

## Germination and vigor of watermelon seeds treated with biostimulant

## Germinação e vigor de sementes de melancia tratadas com estimulante de crescimento

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**ABSTRACT** - Direct sowing is the most used watermelon propagation method in Brazil. Therefore, the use of treated seeds is an alternative for improving uniformity of plant stands at planting. Thus, the objective of this study was to assess the effects of seed treatments with biostimulant on the germination and vigor of watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] seeds of the cultivar Crimson Sweet. A completely randomized experimental design with four replications was used. The seed treatments consisted of six biostimulant (Agressive Desperta<sup>®</sup>) rates (0, 1, 2, 3, 4, and 5 mL kg<sup>-1</sup>). Seed germination speed index, seedling length, seedling dry weight, seedling emergence in the field, and seedling emergence speed index were evaluated. The treatment of watermelon seeds with biostimulant resulted in increases in seed germination speed and seedling length and dry weight as the biostimulant rate was increased. Seedling percentage and emergence speed increased up to the rate of 3 mL kg<sup>-1</sup> under field conditions; however, application of higher rates resulted in phytotoxicity.

**Keywords:** *Citrullus lanatus*. Micronutrients. Amino acids. Cucurbitaceae. Physiological potential.

**RESUMO** - No Brasil, a sementeira direta, é o mais utilizado na propagação de melancia. Para tanto, o uso de sementes tratadas surge como uma alternativa para melhoria da uniformidade de estandes no plantio. Dessa forma, objetivou-se verificar os efeitos do tratamento com estimulante de crescimento em sementes de melancia (*Citrullus lanatus* (Thunb.) Matsum & Nakai), cultivar Crimson Sweet, sobre a germinação e o vigor. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições e seis doses do estimulante de crescimento, Agressive Desperta<sup>®</sup>, 0; 1; 2; 3; 4 e 5 mL kg<sup>-1</sup> de sementes de melancia. Avaliou-se a germinação, índice de velocidade de germinação, comprimento das plântulas, massa seca das plântulas, emergência de plântulas em campo e índice de velocidade de emergência de plântulas. O tratamento de sementes de melancia com estimulante de crescimento proporcionou maior velocidade de germinação, comprimento e massa seca de plântulas, diretamente proporcional ao aumento das dosagens. Em condições de campo, houve melhora na porcentagem e velocidade de emergência das plântulas até a dosagem de 3 mL kg<sup>-1</sup> de sementes. Dosagens superiores a essa ocasionaram fitotoxidez.

**Palavras-chave:** *Citrullus lanatus*. Micronutrientes. Aminoácidos. Cucurbitaceae. Potencial fisiológico.

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### INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] is native to dry regions of Tropical Africa and stands out among Cucurbitaceae species. Watermelon crops were grown on 99,212 ha in Brazil in 2020, with a production of 2,184,907 Mg. The Central-West region was responsible for the highest mean watermelon yield (33.4 Mg ha<sup>-1</sup>), where the state of Goiás stood out with the highest yield: 41.3 Mg ha<sup>-1</sup> (IBGE, 2021).

Watermelon crops can be established through direct sowing or production of seedlings for transplanting. Most watermelon growers in Brazil use direct sowing because it is a relatively easy and low-cost method. However, production of seedlings under protected environment for transplanting has been used due to high losses during the establishment of the crop (NASCIMENTO; SILVA; LIMA, 2014).

One of the main methods to ensure the crop establishment quality is the use of high-quality seeds. The physiological quality of seeds is essential because it is directly connected to the seedling establishment in the field and the obtaining of uniform stands (RODRIGUES et al., 2018).

Seed treatment is a technique used for improving the uniformity of seedling stands under direct sowing. The application of amino acid-based products to seeds emerged as a technology that has been worldwide studied for improving the performance of seedlings under field conditions, mainly when subjected to adverse conditions (MONDAL et al., 2015). They reduce the incidence of pathogens and provide micronutrients and amino acids for seedling initial establishment.

The objective of applying biostimulants containing micronutrients and



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amino acids is not only to supply the plant needs for protein synthesis, but to activate the plant physiological metabolism, focused on antistress responses (SILVA et al., 2017). In addition, amino acids assist in the action of the Krebs cycle, improve the processes of respiration and energy production in the plant (HILDEBRANDT et al., 2015), and are precursors of plant hormones such as tryptophan, as well as precursors of indoleacetic acid (TAIZ et al., 2017), methionine, and ethylene (HILDEBRANDT et al., 2015).

