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**PLANT ABIOTIC STRESS TOLERANCE**



Pantanal Editora

2020

Fábio Steiner  
(Organizador)

# PLANT ABIOTIC STRESS TOLERANCE



Pantanal Editora

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## APRESENTAÇÃO

A obra “Plant Abiotic Stress Tolerance”, uma publicação da Pantanal Editora, apresenta, em seus 9 capítulos, uma ampla gama de assuntos sobre os recentes avanços e conhecimentos científicos nas áreas de ecofisiologia da produção vegetal e conservação dos recursos naturais e meio ambiente. Os temas abordados mostram algumas das ferramentas atuais que permitem o incremento da produção de alimentos, a melhoria da qualidade de vida da população, e a preservação e a sustentabilidade dos recursos disponíveis no planeta. A obra, vem a materializar o anseio da Editora Pantanal na divulgação de resultados e conhecimentos, que contribuem de modo direto no desenvolvimento humano.

Nas últimas décadas, a produção de alimentos tem sido frequentemente limitada por inúmeros fatores de estresse abióticos, dentre os quais, podemos citar a baixa disponibilidade de água (deficiência hídrica), temperaturas extremas (frio, geadas, calor e fogo), salinidade, deficiência de nutrientes minerais e toxicidade. Esses fatores são responsáveis por consideráveis perdas econômicas tanto para os pequenos agricultores quanto para os produtores de commodities como a cultura da soja, entre outras. Além disso, estes danos podem ser potencialmente agravados pelos efeitos das recentes mudanças climáticas globais, sendo, portanto, a sua mitigação um grande desafio para a comunidade científica. O foco principal das pesquisas abordadas neste e-book é compreender os mecanismos de defesa/tolerância dos estresses abióticos em plantas e apresentar tecnologias e práticas de manejo que possibilitem o aumento da tolerância das plantas a esses estresses abióticos.

Temas associados à identificação de cultivares de soja tolerantes à seca e o manejo da salinidade e da restrição hídrica nas culturas de soja, amendoim e pepino são abordados. A tolerância de plantas de pinhão-manso a toxicidade do alumínio (Al<sup>3+</sup>), a tolerância de quatro espécies hortícolas ao estresse térmico causado por altas temperaturas e a tolerância de mutantes de trigo ao estresse salino também é sugerido. Na área de recursos naturais é mostrado os efeitos fitotóxicos dos metais pesados nas plantas cultivadas e o estresse ambiental causado pelo fogo na região do Cerrado. Portanto, esses conhecimentos irão agregar muito aos seus leitores que procuram promover melhorias quantitativas e qualitativas na produção de alimentos e, ou melhorar a qualidade de vida da sociedade. Sempre em busca da sustentabilidade do planeta.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas áreas de ecofisiologia da produção vegetal e conservação dos recursos naturais e meio ambiente, os agradecimentos do Organizador e da Pantanal Editora.

Por fim, esperamos que este e-book possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias. Assim, garantir uma difusão de conhecimento fácil, rápido para a sociedade.

**Fábio Steiner**

## PRESENTATION

The eBook “Plant Abiotic Stress Tolerance”, a publication by Pantanal Editora, presents in its 9 chapters a wide range of questions about recent advances and scientific knowledge in the areas of ecophysiology of plant production and conservation of natural resources and the environment. The topics presented show some of the current tools that allow the increase in food production, the improvement of quality of life in people's and the preservation and sustainability of the resources available on the planet. This eBook materializes Editora Pantanal's desire to disseminate results and knowledge, which directly contribute to the development of society.

In the last decades, food production has often been limited by numerous abiotic stress factors, among which, we can mention the low availability of water (water deficit), extreme temperatures (cold, frosts, heat and fire), salinity, mineral nutrient deficiency and toxicity. These factors are responsible for considerable economic losses, both for small farmers and for producers of commodities such as soybean, among others. In addition, these damages can potentially be aggravated by the effects of recent global climate changes, and therefore, mitigating these damages is a major challenge for the scientific community. The main objective of the research presented in this e-book is to understand the defense or tolerance mechanisms of abiotic stresses in plants and to present technologies and management practices that enable greater tolerance of plants to these abiotic stresses.

Topics associated with the identification of drought-tolerant soybean cultivars and the management of salinity and water restriction in soybean, peanut and cucumber crops are presented. The tolerance of physic nut plants to aluminum toxicity ( $Al^{3+}$ ), the tolerance of four vegetable species to heat stress caused by high temperatures and the tolerance of wheat mutants to salt stress is also suggested. In the area of natural resources, the phytotoxic effects of heavy metals on plant growth and the environmental stress caused by fire in the Cerrado region are shown. Therefore, this knowledge can add much to its readers who seek to promote quantitative and qualitative improvements in food production and, or improve the quality of life in society. Always in search of the planet's sustainability.

To the authors of the chapters, for their dedication and efforts, that made this eBook possible, which exposes the recent scientific and technological advances in the areas of ecophysiology of plant production and conservation of natural resources and the environment, thanks to the Organizer and Pantanal Editora.

Finally, we hope that this e-book can collaborate and instigate more students and researchers in the constant search for new technologies. Thus, ensuring an easy and quick dissemination of knowledge to society.

