

## Hydrogen peroxide as a mitigator of salt stress for melon seed germination

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### Abstract

The effect of hydrogen peroxide has been very studied, but little is known on its effect on seed germination and initial growth of melon trees under salt stress. In this context, the objective of this work was to evaluate the effect of hydrogen peroxide as a mitigator of salt stress on melon seed germination. The experiment was conducted in the Laboratory of Seeds and Seedlings of the Federal University of Campina Grande, in Pombal, PB, Brazil. A completely randomized design was used, in a 4×6 factorial arrangement consisting of times of seed imbibition in H<sub>2</sub>O<sub>2</sub> (0, 8, 16, and 24 hours) and irrigation water salinity levels (0.3, 1.2, 2.1, 3.0, 3.9, and 4.8 dS m<sup>-1</sup>), with four replications. Melon seeds of the commercial variety Imperial-45 were used. The H<sub>2</sub>O<sub>2</sub> solution was prepared from a 1 mmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> solution, which was diluted to reach the concentration of 10 μmol L<sup>-1</sup>. The seeds were placed in a beaker and subjected to imbibition with 100 ml of the solution for the different times studied. The variables evaluated were: germination percentage, first germination counting, germination speed index, mean germination speed, mean germination time, and radicle length. The application of H<sub>2</sub>O<sub>2</sub> solution to seeds for 16 hours mitigates the negative effects caused by salt stresses by up to 2.2 dS m<sup>-1</sup>; however, the application of H<sub>2</sub>O<sub>2</sub> solution for 24 hours is harmful to the germination process of melon seeds.

**Keywords:** electrical conductivity, growth, melon tree, osmopriming, tolerance

### Introduction

Melon (*Cucumis melo* L.) is one of the main fruits in the world, which directly contributes to human feed and nutrition due to the high nutritional value of its composition (Zhang et al., 2020). In 2018, the world melon production reached 27,347.21 Mg; the Northeast region of Brazil was responsible for 555.41 Mg, corresponding to 95% of the country's production (FAOSTAT, 2018).

Melon trees are adapted to high-temperature regions with low annual rainfall depths; this tolerance to produce under such abiotic adversities enables their growth practically all year round (Nóbrega et al., 2018; Andrade et al., 2018). However, the populational growth and increasing demand for foods decreased the quality of irrigation waters; thus, the use of alternatives for irrigation waters is necessary, such as brackish water, which is common in semiarid regions, to which this crop is better adapted (Mbarki et al., 2020; Yan et al., 2020). Despite

its availability, the use of brackish water for irrigation can affect negatively seed imbibition, resulting in decreases in germination (Nóbrega et al., 2020), directly impacting the crop yield and performance in the field (Amini et al., 2016).

The use of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is among the alternatives that has been addressed in the literature as strategies to reduce the effects of stress caused by excess salts in the irrigation water; this molecule belongs to the group of oxygen reactive species (ERO), and it affects several physiological and developmental processes of plants and their resistance to salt stress (Hemalatha, et al., 2017). The application of H<sub>2</sub>O<sub>2</sub> at adequate concentrations improves the plant tolerance to the salt stress, increases germination rates by decreasing endogenous abscisic acid levels (Barba-Espin et al., 2010), regulates genetic expressions through oxidation of proteins (Lariguet et al., 2013), and improves the development of secondary

roots (Chen et al., 2013). However, using it at high concentrations can be toxic and damage cells (Bailly & Bailly, 2004).

The imbibition of seeds in  $H_2O_2$  enables the acclimatization and, consequently, the tolerance to salt stress, resulting in faster responses of the seed germination process and seedling initial growth (Wojtyła et al., 2016). Studies have been conducted to better understand the effects of this acclimatization process by using  $H_2O_2$  on salt stress in seeds of quinoa (Hajjhashemi et al., 2020), barley (Kilic Kahraman, 2016), wheat (Wahid et al., 2007), *Cakile maritima* (Ellouzi et al., 2017), rapeseed (*Brassica napus*) (Kubala et al., 2015), sunflower (Silva et al., 2019), and tobacco (Sousa et al., 2016). However, studies about this acclimatization for melon seeds are still needed.

In this context, the objective of this work was to evaluate the effect of acclimatization with aid of  $H_2O_2$  on seed germination and seedling initial growth of melon (*Cucumis melo*) under salt stress.