Several scientific studies on seed treatments have shown promising results when using micronutrients and amino acids. The application of micronutrients to castor bean seeds resulted in increases in seed germination and vigor (OLIVEIRA et al., 2010). Micronutrients applied through seed treatment also affected positively germination and vigor of green bean seeds (OHSE et al., 2014) and, application of amino acids resulted in better performance of seedlings, mainly under adverse conditions, for broccoli (BETTONI et al., 2013), wheat (HAMMAD; ALI, 2014), *Eustoma grandiflorum* (MONDAL et al., 2015), and soybean (DÖRR et al., 2020) crops.

Plants of the family Cucurbitaceae are still little studied regarding effects of seed treatments. However, promising results were reported by Merzah and Aboohanah (2020) infor zucchini (*Cucurbita pepo* L.) plants subjected to spraying with amino acids and boron, which improved seed viability and vigor. Ramos et al. (2023) evaluated the effect of seed treatment using biostimulant on *Luffa operculata* L. and found better seedling performances.

No information on responses of watermelon seedlings to seed treatments with biostimulant focused on direct sowing is found in the literature. Thus, the objective of this work was to assess the effects of treatments with biostimulant containing micronutrients and amino acids on germination and vigor of watermelon seeds of the cultivar Crimson Sweet.

## MATERIAL AND METHODS

The experiment was conducted at the Laboratory of Seed Analysis of the Federal Institute Goiano, Ipora Campus – Farm School, in Ipora, Goiás, Brazil. The treatments consisted of applications of six biostimulant (Agressiva Desperta®) rates (0, 1, 2, 3, 4, and 5 mL kg<sup>-1</sup>) to watermelon seeds of the cultivar Crimson Sweet, with 100% purity, without other treatments.

The biostimulant used for seed treatments is a product of the Fertilizer Agrosociences company; according to the manufacturer, it is 1.5% sulfur, 0.1% boron, 0.5% cobalt, 0.1% copper, 0.6% manganese, 5% molybdenum, 2% zinc, 5% amino acids (0.69% aspartic acid, 1.68% glutamic acid; 0.89% alanine, 0.67% arginine, 0.01% cystine, 0.26% phenylalanine; 1.58% glycine; 0.13% histidine, 0.39% isoleucine; 0.41% leucine; 1.56% methionine; 0.18% proline;

0.01% tyrosine, 0.01% ornithine, 0.02% methylhistidine, 0.37% tryptophan; 0.31% serine, 0.31% valine, and 0.22% threonine), 5% seaweed extract, and 3.7% carboxylic acid.

The solutions were prepared, adjusting the rates to 10 g of seeds, and diluted in 10 mL of distilled water. The solution was placed in plastic bags containing 400 seeds, which were shaken for three minutes to completely distribute the product and cover the seeds. The treated seeds were then placed on paper towels for absorption of the product, according to the methodology of Nunes (2005).

The following tests were carried to evaluate the effect of the seed treatments with biostimulant:

Seed germination percentage (G): carried out with four replications of 50 seeds per treatment; the seeds were sown in blotter paper rolls moistened with a quantity of water equivalent to 2.5-fold the weight of the dry paper and maintained in a germinator at 25 °C under constant light. The counts were carried out daily for 14 days after sowing and the results were expressed in percentage of normal seedlings (BRASIL, 2009).