**Fábio Steiner**

## PRESENTACIÓN

El trabajo “Plant Abiotic Stress Tolerance”, publicación de Pantanal Editora, presenta, en sus 9 capítulos, una amplia gama de temas sobre avances recientes y conocimientos científicos en las áreas de ecofisiología de la producción vegetal y conservación de los recursos naturales y el medio ambiente. Los temas tratados muestran algunas de las herramientas actuales que permiten el aumento de la producción de alimentos, la mejora de la calidad de vida de la población y la preservación y sostenibilidad de los recursos disponibles en el planeta. El trabajo materializa el afán de Editora Pantanal por difundir resultados y conocimientos, que contribuyan directamente al desarrollo humano.

En las últimas décadas, la producción de alimentos se ha visto a menudo limitada por numerosos factores de estrés abiótico, entre los que podemos mencionar la baja disponibilidad de agua (deficiencia de agua), temperaturas extremas (frío, heladas, calor y fuego), salinidad, deficiencia, nutrientes minerales y toxicidad. Estos factores son responsables de considerables pérdidas económicas tanto para los pequeños agricultores como para los productores de commodities como la soja, entre otros. Además, estos daños pueden verse potencialmente agravados por los efectos de los cambios climáticos globales recientes y, por lo tanto, mitigarlos es un desafío importante para la comunidad científica. El foco principal de las investigaciones cubiertas en este libro electrónico es comprender los mecanismos de defensa / tolerancia contra el estrés abiótico en las plantas y presentar tecnologías y prácticas de manejo que permitan aumentar la tolerancia de las plantas a estos estreses abióticos.

Se abordan temas relacionados con la identificación de cultivares de soja tolerantes a la sequía y el manejo de la salinidad y la restricción hídrica en cultivos de soja, maní y pepino. También se sugiere la tolerancia de las plantas de frutos secos a la toxicidad del aluminio ( $Al^{3+}$ ), la tolerancia de cuatro especies hortícolas al estrés por calor causado por las altas temperaturas y la tolerancia de los mutantes del trigo al estrés por sal. El área de recursos naturales muestra los efectos fitotóxicos de los metales pesados en las plantas cultivadas y el estrés ambiental causado por los incendios en la región del Cerrado. Por tanto, este conocimiento aportará mucho a sus lectores que buscan promover mejoras cuantitativas y cualitativas en la producción de alimentos y, o mejorar la calidad de vida en la sociedad siempre en busca de la sostenibilidad del planeta.

A los autores de los distintos capítulos, por su dedicación y esfuerzo irrestricto, que hizo posible este trabajo, que retrata los recientes avances científicos y tecnológicos en las áreas de ecofisiología de la producción vegetal y conservación de los recursos naturales y el medio ambiente, gracias a la Organización y a Pantanal Editora.

Finalmente, esperamos que este libro electrónico pueda colaborar e instigar a más estudiantes e investigadores en la búsqueda constante de nuevas tecnologías. De esta forma, se garantiza una fácil y rápida difusión del conocimiento a la sociedad.

**Fábio Steiner**



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## Potassium nitrate priming to induce salt stress tolerance in cucumber seeds

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### INTRODUCTION

Cucumber (*Cucumis sativus* L.) is the fourth most cultivated vegetable crop in Brazil, both in outdoor fields and greenhouses (Carvalho et al., 2016). However, the sustainability of vegetable production in many areas of Brazil, especially under greenhouse conditions is at risk due to excess salts in the soil or irrigation water (Pereira et al., 2017). Salinity is one of the most serious stress factors that limit plant growth and yield of cucumber in arid, semiarid, and irrigated areas, mainly due to its dramatic effects on plant physiology and performance (Acosta-Motos et al., 2017). A soil is considered saline when the electric conductivity of the extract (ECe) from the water saturated soil reaches 4.0 dS m<sup>-1</sup> (equivalent to 40 mmol L<sup>-1</sup> NaCl), generating an osmotic pressure of about -0.2 MPa and significantly reducing the yields of most vegetables (Munns; Tester, 2008).

Soil salinity may adversely affect plant growth either through osmotic inhibition of water uptake by plant roots or phytotoxic effects of specific ions that lead to secondary oxidative stress in plants (Acosta-Motos et al., 2017). Salinity generates a low water potential in the soil, making it difficult for plants to acquire water, and resulting in water restriction conditions (Porcel et al., 2012). Therefore, excessive concentration of salt in the soil solution negatively affects the physiology, growth, and yield of crops (Lima et al., 2016; Sá et al., 2018).

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Salinity affects plant growth at all developmental stages; however, sensitivity varies from one growth stage to another. Seed germination is one of the most salt-sensitive plant growth stages and is severely inhibited with increasing salt level (Fuller et al., 2012; Zuffo et al., 2020). Delayed and reduced seedling emergence cause non-uniform stand establishment, which results in reduced crop yields (Lawles et al., 2012). In this regard, several approaches including seed priming, seed soaking, and seed coating have been employed to improve seed germination and plant establishment under salt stress.

Among the strategies used to mitigate the salt stress-induced adverse effects the priming of seeds by soaking in inorganic salt solutions (halopriming) or water (hydropriming) are cited as the most appropriate, efficient and economic techniques to enhance the rate and the uniformity of germination under saline conditions (Singh et al., 2015; Matias et al., 2018). (Nerson; Govers. 1986) suggested that nitrate-containing compounds may function more efficiently than other salts as priming agents. Indeed, the seed priming with potassium nitrate ( $\text{KNO}_3$ ) solution have been shown to have beneficial effects on germination and growth rate of a wide range of vegetable crops under stressful environments, as in eggplant (Nascimento and Lima, 2008), melon (Nerson; Govers, 1986; Oliveira et al., 2019), tomato (Ebrahimi et al., 2014), and pepper (Batista et al., 2015). Ebrahimi et al. (2014) indicated that seed priming with  $\text{KNO}_3$  led to increasing of germination and growth rate of tomato seedlings under salinity conditions. However, Oliveira and Steiner (2017) showed that the priming of cucumber seeds with  $\text{KNO}_3$  had insignificant effect to improve germination capacity and growth rate of seedlings under salt stress conditions.