## Material and Methods

### Experimental area

The experiment was conducted in the Laboratory of Seeds and Seedlings (LABASEM) of the Academic Unit of Agricultural Sciences (UAGRA) of the Center for Agro-Food Sciences and Technology (CCTA) of the Federal University of Campina Grande (UFCG), Pombal campus, Paraíba (PB), Brazil.

### Experimental design

A completely randomized design was used, in a 4x6 factorial arrangement consisting of times of seed imbibition in  $H_2O_2$  (0, 8, 16, and 24 hours) and irrigation water salinity levels (0.3, 1.2, 2.1, 3.0, 3.9, and 4.8 dS  $m^{-1}$ ), with four replications.

### Conduction of the experiment and application of treatments

Melon seeds of the commercial variety Imperial-45 and presenting 6.6% moisture were used; they were acquired from a specialized seed market. The  $H_2O_2$  solution was prepared from a 1 mmol  $L^{-1}$   $H_2O_2$  solution, based on its molecular weight, and diluted to reach the concentration of 10  $\mu mol L^{-1}$ . The seeds were placed in a beaker and subjected to imbibition with 100 ml of the solution for the different times.

After the period of imbibition in the  $H_2O_2$  solution, the seeds were subjected to asepsis with 1% sodium hypochlorite for five minutes and, subsequently, washed in running water with the aid of a sieve.

The brackish waters were prepared by diluting

a stock solution presenting 30 dS  $m^{-1} L^{-1}$  composed of a mixture of sodium chloride (NaCl), dihydrate calcium chloride ( $CaCl_2 \cdot 2H_2O$ ), and hexahydrate magnesium chloride ( $MgCl_2 \cdot 6H_2O$ ) at the ratio of 7:2:1, respectively; the concentrations were measured with the aid of a bench conductivity meter.

### Variables analyzed

*Germination percentage*: four replications of 50 seeds per treatment were placed in Gernitest paper moistened with brackish water, according to the treatment, at the proportion of 2.5-fold the weight of the dry paper. The rolls were placed in plastic bags and kept in a germinator (BOD-Eletrolab®) with 12-hour photoperiod and adjusted to a temperature of 25 °C. The counts were carried out from the fourth to the eighth day after the implementation of the test (BRASIL, 2009).

*First germination counting, germination speed index, mean germination speed, and mean germination time*: the analyses were carried out together with the germination test. The first germination counts were obtained according to the Rules for Seed Analysis (Brazil, 2009) at the fourth day after sowing. Germination speed index was evaluated by daily counting of germinated seeds, from the fourth to the eighth day of evaluation of the germination test, and calculated according to the formula proposed by Maguire (1962). The mean germination time and mean germination speed were obtained by daily counting of germinated seeds and calculations according to the methodology described by Labouriau, (1983) and Labouriau & Valadares (1976).

*Radicle length*: it was determined using four replications of 10 seeds per treatment, which were distributed on a line drawn on the upper third of the Gernitest paper moistened with brackish water. The rolls were maintained in BOD chamber at temperature of 25° C under absence of light. The evaluation was carried out at the seventh day and the normal seedlings were measured with the aid of a ruler (cm), as recommended by BRAZIL (2009), considering the number of seeds sown that developed normal seedlings.

### Statistical analyses

The results were subjected to analysis of variance for the diagnosis of significant effects by the F test, and the means were compared by regression analysis at 5% probability level, using the program Sisvar (Ferreira, 2014).

## Results and Discussion

The results of the analysis of variance for seed germination percentage, first germination counting,

germination speed index, mean germination time, mean germination speed, and seedling radicle length of melon seeds subjected to different times of imbibition in H<sub>2</sub>O<sub>2</sub> and irrigation water salinity levels are shown in Table 1. Both factors evaluated affected simultaneously the melon seed variables analyzed.