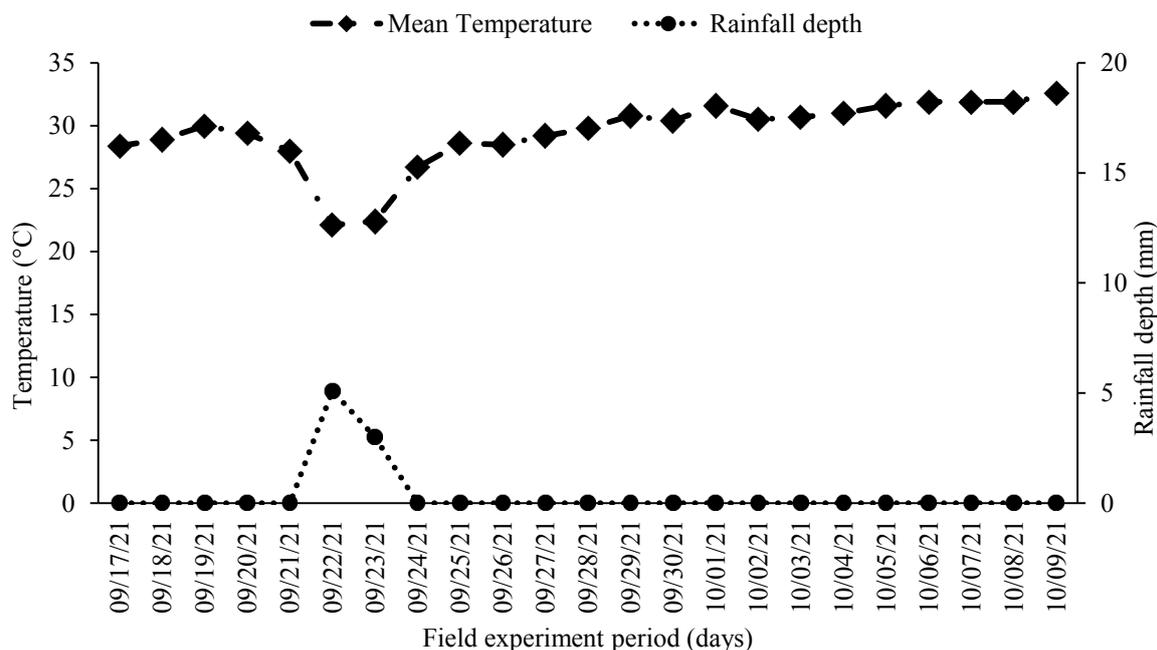
Seed germination speed index: calculated by the sum of number of germinated seeds in each day until the end of the experiment (14 days), divided by the number of days between sowing and germination (MAGUIRE, 1962).

Seedling emergence in the field: seeds were directly sown in beds with four replications of 100 seeds per treatment; they were placed at 2 cm depth in 4-meter-long furrows, with spacing of 15 cm between furrows. The first counting was carried out five days after sowing, followed by daily counts until 14 days after sowing.

Seedling emergence speed index: determined by daily counting of normal germinated seedlings for 14 days. Those with fully expanded cotyledons without adhered integument were considered as normal seedling. The lengths of the seedlings were determined at the end of the test of seedling emergence in the field. The seedlings were then placed in paper bags and dried in an oven at 80 °C for 24 hours. The dried samples were weighed (NAKAGAWA, 1999) to obtain the seedling dry weight.

The tests under field conditions for evaluating seedling emergence in the field and emergence speed index were carried out using a micro-sprinkler irrigation system; the area was irrigated twice a day, in the morning and afternoon, focused on maintaining the soil field capacity. Data on daily minimum, maximum, and mean temperatures and rainfall depths are shown in Figure 1.

A completely randomized experimental design was used, with four replications. The experimental data were subjected to analysis of variance and the means of the variables were subjected to polynomial regression, defining the best fit based on the combination of significance with the highest coefficient of determination. The statistical program SISVAR (FERREIRA, 2014) was used for the statistical analyses.



**Figure 1.** Data on rainfall depth (mm) and temperature (°C) in Ipora, GO, Brazil, in September and October 2021. Meteorological Station of the Federal Institute Goiano.

**RESULTS AND DISCUSSION**

The seed treatment with biostimulant resulted in higher percentages of germination when using the biostimulant rates of 3, 4, and 5 mL kg<sup>-1</sup> of seeds (24.0%, 26.5%, and 32.5% germination, respectively) at the eighth day of counting (Figure 2A).

Seeds treated with the rates of 2, 3, and 4 mL kg<sup>-1</sup> presented 60.0%, 52.0%, and 57.5% germination, respectively, at the tenth first day, denoting higher germination percentages for rates containing 1 mL kg<sup>-1</sup> (Figure 2B). However, the absence of biostimulant and the treatment with the biostimulant rate of 1 mL kg<sup>-1</sup> resulted in a slow germination (Figure 3B), presenting high germination percentage only eight days after sowing (Figures 2B and 2C), i.e., most seeds needed six more days to finish the germination process.

The seed ability of emerge quickly and uniformly ensures the establishment of an adequate plant population under field conditions; from the practical point of view, faster germination speeds promote adequate emergences, avoiding losses and increases in production costs due to the need of new sowing (BERTOLIN; SÁ; MOREIRA, 2011).