On the other hand, Matias et al. (2018) recommended the soaking of seeds in water to improve plant tolerance under salt stress conditions. These authors argue that the hydropriming stands out for its practicality and requires low investments in reagents, compared with the other methods, besides avoiding interference of substances that are undesirable or harmful to the seeds during soaking. Therefore, these and other contradictory results justify the need of conducting more research to investigate the effectiveness of seed pretreatment with  $\text{KNO}_3$  or water in the induction of salt stress tolerance in cucumber plants.

The study was carried out to investigate the effects of halopriming and hydropriming as inducers of salt stress tolerance in cucumber seeds (*Cucumis sativus* L., cv. Aodai Melhorado) exposed to different salinity levels.

## **MATERIAL AND METHODS**

Seeds of cucumber (*Cucumis sativus* L., cv. Aodai Melhorado) were surface-sterilized in 2% (v/v) sodium hypochlorite solution for 5 minutes and rinsed three times in distilled water. The “Aodai Melhorado” cucumber cultivar is considered a moderately tolerant genotype to salt stress during the

germination and seedling establishment stage (Oliveira; Steiner, 2017). The sterilized seeds were then primed by soaking with 5 g L<sup>-1</sup> KNO<sub>3</sub> solution (halopriming) or distilled water (hydropriming) for 22 hours at 25 °C. After priming, seeds were removed and washed with tap water and then put to dry at room temperature (23–28 °C) for 48 hours. A set of unprimed dry seeds were taken as control.

To compare the effect of priming techniques on plant tolerance to saline stress, the seeds were exposed to 0.0, -0.30 and -0.60 MPa iso-osmotic solutions with NaCl. The concentration of NaCl required to obtain the osmotic potential of -0.3 and -0.6 MPa was calculated by the van't Hoff equation (Hillel, 1971):  $\Psi_s = -RTC_i$ , where  $\Psi_s$  is the osmotic potential (MPa);  $R$  is the ideal gas constant (0.008314 MPa mol<sup>-1</sup> K<sup>-1</sup>);  $T$  is the absolute temperature (273.15 + °C);  $C_i$  is the concentration in molarity of the solute (mol L<sup>-1</sup>); and  $i$  is the van't Hoff factor, the ratio of amount of particles in solution to amount of formula units dissolved [i.e., for NaCl this value is 2.0 (Na<sup>+</sup> and Cl<sup>-</sup>)].

Treatments were arranged in a completely randomized design in a 3 × 3 factorial: three priming techniques [hydropriming (water), halopriming (0.5% KNO<sub>3</sub>) or unprimed seeds] and three salt stress levels [0 MPa (control), -0.3 MPa (mild stress) and -0.6 MPa (severe stress)], with four replicates.

Four replicates of 30 seeds were evenly distributed in plastic boxes type Gerbox<sup>®</sup> (11.0 × 11.0 × 3.5 cm) with blotter paper, properly moistened with the salt solution of each treatment, in a volume equivalent to three times the weight of dry paper. The boxes were then closed with lids to prevent evaporation and maintain the relative humidity close to 100%. Germination was carried out in a germination chamber under 12/12 h photoperiod (light/darkness), photosynthetic photon flux density (PPFD) of 180 μmol m<sup>-2</sup> s<sup>-1</sup> and temperature of 25 °C for 14 days. Thiram was added to the solutions at a concentration of 0.2% (v/v) to control the fungi infection. Seeds were considered germinated when radicle was longer than 10.0 mm. Germinated seeds were recorded every 24 h for 14 days.

The number of germinated seeds was recorded daily, and the final germination percentage was determined after 14 days. The germination rate index (GRI) was calculated using Maguire's equation (Maguire, 1962):  $GRI = \sum (n_i/t_i)$ , where  $n_i$  is the number of germinated seeds on a given day, and  $t_i$  is the time in days from the starting/sowing day (0). The mean germination time (MGT) was calculated using the equation of Labouriau (1983):  $MGT = (\sum n_i t_i) / \sum n_i$ , where  $n_i$  is the number of germinated seeds on a given day, and  $t_i$  is the time in days from the starting/sowing day (0).

The hypocotyl length (HL, in cm) and radicle length (RL, in cm) was measured in ten normal seedlings randomly obtained after count of the total germination (14th day) using meter scale. To determine the dry matter partitioning into shoot and roots, all seedlings obtained at the end of the germination test (14 days) were separated into shoots and roots, dried in an oven for three days at 65 °C, and then weighed. The results were expressed in mg seedling<sup>-1</sup>. To determine root: shoot ratio (RSR), root dry matter obtained was divided by the shoot dry matter.

The data of germination percentage, seedling length, and dry matter accumulation were used to calculate the seedling vigor and drought tolerance indices. Seedling weight and length vigor indices in each treatment were calculated using following equations, as suggested by Abdul-Baki and Anderson (1973):  $SLVI = [\text{seedling length (cm)} \times \text{seed germination (\%)}]$  and  $SWVI = [\text{seedling dry weight (mg)} \times \text{seed germination (\%)}]$ .

