Pre-germination treatments with plant growth regulators and bioactivators attenuate salt stress in melon: effects on germination and seedling development

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ABSTRACT. The scarcity of surface water has led to the use of underground sources as an alternative for crop irrigation by farmers in semi-arid regions. However, these water sources generally have high salinity, which prevents agricultural production. The objective of this study was to determine the effects of pre-germination treatments with plant growth regulators and bioactivators on melon seeds to attenuate salt stress caused by irrigation water during germination and seedling development. Two trials were carried out separately with the hybrids, Goldex and Grand Prix. The design was completely randomized in a 4 × 3 factorial scheme (four seed treatments and three dilutions of irrigation water). Seeds were treated with salicylic acid and gibberellic acid and the insecticide, thiamethoxam, in addition to the control. Local supply water, artesian well groundwater, and dilution of these waters at a 1:1 ratio were employed for irrigation. Fourteen days after sowing, morphological and physiological analyses were performed, and the material was collected for biochemical determination. The use of saline well water affected the initial development of melon seedlings of the Goldex and Grand Prix hybrids. Pre-germination treatment of Goldex hybrid seeds with gibberellic acid was inefficient at mitigating salt stress. However, the effects of irrigation water salinity on Grand Prix melon seeds pretreated with salicylic acid and thiamethoxam were attenuated.

Keywords: Cucumis melo; Cucurbitaceae; salicylic acid; gibberellic acid; thiamethoxam; saline water.

Received on August 11, 2021.
Accepted on April 17, 2022.

Introduction

Melon (Cucumis melo L.) is a fruit appreciated on all continents, with a cultivated area of approximately 1.3 million hectares and an estimated production of 51 million tons (FAOSTAT, Retrieved on Dec. 15, 2019 from http://www.fao.org/). In Brazil, Rio Grande do Norte, Ceará, and Bahia, located in the semi-arid region of the Northeast, are the main states that produce this fruit (Kist, Carvalho, & Beling, 2021). In this region, semi-arid, the climate is characterized by high evapotranspiration rates and low rainfall, which are aggravated by irregular distribution throughout the year. Accordingly, the scarcity of surface water and the increase in soil salinity are recurrent, resulting in problems for agricultural crops (Bezerra, Araújo, Azevedo, Pereira Filho, & Lima, 2020).

Salinization of agricultural areas reduces the soil water potential and toxicity of ions, leading to stress (Nóbrega et al., 2020). Toxicity is a problem that occurs when certain ions, especially chlorine, sodium, and boron, even at low concentrations and mainly absorbed via the roots, accumulate in the leaves through transpiration, producing toxic effects in plants (Muscolo, Panuccio, & Heshel, 2013). This abiotic stress is even more harmful to the establishment of annual agricultural crops, highlighting the need for using technologies that promote and/or enhance the germination and establishment of seedlings in the field (Dourado et al., 2020).

Among the means to mitigate the harmful effects of abiotic stresses, the use of phytohormones, such as gibberellic acid and salicylic acid, which have been demonstrated to attenuate these stresses, has been highlighted. Stress effects were attenuated in beet and sesame seeds treated with salicylic acid and subjected to salinity and water restriction, respectively (Kandil, Sharief, Abido, & Awed, 2014; Silva et al., 2017). Similarly, the use of salicylic acid in the treatment of onion and pumpkin seeds promoted higher germination and initial development, even under salinity conditions (Guirra et al., 2020). These benefits can be explained...
by the mode of action of these regulators. Gibberellins act in the mobilization of reserves during germination and cell elongation, whereas salicylic acid is related to responses in stressful situations through secondary metabolism (Taiz, Zeiger, Moller, & Murphy, 2017).