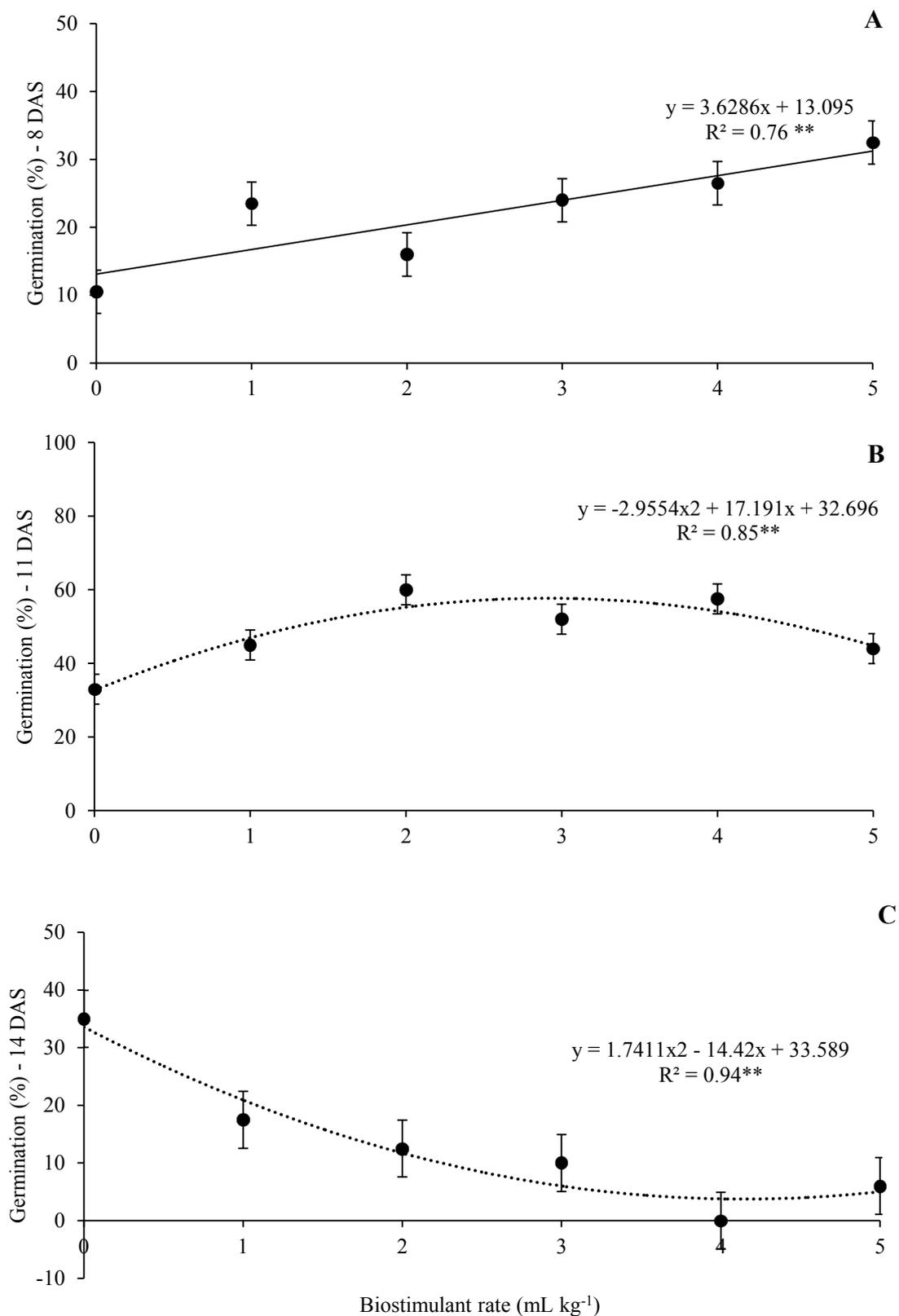
The seed treatment with micronutrients and amino acids improves the seedling emergence speed and reduces the exposure of seeds to unfavorable factors under field conditions. The treatment of green bean seeds with micronutrients resulted in increases of 38% in germination with increased number of normal seedlings, compared to the control, when using the maximum efficiency rate: 9.9 mL kg<sup>-1</sup> (OHSE et al., 2014). Watermelon seeds treated with amino acids were significantly affected in the first counting, with increases in germination percentage (RADKE et al., 2017).

The seed treatments had no effect on the germination percentage (Figure 3A); however, the germination speed index increased as the biostimulant rate was increased, fitting to a linear function (Figure 3B).