The salt tolerance index was calculated using the equation of Bouslama and Schapaugh (1984):  $YSI = Y_s/Y_c$ , where YSI is the yield stability index,  $Y_s$  and  $Y_c$  are the total dry matter yield (mg per seedling) under saline stress and non-stress conditions (control), respectively.

The normality of data was previously tested by the Kolmogorov-Smirnov test at the 5% level and then data were submitted to analysis of variance (ANOVA), and means of seed priming and stress level were compared by Fisher's Least Significant Difference (LSD) test at the 0.05 level of confidence. For statistical analysis, the data expressed in percentage were previously transformed into  $\arcsin\sqrt{(x/100)}$ . The analyses were performed using Sisvar<sup>®</sup> version 5.6 software for Windows (Statistical Analysis Software, UFPA, Lavras, MG, BRA).

## RESULTS AND DISCUSSION

The results of the analysis of variance showed significant effects ( $p < 0.05$ ) for the main effects of seed priming with  $KNO_3$  and salinity levels, as well as for interaction, for the majority of the traits measured (Table 1). The significant interaction between the main effects of seed priming and salt stress for most of the evaluated traits indicates germination and initial growth of cucumber seedlings from seeds submitted to different priming techniques has a distinct response when exposed to different saline stress levels.

**Table 1.** Summary of the analysis of variance for the measurements of germination traits, initial growth, dry matter partitioning and vigor indexes of cucumber seedlings for the effects of seed priming and saline stress

Causes of variation	Probability > F										
	GC	GRI	MGT	HL	RL	SDM	RDM	TDM	RSR	SLVI	SWVI
Priming (P)	0.004	<0.000	0.181	0.538	<0.000	0.005	<0.000	0.001	0.215	0.134	0.442
Salinity (S)	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	0.018	<0.000	<0.000	0.002
P × S	0.005	<0.000	0.002	0.067	0.031	0.035	0.003	0.043	0.055	0.042	0.003
CV (%)	7.92	7.80	5.23	10.54	10.82	12.73	11.06	8.00	11.93	12.88	12.32

GC: germination capacity. GRI: germination rate index. MGT: mean germination time. HL: Hypocotyl length. RL: radicle length. SDM: shoot dry matter. RDM: Root dry matter. TDM: Total dry matter. RSR: Root: shoot ratio. SLVI: Seedling length vigor index. SWVI: Seedling weight vigor index. Source: The authors.

### Effect of seed priming and salt stress on germination

The germination percentage of cucumber seeds in the salt-free control treatment was higher than the standard value used as reference for the commercialization of cucumber seeds in Brazil (i.e., 80%) (Figure 1A). This indicates that the seeds used in this study were of high physiological quality.

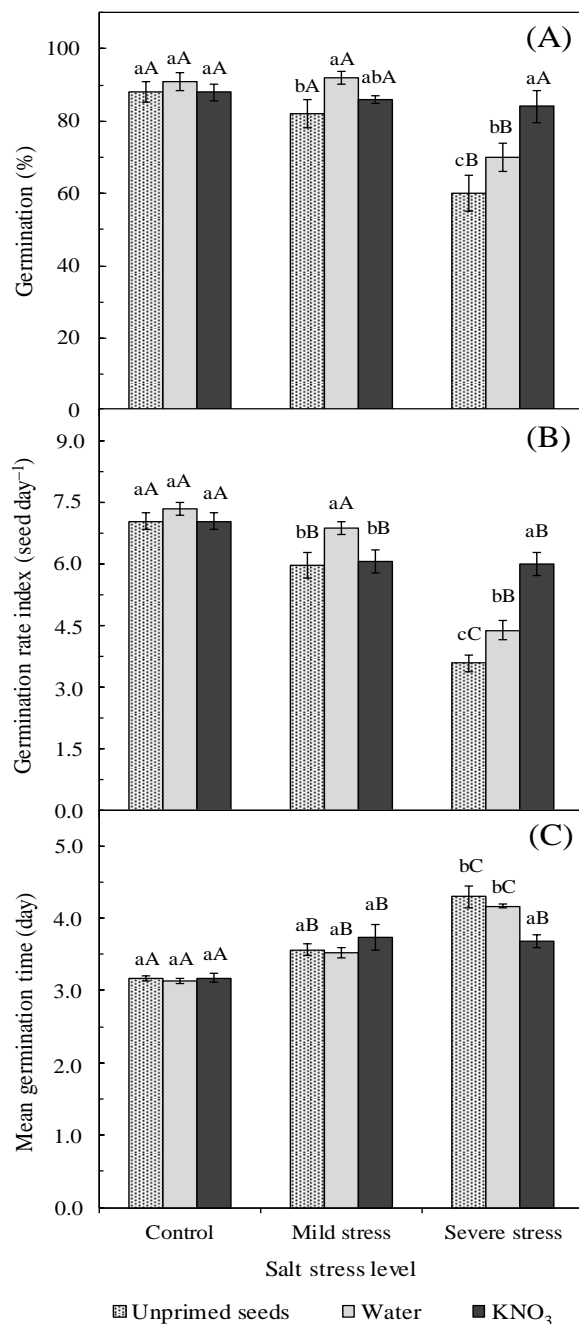
The different priming techniques did not interfere in the germination capacity, germination rate index and mean germination time when the cucumber seeds were submitted to the salt-free control conditions (Figure 1). These results indicate that the germination response of cucumber seeds has not been improved by hydropriming and halopriming when the seeds are exposed to non-stressful environments. However, when the seeds were submitted to mild salt stress the germination percentage was significantly greater with hydropriming, and lower for unprimed seeds (Figure 1A). Under conditions of severe salt stress, the germination ranged from 60 to 84% and was significantly greater when the seeds were primed with KNO<sub>3</sub>, followed by hydropriming, and lower for unprimed seeds (Figure 1A).