In addition to gibberellic and salicylic acids, thiamethoxam, which is a systemic neonicotinoid insecticide, has been used to attenuate abiotic stress. In carrots, thiamethoxam promoted increments of up to 30% in the physiological performance and quality of seedlings (Almeida et al., 2014). The application of thiamethoxam also enabled the attenuation of the effects of abiotic stresses on rice (Grohs, Marchesan, Roso, & Moraes, 2016), cotton (Almeida et al., 2020), and millet (Cazarim et al., 2021). Therefore, this insecticide has potential as a promoter of germination and the retrieval of more vigorous seedlings and is considered even as a bioactivator (Macedo & Castro, 2011).

The objective of this study was to determine the effects of pre-germination treatments with plant growth regulators and bioactivators on melon seeds to attenuate salt stress induced by irrigation water during germination and seedling development.

**Material and methods**

**Location, experimental design, and water quality**

The study was conducted in two stages, according to the melon hybrid (Goldex and Grand Prix), in the laboratory and greenhouse environments of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, Rio Grande do Norte State, Brazil (5°20'31,65" S, 37°32'50,84" W; altitude of 16 m). The region presents a BSw‘h’, tropical semiarid hot climate, according to the Köppen classification. During the experiment, the mean temperature and relative humidity of the air inside and outside the greenhouse were 26°C and 60%, and 24.5°C and 55%, respectively, with 3 mm of rainfall.

The design used was completely randomized in a 4 × 3 factorial scheme corresponding to four seed treatments (control, salicylic acid, gibberellic acid, and thiamethoxam) and three water dilutions (local supply water, dilution of local supply water in 50% of well water, and well water), with four replicates of 25 seeds using the hybrids, Goldex (yellow) and Grand Prix (Piel de Sapo).

The saline groundwater used in the treatments was collected from an artesian well located on the central campus of UFERSA, Mossoró, Rio Grande do Norte State, Brazil. Accordingly, the water was differentiated as follows: W1 = urban supply water, W2 = 50% supply water + 50% saline well water, and W3 = saline well water. Water analysis was carried out at the Soil and Water Laboratory (UFERSA) (Table 1).

<table>
<thead>
<tr>
<th>Water dilutions</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>CO₃²⁻</th>
<th>HCO₃⁻</th>
<th>Cl⁻</th>
<th>CE 25°C</th>
<th>pH</th>
<th>SAR*</th>
<th>WQR**</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>0.9</td>
<td>0.4</td>
<td>3.3</td>
<td>0.2</td>
<td>0.6</td>
<td>3</td>
<td>2.8</td>
<td>0.55</td>
<td>8.4</td>
<td>4.2</td>
<td>C2S1</td>
</tr>
<tr>
<td>W2</td>
<td>10.2</td>
<td>11.4</td>
<td>12.0</td>
<td>0.5</td>
<td>0.8</td>
<td>4.1</td>
<td>19.0</td>
<td>3.2</td>
<td>8.2</td>
<td>3.7</td>
<td>C4S1</td>
</tr>
<tr>
<td>W3</td>
<td>18.5</td>
<td>20.6</td>
<td>21.9</td>
<td>0.7</td>
<td>1.4</td>
<td>5.2</td>
<td>6.4</td>
<td>5.3</td>
<td>8.1</td>
<td>13.0</td>
<td>C4S2</td>
</tr>
</tbody>
</table>

*W1 = supply water; W2 = 50% supply water + 50% well water; W3 = well water *Sodium adsorption ratio. **Water quality rating (Richards, 1954).

**Seed treatment and seedling production**

Seeds with water contents of 8% (Goldex) and 8.5% (Grand Prix) were stored in an air-conditioned environment (± 15°C and 60% relative humidity) for six months before the beginning of the experiment.

Initially, pre-tests were conducted to determine the appropriate dose for each regulator. Thus, the solutions of salicylic acid (50 μM) and gibberellic acid (50 mg L⁻¹) for hydrating the paper towel substrate were determined using the volume of solution equivalent to twice the mass of the dry substrate. The seeds were placed in a paper towel roll for 20h in a germination chamber at 25°C. This period was determined based on the seed imbibition curve performed during the pretest.