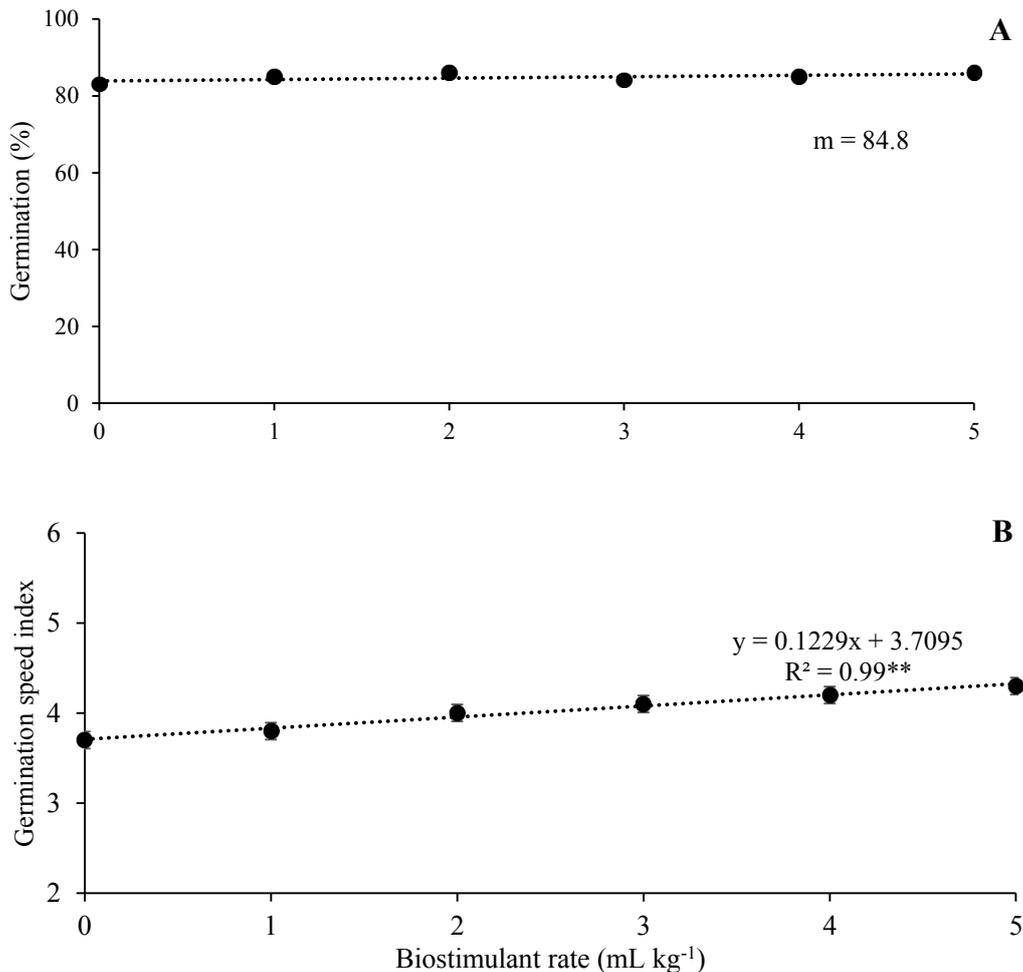
The micronutrients zinc (Zn), cobalt (Co), molybdenum (Mo), boron (B), and manganese (Mn), which are in the composition of the biostimulant used, have essential functions for plant metabolism processes. Zinc and boron are nutrients required for basic seedling functions connected to formation of auxins, RNA, and ribosomes and are connected to cell division and development of tissues; manganese participates in energy connections between ATP and the enzymatic complex of photosynthesis; and molybdenum participates as a cofactor of enzymes (TAIZ et al., 2017). All these processes occur during the mobilization process of reserve materials from the cotyledons to the embryonic axis in the germination process.

In the initial process of imbibition, the seed ability to reorganize membranes and repair possible physical and biological damages occurred during the production process significantly affects the selectivity of membrane systems. Therefore, the faster the germination speed, the higher the seed ability to reestablish the integrity of cell membranes (VIEIRA; KRZYZANOWSKI, 1999). The ability to reorganize cell membranes and repair damages is higher in vigorous seeds than in low-vigor seeds.

In addition to micronutrients, amino acids are essential to potentiate germinability. The application of amino acids through seed treatment can be connected to the processes of protein synthesis for embryo growth and, additionally, amino acids are precursors of other substances that regulate plant metabolism (DÖRR et al., 2020).