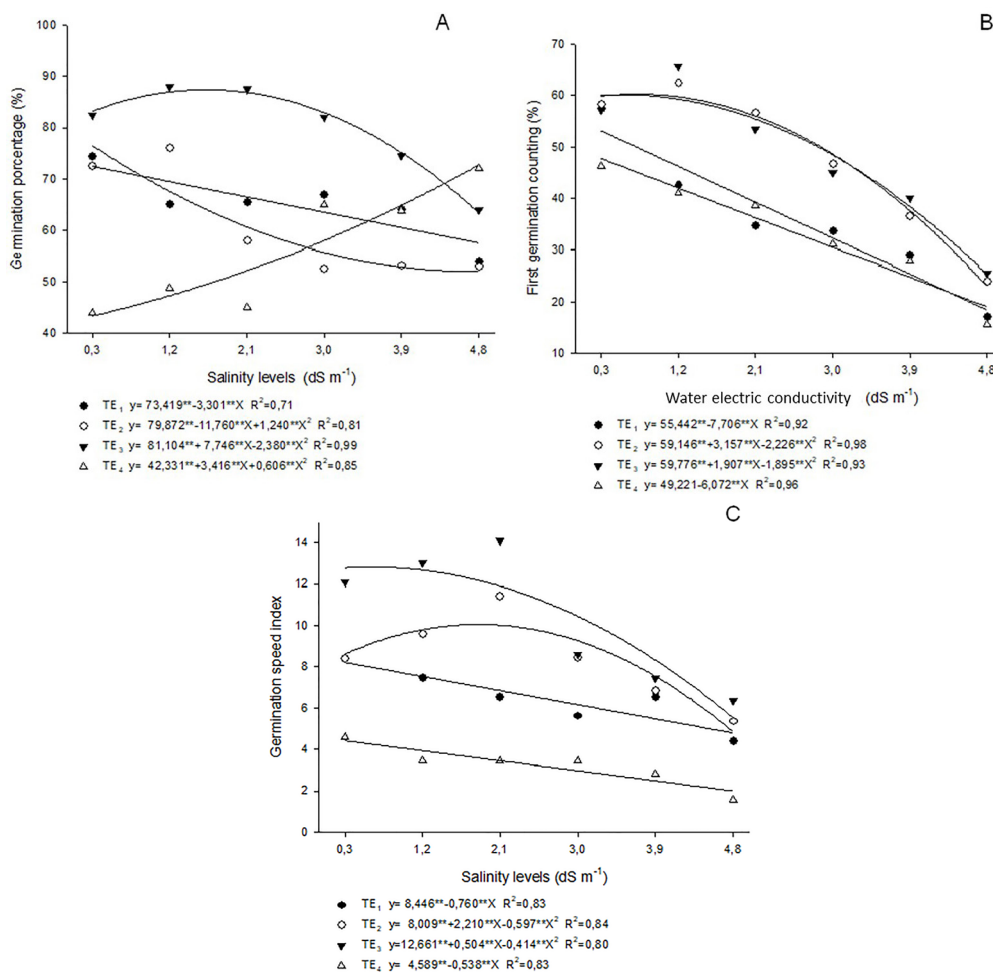
The germination percentage found showed that seeds that were not subjected to imbibition in H<sub>2</sub>O<sub>2</sub> solution (0 hours; TI<sub>0</sub>) presented linear results, decreasing

as the salinity level was increased, and decreasing the germination from 72.4% to 57.5% from the conductivities of 0.3 to 4.8 dS m<sup>-1</sup>, representing a 20.5% decrease. The seed imbibition times of 8 (TI<sub>2</sub>), 16 (TI<sub>3</sub>), and 24 (TI<sub>4</sub>) hours fitted to a second-degree polynomial equation; TI<sub>2</sub> presented the lowest estimate of 51.9% for the electrical conductivity of 4.7 dS m<sup>-1</sup>, and TI<sub>3</sub> and TI<sub>4</sub> presented the highest estimates of 87.4% and 72.6% for the electrical conductivities of 1.6 and 4.8 dS m<sup>-1</sup>, respectively (Figure 1A).

**Table 1.** Analysis of variance for germination percentage (GP), first germination counting (FGC), germination speed index (GSI), mean germination time (MGT), mean germination speed (MGS), and radicle length (RL) of melon seeds subjected to different seed imbibition times in H<sub>2</sub>O<sub>2</sub> and to different electrical conductivities of the irrigation water. UFCG, 2022.