In the treatment with thiamethoxam, 1 mL of Cruiser 350® was diluted in 8 mL of distilled water, sufficient for the treatment of 1 kg of seeds. This mixture was kept in contact with the seeds for 30 min. to promote greater adhesion of the product to its surface, according to the recommendations of Syngenta®. Seeds in the control group were not treated.

Sowing was performed in polystyrene trays with 200 cells (18 cm³) randomly arranged and filled with the commercial substrate, Carolina Soil®. Irrigation was performed daily with 2.5 L of the water evaluated for each tray for 14 days.

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Silva et al.
Physiological and biometric analyses

During the evaluation period, daily counts of emerged seedlings (exposed hypocotyl) were performed until 14 days after sowing. Accordingly, the emergence speed index (Krzyzanowski, França-Neto, Gomes-Junior, & Nakagawa, 2020) and the percentage of emerged seedlings were obtained.

Shoot length was measured by considering the part between the insertion of the root and that of cotyledons; however, for root length, the main root was considered. This evaluation was performed using 10 random seedlings per replicate, 14 days after sowing, with a millimeter ruler.

The plant parts used to evaluate seedling length were placed in a paper bag and dried in a forced circulation oven at 65°C for 72h to determine the dry mass on a precision scale (0.001 g) (Krzyzanowski et al., 2020).

Organic solutes

Total soluble sugars, total amino acids, and proline were quantified using samples of 0.2 g of fresh tissue from the shoots of normal seedlings. These samples were automatically macerated in hermetically sealed tubes containing 5 mL of 80% ethanol. Subsequently, the tubes were kept in a water bath at 60°C for 20 min. Thereafter, the samples were centrifuged at 10,000 rpm for 8 min. at 4°C, and the supernatant was collected.

The total soluble sugars were determined using the anthrone method (Yemm & Willis, 1954), and the results are expressed in mg·g⁻¹ of fresh mass. Total amino acids were analyzed by the ninhydrin method (Yemm & Cocking, 1955), and the results are expressed in μmol g⁻¹ of fresh mass. Proline was determined using the methodology proposed by Bates, Waldren, and Teare (1973), and the results are expressed in μmol g⁻¹ of fresh mass.

Statistical analysis

The collected data were subjected to analysis of variance (p ≤ 0.05). If significance was found, the means were subjected to the Scott-Knott test. The statistical program, SISVAR®, was used (Ferreira, 2011).

Results and discussion

Physiological and biometric analyses

The interaction between the factors (water dilution × seed treatment) was only significant (p ≤ 0.05) for the variables of shoot length and root length for the Goldex hybrid. In this hybrid, total amino acids, shoot dry mass, and root dry mass only displayed differences for the water factor. The emergence speed index and total soluble sugars showed differences between the two factors separately. In addition, for Goldex, seedling emergence and proline showed no differences induced by the single factors. As a result, the data were not presented.

An interaction between factors for emergence speed index and seedling emergence was found for the Grand Prix hybrid. Further, no interaction was found between the factors (p ≤ 0.05) for shoot dry mass; however, there was a significant difference in the water factor. The root dry mass and proline content showed differences caused by both factors. Further, there was no interaction between total soluble sugars and the factors for this hybrid, and no differences were caused by these factors individually.

The highest emergence speed index for seedlings of the Goldex hybrid as a function of irrigation water was obtained in the treatment with artesian well water (W3), which was 6% higher than that obtained in treatments with supply water (W1) and dilution of artesian well water (W2), respectively (Figure 1a). Regarding seed treatments, gibberellic acid promoted the best emergence speed index, which was 30% higher than that of the control (Figure 1b).

Increments in the speed of emergence and establishment of seedlings in the field are positive factors, especially under stressful conditions. These results are closely linked to the mode of action of gibberellic acid, which is responsible for the onset of germination and cell elongation (Paixão et al., 2021). In addition, this regulator can act as a signal for the synthesis of hydrolytic enzymes, which are responsible for mobilizing seed reserves that directly influences the growth and development of the embryonic axis (Tsegay & Andargie, 2018). As observed in this study with Goldex melon seeds, treatment with gibberellic acid promoted positive results in alfalfa seeds (Younesi & Moradi, 2014) and beet (Kandil, Sharief, Abido, & Awed, 2014), both under salt stress (200 mM NaCl).