The hydropriming was efficient to improve the germination capacity and germination rate index only under conditions of mild salt stress (Figure 1). However, under severe salt stress conditions, seeds primed with KNO<sub>3</sub> must be used to obtain higher germination percentage and germination rate index and lower mean germination time. These results show that priming of cucumber seeds with KNO<sub>3</sub> can have significant improvements of germination under conditions of high salt levels. Ebrahimi et al. (2014) also reported that the use of priming with KNO<sub>3</sub> may enhance the germination and growth rate of tomato seedlings under high salinity conditions. These findings agree with Kubala et al. (2015) who

found that halopriming may improve germination performance through metabolic activation involving the synthesis of proteins, nucleic acids, and enzymes, and increasing water uptake, respiratory activity, and reserve mobilization. Therefore, in field or greenhouse conditions where the soil or substrate is affected by salinity, the use of cucumber seeds primed with  $\text{KNO}_3$  could make the difference between successful field germination and establishment or substantial crop failure (Ashraf et al., 2008).

The exogenous application of  $\text{KNO}_3$  can stimulate seed germination at salt stress conditions due to the production of substances that release nitric oxide (NO) (Kaiser et al., 2016). These substances act in membrane permeability, preventing or reversing the damage caused by osmotic stresses (Pereira et al., 2010). Therefore, as the process of saline stress involves changes in osmotic pressure and tension on the cell membrane, it is possible that substances NO-liberating improves the germination process under salt stress (Kaiser et al., 2016). Nitric oxide is a molecule that acts as a signaler in higher plants and studies on their functions in the physiological processes of plants indicate that NO is involved in the regulation of plant growth and development, defense against pathogen and responses to abiotic stress (Sanz et al., 2015).

The germination rate index (GRI) of cucumber seeds is reduced with the increase of the salt stress level; however, this effect can be partially reversed with the  $\text{KNO}_3$  priming under severe salt stress (Figure 1B). The decrease of the GRI was due to lower capacity of water uptake by the seeds with highly negative osmotic pressure. According to Feijão et al. (2011), the lower germination rate and growth inhibition due to salinity are caused by low external water potential, ion imbalance, and specific ion toxicity. Under severe salt stress conditions, there is a decrease in water uptake and an excessive uptake of ions (Akram et al., 2010). Osmotic stress affects the starch synthesis reactions and energy production process (adenosine triphosphate – ATP) through respiration, resulting in reduced of germination (Figure 1A), germination rate index (Figure 1B) and thus in delay of germination time (Figure 1C).



**Figure 1.** Effects of salt stress levels on germination capacity (A), germination rate index (B), and mean germination time (C) of cucumber (*Cucumis sativus* L., cv. Aodai Melhorado) from seeds submitted to priming with water (hydropriming) or 0.5% KNO<sub>3</sub> solution (halopriming) and unprimed seeds. Bars followed by distinct lower-case letters, between the priming techniques or upper-case letters, for the salt stress levels, show significant difference (LSD test,  $p \leq 0.05$ ). Data refer to mean values ( $n = 4$ )  $\pm$  mean standard error. UEMS/Cassilândia-MS, 2018. Source: The authors.

The mean germination time (MGT) was delayed with the rise of salt stress levels (Figure 1C). Under severe salt stress, the MGT was delayed in 1.1 days (from 3.17 to 4.30 days) for the unprimed



seeds, in 1.0 days (from 3.14 to 4.17 days) for the seeds primed with water, against 0.5 days (from 3.17 to 3.68 days) for the  $\text{KNO}_3$  priming compared to the NaCl-free treatment (control). A delay in the time to seed germination may be disadvantageous for successful establishment, since the delayed germination leaving the seeds more vulnerable to attack from predators (pests and pathogens) and, therefore, compromise the establishment of a uniform stand.

The delay of germination was due to salinity affect the water uptake of the seeds, which is the first step to occur germination process (i.e., imbibition). According to Marcos-Filho (2005), it is necessary that the seeds reach an adequate level of hydration during the imbibition phase, to occur reactivation of seed metabolic processes and growth of embryonic axis. Seeds subjected to osmotic stress require more time to adjust the internal osmotic potential in accordance with the external environment (Parida; Das, 2005; Munns; Tester, 2008). Meneses et al. (2011) reported that highly negative osmotic pressure may affect the seeds water uptake, making germination not possible. Additionally, the osmotic potential of the external medium can affect the enzymatic reactions in the seed, therefore, the delay in germination is due to delay of enzymatic reactions (Marcos-Filho, 2005), caused by the break of the imbibition period. The most common responses of plants to the reduction of osmotic pressure are a delay in initial germination and a reduction in the rate and total germination (Oliveira; Gomes-Filho, 2009). The result of these changes is an unevenness in the germination process and stand establishment.