Quadrado médio							
FV	GL	PG (%)	PCG (%)	IVG	TMG (dia <sup>-1</sup> )	VMG (dia <sup>-1</sup> )	CR (cm)
CEa	5	167.96**	2611.67**	49.05**	1.31**	0.003**	30.74**
TE	3	2457.67**	1244.85**	216.41**	34.91**	0.15**	111.61**
CEa x TE	15	453.06**	76.59**	7.14**	1.10**	0.006**	12.86**
Residue	72	5.82	3.41	0.13	0.07	0.0004	0.20
Total	95						
CV (%)		3.68	4.48	5.19	5.68	9.48	2.77
Average		65.51	41.20	7.08	4.67	0.23	16.34

\*\* significant at 1% probability; \* significant at 5% probability by the Fisher test; SV = source of variation; CV = coefficient of variation; ECw = water electrical conductivity; and TI = time of imbibition in H<sub>2</sub>O<sub>2</sub>.



**Figure 1.** Germination percentage (A), first germination counting (B) and germination speed index (C), of melon seeds subjected to different seed imbibition times in H<sub>2</sub>O<sub>2</sub> and to different electrical conductivities of the irrigation water. UFCG, 2022.

This effect can be explained by the production or external uptake of  $H_2O_2$  during germination, as the mobilization of reserves is favored due to oxidative changes in stored proteins, which are recognized by storage organs as signs to mobilize reserves for the embryonic axis, thus favoring the germination. The seed imbibition in  $H_2O_2$  solution can increase  $O_2$  production and concentration, favoring mitochondrial respiration and, consequently, the germination process (Verma et al., 2015).

Similar results were found by Hasanuzzaman et al. (2018), Silva et al. (2019), and Hajjhashemi et al. (2020), who reported that the application of  $H_2O_2$  decreased deleterious effects caused by excess salts on the germination of wheat seeds, as low concentrations of  $H_2O_2$  act as signaling molecules, resulting in certain tolerance to environmental and salt stresses. However, high  $H_2O_2$  concentrations can induce oxidative stress, directly damaging cell functions (Barba-Espin et al., 2011; Wojtyła et al., 2016).

Considering the vigor of melon seeds under different times of seed imbibition in  $H_2O_2$  and different salinity levels of the irrigation water, the first germination counting showed that the treatments  $Tl_1$  and  $Tl_4$  presented linear results, decreasing the germination from 65.2% to 57.6% from the highest to the lowest water electrical conductivities evaluated.  $Tl_2$  and  $Tl_3$  presented the highest estimates for the conductivity of  $0.7 \text{ dS m}^{-1}$ , significantly decreasing the first germination counting in both seed imbibition times, representing decreases of 61.8% and 58%, respectively, as the irrigation water salinity level was increased (Figure 1B).

The germination percentage and first germination counting showed that increases in the irrigation water salinity level significantly affect these parameters when seeds are not subjected to imbibition in a  $H_2O_2$  solution; it is connected to excess salts because this stress reduces the water availability to seeds, hindering the nutrition process and making K absorption difficult, which is a co-factor of several enzymes that are important for essential metabolic processes to plants, as photosynthesis and respiration, which induce the germination process (Taiz et al., 2017).

Similar results were found by Castañares & Bouzo, (2020) for seeds of *Cucumis melo*, as well as for other crops, such as *Oryza sativa* (Hemalatha et al., 2017), *Annona muricata* (Silva et al., 2019), *Capsicum annuum* (Gammoud et al., 2020), *Chenopodium quinoa* (Hajjhashemi et al., 2020), and *Triticum aestivum* (Panhwar et al., 2021). These studies evaluated the effect of  $H_2O_2$  as

a mitigator of deleterious effects caused by excess salts on seed germination and showed that seeds subjected to imbibition in  $H_2O_2$  solutions present higher germination percentage and germination speed than non-treated seeds.

The treatments  $Tl_1$  and  $Tl_4$  presented linear results for germination speed index, which decreased as the water salt concentration was increased, presenting decreases of 41.6% and 54.6% as the electrical conductivity was increased from  $0.3$  to  $4.8 \text{ dSm}^{-1}$ , denoting that no exogenous application or high concentrations of  $H_2O_2$  can affect negatively the germination speed index of melon seeds (Figure 1C). This decrease can be explained by the increasing salt concentration, which decreases germination rate and germination speed index, causing delays in the seed germination process and initial seedling development (Gammoudi et al., 2020). According to Silva et al. (2019), high salinity levels can cause adverse effects on the permeability of cell membranes, decreasing germination speed index and, consequently, seed germination.