The use of saline well water hampered the emergence of Grand Prix melon seedlings (Figure 2a). Nevertheless, treatment with salicylic acid and thiamethoxam promoted a higher percentage of seedling emergence (108 and 68%, respectively, compared to the control), when irrigated with water of higher salinity (W3).
Salicylic acid promoted a higher emergence speed index for seedlings of the hybrid Grand Prix compared to the other treatments. However, when thiamethoxam, supply water (W1), and water diluted to 50% (W2) were used, the seedlings showed similar emergence speed indices (4.5). Treatment with salicylic acid and saline well water (W3) resulted in a speed index of 7.6, a value four-fold higher than that obtained with the control and the same irrigation water (Figure 2b).

Figure 1. Emergence speed index of the melon seedling, Goldex hybrid, irrigated with well water dilutions (a) and subjected to different seed treatments (b). W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. *Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05).

Figure 2. Emergence (a), emergence speed index (b) of the melon seedling, Grand Prix hybrid, irrigated with well water dilutions and subjected to different seed treatments. W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. *Averages followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05). ¯Means followed by the same uppercase letter do not differ in the seed treatment factor by the Scott-Knott test (p ≤ 0.05).

Salinity can cause ionic toxicity, resulting in delays in emergence, mobilization of seed reserves, and even reduction in seed viability (Nóbrega et al., 2020). Ionic toxicity may also be induced as irrigation water comprises sodium, calcium, and magnesium salts, with a higher proportion of the first element, which results in its accumulation (Muscolo et al., 2013). Sodium in irrigation water is associated with plant toxicity. However, the interaction of salicylic acid with salinity can induce the activation of stress-resistance genes and increase seedling emergence (Jini & Joseph, 2017). Such occurrence may be related to the participation of this acid in plant defense metabolism and the synthesis of gibberellic acid, thereby acting directly on seedling emergence (Anaya, Fghire, Wahbi, & Loutfi, 2018). The beneficial action of salicylic acid was verified by the speed of seedling establishment in species, such as sesame, under water deficit (Silva et al., 2017) and melon seedlings subjected to thermal stress (Kaur & Gupta, 2017).

The use of saline well water (W3) negatively affected the shoot length of Goldex melon seedlings (Figure 3a). Similarly, treatments with gibberellic acid and thiamethoxam in W2 and W3 hampered this variable. However, treatment with salicylic acid and irrigation with W2 resulted in seedlings with longer shoot length
than treatment with the other waters, with increments of 50% for those irrigated with supply water and 73% for those irrigated with saline well water. These results were similar for plants administered the control and better than those obtained with gibberellic acid and thiamethoxam under the same water conditions (Figure 3a).

The mitigation of the deleterious effects resulting from salinity is related to the production of secondary compounds and the metabolic pathways of plant defense (Gomes, Assis, Alves, & Reis, 2018). The correlated action of salicylic acid has been verified in barley (Habibi, 2012) and pepper (Prabha & Kumar, 2014) under water stress.

Figure 3. Shoot length (a) and root length (b) of the melon seedling, Goldex hybrid, irrigated with well water dilutions and subjected to different seed treatments. W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. ¹Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05). ²Means followed by the same uppercase letter do not differ in the seed treatment factor by the Scott-Knott test (p ≤ 0.05).

An effect similar to that obtained for shoot length was found for the root length of Goldex melon seedlings, with growth reduction under salinity conditions and without seed treatment. However, treatment with gibberellic acid prevented negative effects on root length compared to the results obtained with the other waters. The use of salicylic acid and thiamethoxam led to higher root growth (approximately 13 cm) with diluted water (W2). Further, compared to the other waters, root growth was 30% higher for those subjected to supply water and 18% higher for those subjected to saline well water (W3) (Figure 3b).