### Effect of seed priming and salt stress on early seedling growth

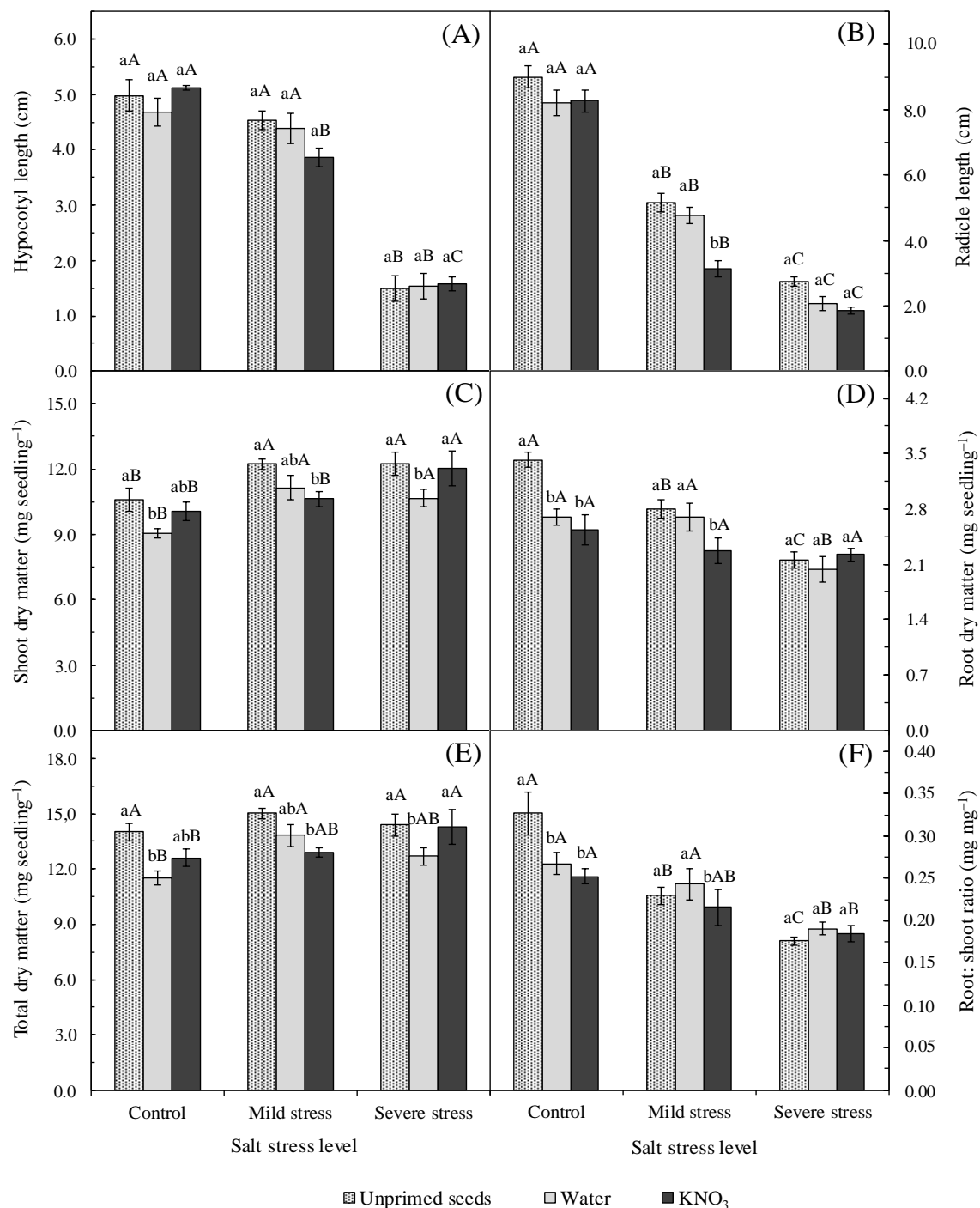
The initial growth of cucumber seedlings was little influenced by the priming or not of the seeds with water or  $\text{KNO}_3$  (Figure 2). These results show that although  $\text{KNO}_3$  priming can improve seed germination performance, it has an insignificant effect on improving the growth of cucumber seedlings. Equivalent results were reported by Oliveira and Steiner (2017), who verified that the halopriming of cucumber seeds had little effect to improve germination and growth rate of seedlings under salt stress conditions.

The length of hypocotyl and primary root were severely inhibited by the increase of salt stress level (Figures 2A and 2B). The hypocotyl length of cucumber seedlings ranged from 4.7 to 5.1 cm in the NaCl-free control treatment, whereas under severe salt stress the hypocotyl length ranged from 1.5 to 1.6 cm (Figure 2A). In turn, the primary root length decreased from 8.2–9.0 cm in the control treatment to 1.9–2.7 cm under severe salt stress conditions (Figure 1B). These results indicate that there was a mean reduction in hypocotyl and root length of 216% and 274%, respectively.

Under high salinity level an irreversible impairment of the photosynthetic apparatus, associated with a reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, occurs when the stress is prolonged, and salt continues to accumulate in the leaves (Acosta-Motos et al., 2017). On the other hand, the noticed decrease in the length of the hypocotyl and primary root could be due to the negative effect of this salt on the changes in enzyme activity (that subsequently affects protein synthesis), and also the decrease in the level of carbohydrates and growth hormones, both of which can lead to inhibition of the growth (Mazher et al., 2007).

The dry matter yield of the shoots was increased with mild and severe salt stress levels (Figure 2C). This increase in shoot dry matter may be because the salt added in the solution may induce osmotic adjustment activity in the plants, which improved seedling growth. (Memon et al., 2010) reported that the addition of low NaCl concentrations led to increases in the growth of pak choi seedlings, however, higher NaCl concentrations caused inhibition.