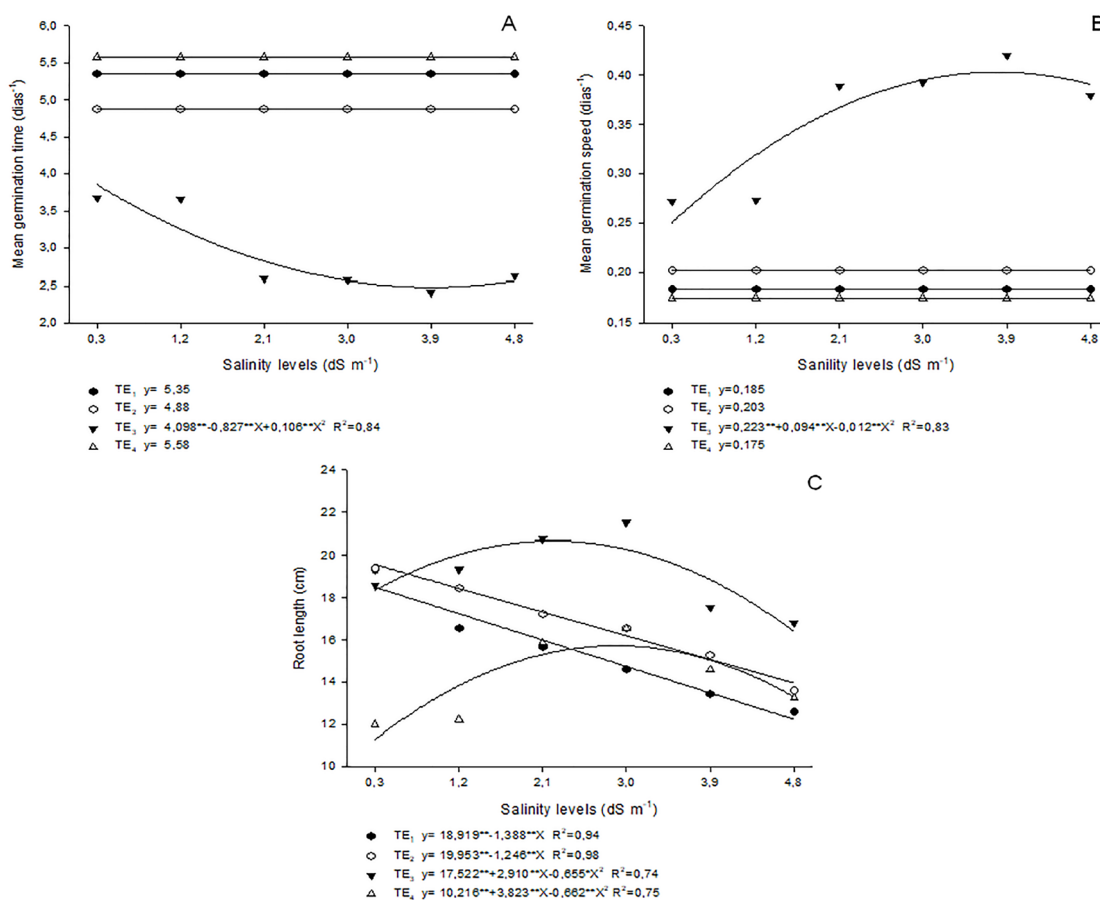
However, when the melon seeds were exposed to  $H_2O_2$ , the treatments  $Tl_2$  and  $Tl_3$  fitted to a quadratic equation, with the highest germination speed index estimates of 10 and 12.6 for the electrical conductivities of  $1.9$  and  $0.7 \text{ dS m}^{-1}$ , respectively (Figure 1C). This denotes the efficiency of seed imbibition in  $H_2O_2$  as a mitigator of salt stress for melon seeds, as  $H_2O_2$  has osmoprotective function and acts on the absorption and transfer of nutrients, ensuring an osmotic balance and reducing the harmful effects of salt stress on seed germination and seedling initial growth (Shahid et al., 2014; Kaya et al., 2010).