**Figure 2.** Germination of watermelon seeds of the cultivar Crimson Sweet treated with biostimulant rates (mL kg<sup>-1</sup>) at eight (A), eleven (B), and fourteen (C) days after sowing (DAS).



**Figure 3.** Germination (A) and germination speed index (B) of watermelon seeds of the cultivar Crimson Sweet treated with biostimulant (mL kg<sup>-1</sup>).

Considering the specificities of each micronutrient or amino acid, they can be incorporated into the seeds and assist in seedling development, as observed by the higher seed germination speed, i.e., increases in the physiological potential as the biostimulant rate was increased (Figure 3B).

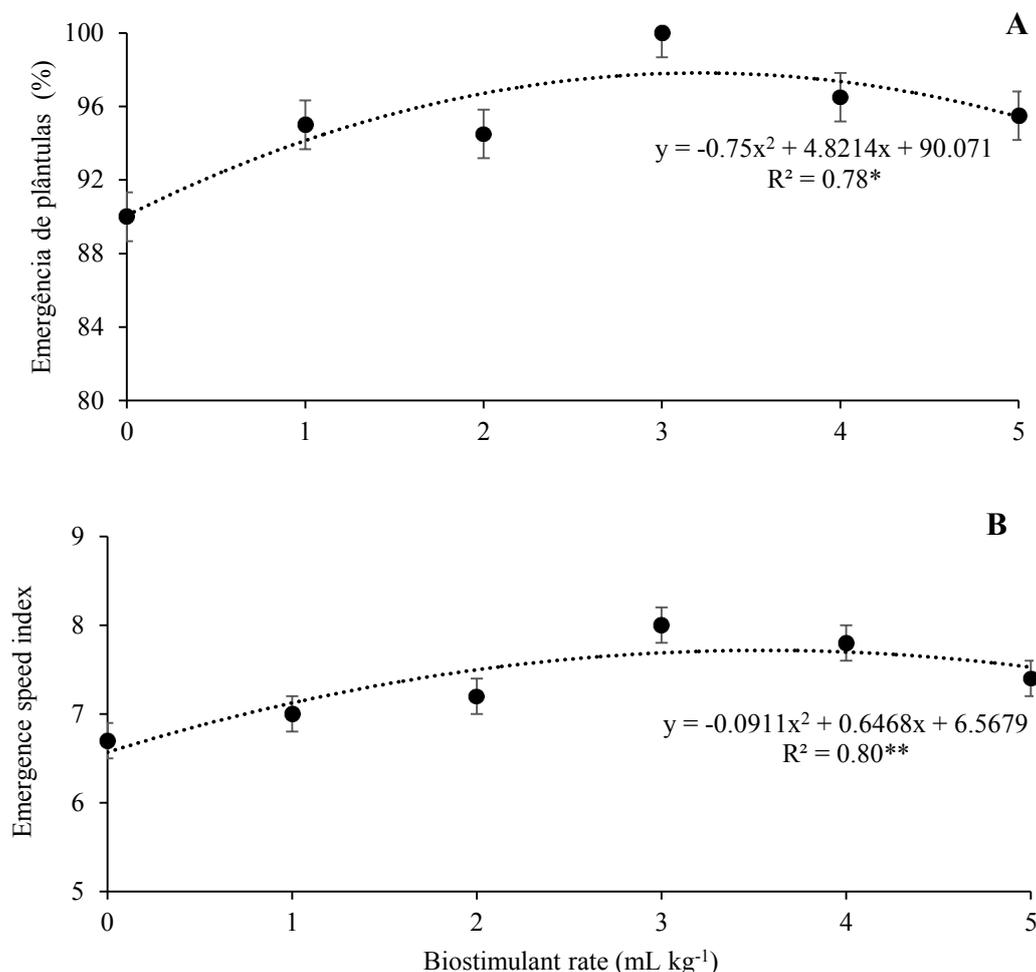
The results obtained for seedling emergence in the field and emergence speed index under field conditions were different from those found in laboratory conditions, probably due to the interaction of the treatments with environmental variables. The emergence percentages of watermelon seedling in the field increased up to the biostimulant rate of 3 mL kg<sup>-1</sup> (10%), with decreases of 3.5% and 4.5% when using higher rates (4 and 5 mL kg<sup>-1</sup>, respectively) (Figure 4A). The seedling emergence speed index presented similar results, with increases up to the rate of 3 mL kg<sup>-1</sup>, followed by decreases of 0.2 and 0.6 when using higher rates (4 and 5 mL kg<sup>-1</sup>, respectively) (Figure 4B).