On the other hand, the dry matter yield of roots was reduced with salt stress levels, except for seeds primed with  $\text{KNO}_3$  (Figure 2D). The lower dry matter yield of roots due to increasing salinity level was caused by low external water potential, ion imbalance and specific ion toxicity (Feijão et al., 2011). One of the initial effects of salt stress on the plant is the reduction of growth rate and dry matter accumulation. (Oliveira; Steiner, 2017) reported that salinity decreases the dry matter accumulation of cucumber plants, and the salt level of  $100 \text{ mmol L}^{-1}$  decreased the root dry matter at around of 40%.



**Figure 2.** Effects of salt stress levels on hypocotyl length (A), radicle length (B), shoot dry matter (C), root dry matter (D), total dry matter (E) and root: shoot dry matter ratio (F) of cucumber (*Cucumis sativus* L., cv. Aodai Melhorado) from seeds submitted to priming with water (hydropriming) or 0.5% KNO<sub>3</sub> solution (halopriming) and unprimed seeds. Bars followed by distinct lower-case letters, between the priming techniques or upper-case letters, for the salt stress levels, show significant difference (LSD test,  $p \leq 0.05$ ). Data refer to mean values ( $n = 4$ )  $\pm$  mean standard error. UEMS/Cassilândia-MS, 2018. Source: The authors.

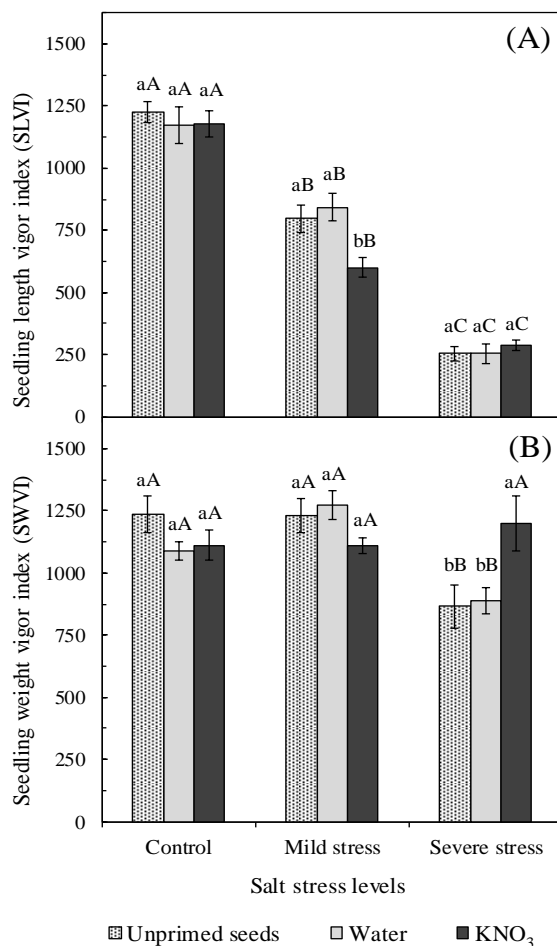
In general, the highest total dry matter yield at most salt stress levels was obtained with the use of unprimed seeds (Figure 2E). These results show that the priming of seeds with water or  $\text{KNO}_3$  did not improve the growth of cucumber seedlings. However, under mild and severe salt stress conditions the priming of water and  $\text{KNO}_3$  resulted in the highest total dry matter compared to the control (Figure 2E). Contrary results were reported by (Oliveira; Steiner, 2017), who found that inhibiting action of salt stress on early seedling growth was increased with the rise of salinity levels, and the exposure of seeds to severe salt stress resulted in the decrease of total dry matter in 34% and 41% compared to the NaCl-free treatment, respectively, for the seeds primed with water or  $\text{KNO}_3$ .

The root: shoot ratio is one of several ratios, which give estimates of dry matter partitioning into roots and shoots, and it is a good indicator for abiotic stress effects on root and shoot dry matter (Boutraa et al., 2010). The root to shoot ratio of cucumber seedlings was reduced with the rise of saline stress levels in all priming treatments (Figure 2F). An increase in the root to shoot ratio was expected to be a common response of plants to salt stress (Acosta-Motos et al., 2017). The greater root proportion under salt stress can favor the retention of toxic ions in this organ, controlling their translocation to the shoots. This response can constitute a typical mechanism of plant resistance or survival under saline conditions (Munns; Tester, 2008; Acosta-Motos et al., 2017). The results presented here suggest that the genotype of cucumber used in this study does not have the capacity to prioritize its allocation of photoassimilates to the roots in detriment to the aerial part and, thus, to improve its tolerances to saline stress. Assimilate partitioning is a complicated process that can be controlled simultaneously by sources and sinks (Taiz et al., 2017).

The length vigor index (SLVI) of cucumber seedlings ranged from 255 to 1,227 units and was drastically reduced with the rise of salt stress levels (Figure 3A). These results evidenced the harmful effect of salt stress on the elongation of cucumber seedlings, as reported by the lower length of the hypocotyl (Figure 2A) and primary root (Figure 2B). The priming treatments only had a significant effect under conditions of mild salt stress, and the greater length vigor index was obtained with the use of hydropriming and unprimed seeds (Figure 3A). On the other hand, the weight vigor index (SWVI) of cucumber seedlings ranged from 865 to 1,273 units and was little affected by salinity levels and priming treatment (Figure 3B). The lowest weight vigor indexes were obtained under severe stress condition with the use of hydropriming and unprimed seeds.

