reactions, such



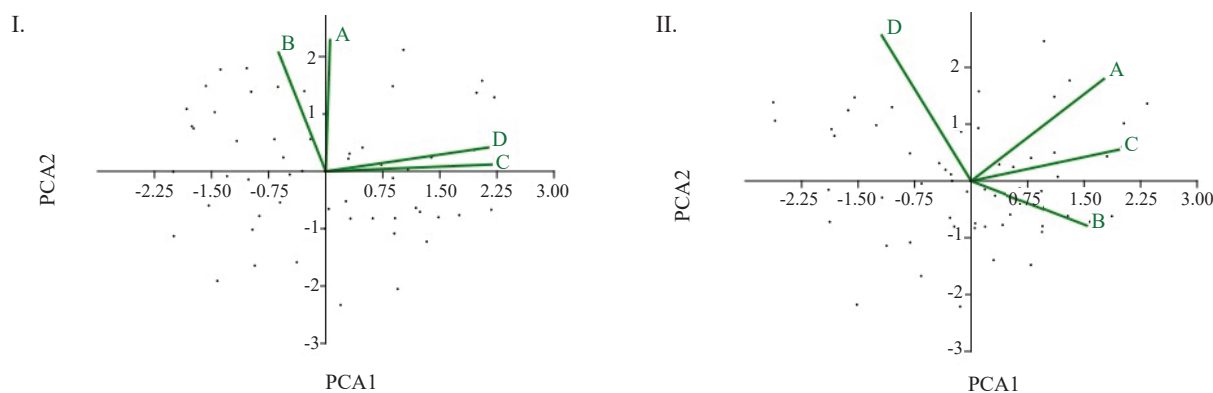


Figure 3. Principal component analysis (PCA) of carrot cultivars treated with biostimulant (Stimulate®) and bioactivator (thiamethoxam), under temperatures of 25 (I) and 30 °C (II). A) Germination; B) first count; C) seedling length; D) seedling dry mass.

as the expression of proteins, thereby acting as a bioactivator. It interacts with the stress defense mechanisms (drought, low pH, high temperature and high soil salinity) of plants and has an overall tonic effect (Morzelle et al. 2017). Thus, plants develop faster with a higher expression of vigor, enzyme activity, increase in nutrient levels and agricultural gains (Seraguzi et al. 2018). In addition, the bioactivator increases the expression of genes involved in the elimination of  $H_2O_2$ , an important strategy of the plant in metabolic recovery. Moreover, it improves the antioxidant performance, thereby maximizing energy for growth and maintenance of plant tissues (Afifi et al. 2015).

At high temperatures, increases in dry mass are not always associated with the components of germination and vigor. Moreover, the behavior of the variable seedling length was similar to that of the seedling dry mass (Figure 3I), demonstrating that temperature interfered with the whole development of the seedling. It was also observed that heat stress exceeded the adjustment capacity of the carrot cultivars, as manifested by the fact that germination had a greater proximity with growth, while accumulation of dry mass seedling has a negative interaction with the initial development (Figure 3II).

At 35 °C, the germination of carrot seeds of all cultivars was inhibited. Previous studies elucidated that this temperature is not recommended for the germination of seeds of this species (Pereira et al. 2007). The heat stress effects are further intensified in cases of heterogeneous lots and low initial vigor. These results indicate that collecting carrot umbels separately is necessary to guarantee that the lots

are more homogeneous. This idea is in line with Bukharov et al. (2021), who stated that seeds from primary umbels are more tolerant to heat stress.

When submitted to higher temperatures, some carrot cultivars are known to germinate at 35 °C, such as Alvorada and Brasília (Pereira et al. 2007). However, these cultivars did not germinate in our study, nor did the others (BRS Esplanada, Tellus and Tropical). The effect of high temperature on germination is harmful owing to the fluidity of membrane lipids (Santos et al. 2020). This causes modifications in the composition and structure of membranes, resulting in different arrangements that make the membrane more permeable (Nelson & Cox 2019).

In general, the use of the biostimulant at 25 °C did not boost the germination and vigor of seeds of the carrot cultivars. However, the application of plant growth regulators (biostimulant and bioactivator) increased the germination and vigor for BRS Esplanada and Tellus at 30 °C, thereby setting a standard for treatments to boost agricultural productivity.

## CONCLUSIONS

1. The treatment of carrot seeds with the biostimulant Stimulate® and the bioactivator thiamethoxam did not have beneficial effects on the germination and initial development of seedlings at 25 °C, except for the Tellus cultivar, when treated with the bioactivator;
2. The use of the biostimulant at 30 °C resulted in an increase in the germination and initial development of the seedlings of BRS Esplanada and Tellus;

3. The germination of the carrot cultivars Alvorada, Brasília, BRS Esplanada, Tellus and Tropical was disrupted at 35 °C.

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