However, the results showed the importance of micronutrients for mineral nutrition of plants, and mainly the narrow margin between adequate nutrition and phytotoxicity due to the excess of some nutrients, generating losses. The decreases in the means of seedling emergence in the field and emergence speed index (4.5% and 0.6, respectively) found for

the treatments with the highest fertilizer rates occurred because of the excess of some micronutrients; many micronutrients are highly available in acids soils with pH between 4.5 and 5.5, as is the case of Zn, Mn, and Cu.

A study on rice showed phytotoxicity for a high Zn rate (45% of Zn weight/volume at the rate of 200 mL per 100 kg of seeds), resulting in decreases in seedling emergence in the field and germination speed index (ARAUJO et al., 2013). Watermelon seeds treated with B (rates from 0 to 34 g kg<sup>-1</sup>) resulted in increases of approximately 2.8 cm in seedling length (B rate of 10.5 g kg<sup>-1</sup>), compared to the control, with decreases when using more concentrated rates (SILVAMATOS et al., 2017).

In addition to the importance of micronutrients, amino acids are important plant metabolites for proteins synthesis and other important cell functions. A study reported that amino acids such as arginine and glycine are essential, as they are fundamental for physiological processes, as photosynthesis (MATYSIAK et al., 2020). The higher the plant photosynthetic rate, the higher the growth and physiological performance in the field, ensuring a better crop uniformity.



**Figure 4.** Emergence in the field (A) and emergence speed index (B) of watermelon seedlings of the cultivar Crimson Sweet from seeds treated with biostimulant (mL kg<sup>-1</sup>).

Thus, the biostimulant rates higher than 2 mL kg<sup>-1</sup> resulted in higher seedling emergence speed index (Figure 4B) and, consequently, higher seedling performance in the field, with higher shoot development (Figures 5 and 6). This increase was probably due to the uptake of biostimulant.

Seedling length and dry weight presented similar results, with direct increases proportional to the biostimulant rates, fitting to linear equations (Figures 5 and 6).

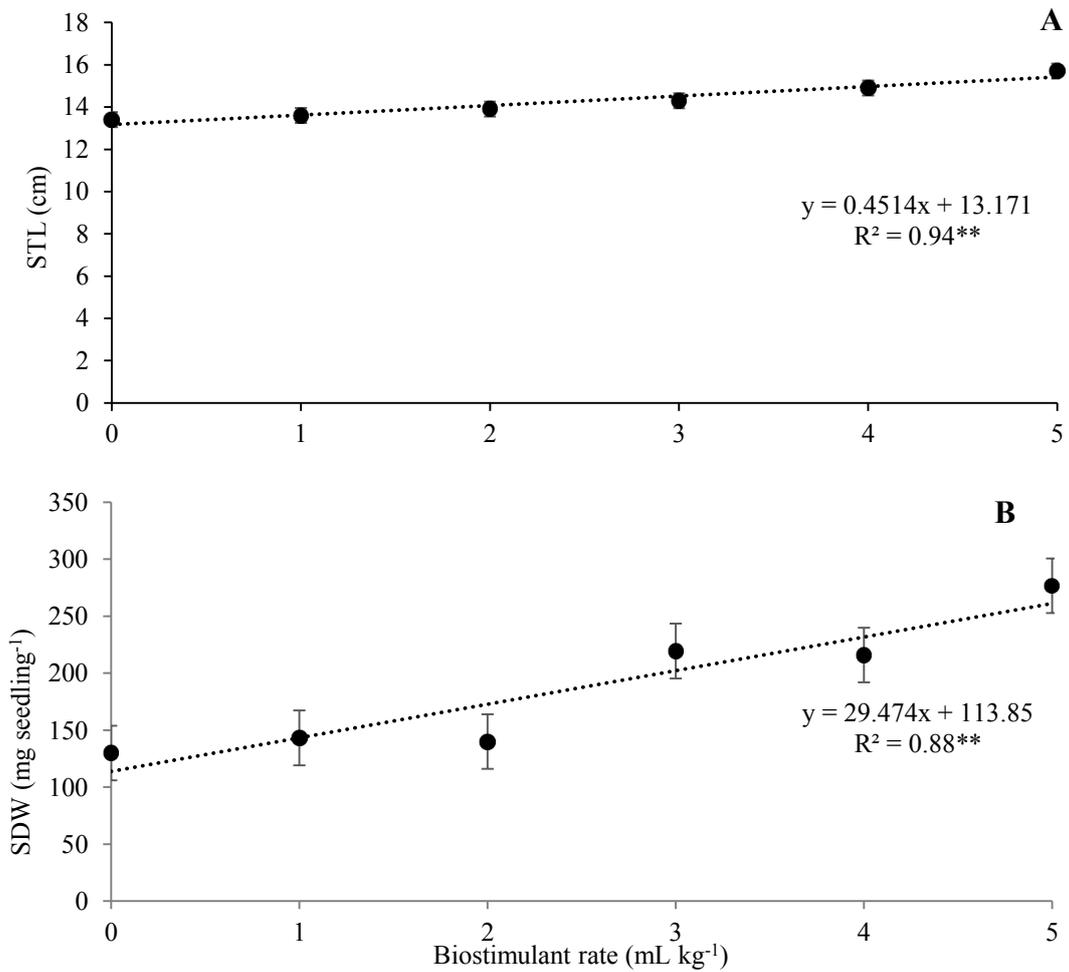
Thus, the beneficial effect of the treatments was evident when considering seedling length, as the seedlings presented increasing results in size as the biostimulant rate was increased (Figure 5A). Considering the seedling dry weight, the macro and micronutrients and amino acids in the biostimulant directly affected the germination process through a positive effect on seedling growth (Figure 5B).