The seedling vigor index has been used as a tolerance index to evaluate the effect of salt stress on seedling growth (Ashkan; Jalal, 2013). Seedling vigor is a measure of the extent of damage that accumulates as viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die (Marcos-Filho, 2005). The lower seedling vigor index obtained under severe salt stress was due to the salinity inhibit the initial growth of seedlings. The reduction in vigor

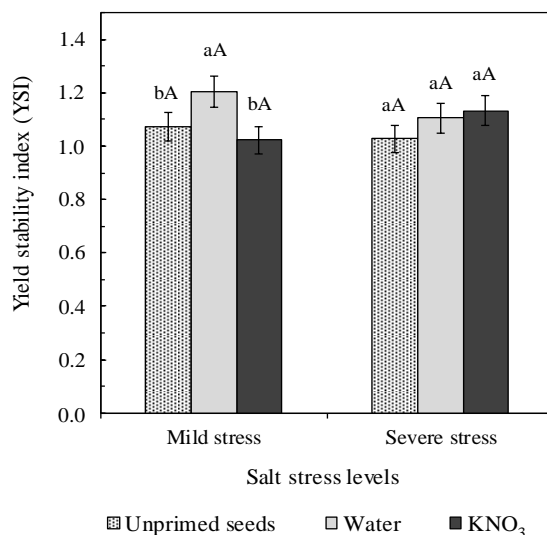
index of seedlings under salinity or water restriction conditions is usually reported by other research (Ashkan; Jalal, 2013; Singh et al., 2015; Oliveira; Steiner, 2017).



**Figure 3.** Effects of salt stress levels on length vigor index (A) and weight vigor index (B) of cucumber seedlings (*Cucumis sativus* L., cv. Aodai Melhorado) from seeds submitted to priming with water (hydropriming) or 0.5% KNO<sub>3</sub> solution (halopriming) and unprimed seeds. Bars followed by distinct lower-case letters, between the priming techniques or upper-case letters, for the salt stress levels, show significant difference (LSD test,  $p \leq 0.05$ ). Data refer to mean values ( $n = 4$ )  $\pm$  mean standard error. UEMS/Cassilândia-MS, 2018. Source: The authors.

The yield stability index of cucumber seedlings ranged from 1.02 to 1.20 and 1.03 to 1.13 for the seeds exposed to mild and severe saline stress, respectively (Figure 4). The yield stability index was suggested by Bouslama and Schapaugh (1984) and has been considered a good salt tolerance index. When yield stability index (YSI) in response to saline conditions is greater than 1.0 indicates that the plant genotype is tolerant to salt stress. These results confirm the assertions of (Oliveira; Steiner, 2017), which reported that the cultivar “Aodai Melhorado” is moderately tolerant to saline stress. Three main

salinity tolerance mechanisms have been proposed by (Munns and Tester, 2008): ion exclusion – the net exclusion of toxic ions from the shoot; tissue tolerance – the compartmentalization of toxic ions into specific tissues, cells and subcellular organelles; and shoot ion-independent tolerance – the maintenance of growth and water uptake independent of the extent of  $\text{Na}^+$  accumulation in the shoot.



**Figure 4.** Effects of salt stress levels on the yield stability index (YSI) of cucumber seedlings (*Cucumis sativus* L., cv. Aodai Melhorado) from seeds submitted to priming with water (hydropriming) or 0.5%  $\text{KNO}_3$  solution (halopriming) and unprimed seeds. Bars followed by distinct lower-case letters, between the priming techniques or upper-case letters, for the salt stress levels, show significant difference (LSD test,  $p \leq 0.05$ ). Data refer to mean values ( $n = 4$ )  $\pm$  mean standard error. UEMS/Cassilândia-MS, 2018. Source: The authors.

## FINAL CONSIDERATIONS

The seed priming with  $\text{KNO}_3$  (halopriming) may be successfully applied on cucumber seeds to improve germination performance in saline conditions, especially under severe salt stress. Under mild salt stress conditions, the hydropriming is enough to improve the germination rate of cucumber seeds. The priming of seeds with water or  $\text{KNO}_3$  had an insignificant effect on the initial growth and vigor of cucumber seedlings.

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## Respostas Ecofisiológicas de Plantas ao Lodo de Esgoto


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### INTRODUÇÃO

Inerente ao crescimento dos centros urbanos, o lodo de esgoto (LE) sanitário é derivado das atividades das empresas de tratamento de água e esgoto. Implica no resíduo gerado a partir do tratamento biológico de efluentes oriundos de esgotos domésticos, sendo acumulado temporariamente nas próprias instalações destas empresas, em diferentes tipos de tanques, até seu destino final, que, de modo geral, são os aterros sanitários.

Atualmente, um dos maiores passivos ambientais associados às populações humanas implica no resíduo proveniente do tratamento dos esgotos sanitários (Abreu et al., 2019). Este material é denominado, popularmente, como lodo de esgoto quando proveniente do tratamento final das estações de tratamento de esgoto (ETE), e biossólido quando apresenta características que permitem sua reutilização em meio agrícola (Andreoli et al., 2006; Nobrega et al., 2017).

O passivo ambiental representado pelo LE implica na necessidade crescente de alternativas para seu uso, como forma de redução do volume de descarte deste resíduo no meio ambiente. A descarga de LE compreende importante fonte de poluição ambiental (Li et al., 2018), além de constituir em custos para as empresas de saneamento quanto ao transporte e deposição de um volume crescente de resíduo sólido. Dados da produção anual média de LE em 16 países da União Europeia (UE), por exemplo, registraram valores em torno de 290 mil toneladas em 2017 (Tabela 1).

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