A similar study was carried out by Rodrigues et al. (2021), who evaluated the potential of  $H_2O_2$  as a mitigator of salt stress effects on germination of seeds of *Myracrodruon urundeuva* and found germination speed index of 5.63 for seeds immersed in  $H_2O_2$  at  $14.0 \mu\text{mol L}^{-1}$  and germinated under a salt concentration of  $1.4 \text{ dS m}^{-1}$ , denoting the efficiency of  $H_2O_2$  under the adverse effects of high salinity levels.

The mean germination time of melon seeds in the treatments  $Tl_1$ ,  $Tl_2$ , and  $Tl_4$  was, respectively, 5.35, 4.88, and 5.58  $\text{days}^{-1}$  due to the increase in water salts; however, the results found in  $Tl_3$  fitted to a quadratic equation, reaching the lowest mean estimate of 2.48  $\text{days}^{-1}$  for the electrical conductivity of  $4.0 \text{ dS m}^{-1}$ , once again denoting that the use of  $H_2O_2$  can affect the melon seed germination process when applied at adequate concentrations and immersion times (Figure 2A). The

mean germination speed of melon seeds in  $TI_1$ ,  $TI_2$ , and  $TI_4$  was, respectively, 0.185, 0.203, and 0.175 days<sup>-1</sup>; however, the results found for seeds in the treatment  $TI_3$  fitted to a quadratic equation as the water salt concentration was

increased, reaching the highest estimated time of 0.375 days<sup>-1</sup> with the electrical conductivity of 3.7 dS m<sup>-1</sup> (Figure 2B).



**Figure 2.** Mean germination time (A), mean germination speed (B), and radicle length (C) of melon seeds subjected to different seed imbibition times in H<sub>2</sub>O<sub>2</sub> and to different electrical conductivities of the irrigation water. UFCG, 2022.

High salinity can delay the seed germination, but this can be overcome with the use of H<sub>2</sub>O<sub>2</sub> solution, as found for the treatment consisting of imbibition of seeds in H<sub>2</sub>O<sub>2</sub> solution for 16 hours, in which the H<sub>2</sub>O<sub>2</sub> caused changes in physiological mechanisms that assist in overcoming adverse effects of salinity, enabling a shorter mean germination time and a higher mean germination speed (Bagheri et al., 2019). Hajjhashemi et al. (2020) evaluated the cross-talk effect of application of H<sub>2</sub>O<sub>2</sub> and calcium on the germination of seeds of *Chenopodium quinoa* subjected to salt stress and found that the application of H<sub>2</sub>O<sub>2</sub> decreased the effect of this abiotic stress.

The results found for radicle length of melon seeds in the treatments  $TI_1$  and  $TI_2$  fitted to a linear equation (Figure 2C), decreasing the radicle length from 33.7% to 28.6% as the water electrical conductivity was increased from 0.3 to 4.8 dS m<sup>-1</sup>. The results found for seeds in  $TI_3$  and

$TI_4$  gradually increased, reaching the highest estimates of 20.7 and 15.7 cm with the electrical conductivities of 2.2 and 2.9 dS m<sup>-1</sup>, respectively. The positive effect of mechanisms of action of low concentrations of H<sub>2</sub>O<sub>2</sub> on the acclimatization and mitigation of harmful effects of high salinity levels results in benefits for root development and seedling growth (Silva et al., 2019). Similar results were found by Panhwar et al. (2021), who evaluated seeds subjected to imbibition in H<sub>2</sub>O<sub>2</sub> and found decreases in deleterious effect of salts on root growth of wheat seeds. Therefore, the efficiency of using small concentrations of H<sub>2</sub>O<sub>2</sub> solution for acclimatization of seeds subjected to salt stress is clear, as this effect is efficient on melon seeds subjected to imbibition in H<sub>2</sub>O<sub>2</sub> solution for 16 hours to significantly reduce the deleterious effects caused by high salinity levels on seed germination and seedling initial growth.



## Conclusions

The use of H<sub>2</sub>O<sub>2</sub> solution for imbibition of melon seeds for 16 hours mitigate the negative effects from salt stresses by up to 2.2 dS m<sup>-1</sup>; however, the imbibition of seeds for 24 hours in H<sub>2</sub>O<sub>2</sub> solution is harmful to the germination process of melon seeds.

## Acknowledgements

The authors thank the Federal University of Campina Grande and its Laboratory of Seeds and Seedlings (LABASEM), Pombal campus, Paraíba, Brazil, for the support and incentives for this research.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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