Researches on wheat, cauliflower, and soybean seeds subjected to application of amino acids containing arginine showed that it is a viable strategy to improve tolerance to abiotic stresses (KARPETS et al., 2018; COLLADO-GONZÁLEZ et al., 2021; CALZADA et al., 2022). For

example, plants with vigorous root system tend to resist stress conditions caused by low water availability (dry spells) because they have easy access to water in deeper layers of the soil profile.

In addition to arginine, other precursors of hormones, as tryptophan (precursor of indoleacetic acid) and methionine (precursor of ethylene) (HILDEBRANDT et al., 2015) also assist in processes of cell stretching and division and formation of adventitious and lateral roots, improving seedling germinability and growth rate (TAIZ et al., 2017). Both amino acids were present in the applied biostimulant.

Micronutrients also contribute to seedling development. A study on castor bean seeds showed that Mn and Mo are the most important micronutrients to increase plant dry weight (OLIVEIRA et al., 2010). These micronutrients are present in the constitution of enzymes and participate in the transport of electrons and in the photosynthesis, thus, contributing to increases in plant dry weight. Therefore, the supply of Mo is highly connected to plant growth (KIRKBY; RÖMHELD, 2007).



**Figure 5.** Total length (STL) (A) and dry weight (SDW) (B) of watermelon seedlings of the cultivar Crimson Sweet, from seeds treated with biostimulant (mL kg<sup>-1</sup>), grown under field conditions.



Germination and vigor of watermelon seeds treated with biostimulant

**Figure 6.** Watermelon seedlings, from seeds treated with biostimulant, grown under field conditions, at 20 days after sowing. Control (0), biostimulant rates of 1 mL kg<sup>-1</sup> (1), 2 mL kg<sup>-1</sup> (2), 3 mL kg<sup>-1</sup> (3), 4 mL kg<sup>-1</sup> (4), and 5 mL kg<sup>-1</sup> (5).

Ohse et al. (2012) evaluated watermelon seed treatments with Zn and found divergent results, with decreases in seedling dry weight and length as the Zn rate was increased (from 0 to 1.52 g kg<sup>-1</sup>); the means of shoot and root lengths of watermelon seedlings from seeds not treated with Zn (control) were 27.75% and 37.77% higher, respectively, than those found for seedlings from seeds treated with a Zn rate of 1.52 g kg<sup>-1</sup>. Zn phytotoxicity decreases dry weights of shoots and roots and inhibits plant growth (LI et al., 2011).

Thus, the results shown the importance of using biostimulants focused on improving the performance of watermelon seedlings. These biostimulants can enable a shorter response time of plants when subjected to stressful conditions, since climate conditions during the initial stages of plant establishment in the field may negatively affect the stand establishment and compromise the crop success.

## CONCLUSION

The treatment of watermelon seeds with biostimulant results in a higher germination speed index (4.3) and seedling length (15.7 cm) and dry weight (277 mg), with increases directly proportional to the increase in biostimulant rate. It improves seedling emergence (100%) and emergence speed (8.0) under field conditions up to the rate of 3 mL kg<sup>-1</sup>. However, biostimulant rates higher than 3 mL kg<sup>-1</sup> cause phytotoxicity.

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