Shoot length was affected in seedlings of the Grand Prix hybrid irrigated with artesian well saline water (W3), except for those treated with gibberellic acid. When treated with this acid, even under W1 water, shoot length was reduced (10% compared to the control), with no difference in the water within this treatment. When the use of W2 water was considered, no differences were found between the control, salicylic acid, and thiamethoxam. Under the condition of maximum salinity (W3), treatments with salicylic acid and thiamethoxam increased shoot length by 46 and 38%, respectively, compared to the control (Figure 4a).

Figure 4. Shoot length (a) and root length (b) of the melon seedling, Grand Prix hybrid, irrigated with well water dilutions and subjected to different seed treatments. W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. ¹Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05). ²Means followed by the same uppercase letter do not differ in the seed treatment factor by the Scott-Knott test (p ≤ 0.05).
Salinity directly affects the development of seedling shoots due to the osmotic stress of the water and the toxic effects of ions, which interfere with cell multiplication and division, hampering growth (Lima et al., 2015). However, in rice seedlings under saline conditions, treatment with gibberellic acid resulted in an increase in seedling length (Chunthaburee, Sanitchon, Pattanagul, & Theerakulpisut, 2014). The exogenous inclusion of this hormone may also be decisive in the process of cellular elongation for the Grand Prix hybrid.

When W2 water was used, treatment with thiamethoxam favored root length, outperforming the others by approximately 60%. With the use of saline well water (W3), treatment with salicylic acid and thiamethoxam promoted greater root length (Figure 4b). In saline substrates, the metabolism of seedlings is stimulated to allow root growth to overcome the effects of this stress, mainly the osmotic effect (Taiz et al., 2017). Thus, when dilution was carried out (W2), salinity promoted an eustress, as it stimulated the metabolism and physiological activity of the seedlings in a positive manner (Lichtenthaler, 2004). Thiamethoxam can be considered a plant bioactivator even under salinity conditions; this is because it promoted greater root growth under W2 and W3 than the control. This bioactivator function was also verified under stress-free conditions by Lemes et al. (2015), who identified better development of pumpkin seedlings from seeds treated with this product.

Seedlings of the Goldex hybrid had shoot and root dry mass accumulation hampered by the salinity of artesian well water (W3). Shoot dry mass and root dry mass were reduced by 17 and 23%, respectively, compared to those obtained with supply water (Figure 5).

![Figure 5. Shoot dry mass (a) and root dry mass (b) of the melon seedling, Goldex hybrid, irrigated with well water dilutions. W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. *Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05).](image)

The shoot dry mass of Grand Prix melon seedlings was negatively affected by the use of water of higher salinity (W3) (Figure 6). These results follow the basic principle of the action of the salt that hampers dry mass accumulation; this is due to the osmotic and toxic effects of the salts in the solution, which cause metabolic and physiological changes, resulting in a decrease in biomass accumulation (Lima et al., 2015).

![Figure 6. Shoot dry mass of the melon seedling, Grand Prix hybrid, irrigated with dilutions of well water. W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. *Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05).](image)

In relation to the root dry mass of Grand Prix hybrid seedlings, a higher biomass accumulation was obtained when the mixture of supply water with saline well water (W2) was employed (Figure 7a). Further,
Mitigation of salt stress in melon

Treatment with salicylic acid and thiamethoxam promoted greater accumulation of root dry mass (60.8 mg) compared to the other treatments (Figure 7b). These results demonstrate that the growth of roots of seedlings of this hybrid was not only obtained by cell elongation, but also by the division and biomass accumulation, which are effects related to the mode of action of salicylic acid and thiamethoxam (Grohs et al., 2016). Such greater accumulation of root dry mass has been verified in barley (Habibi, 2012) and pepper (Prabha & Kumar, 2014) treated with salicylic acid and subjected to water stress.

In general, the physiological and biometric results of melon seedlings were favored by the use of salicylic acid and thiamethoxam. Thus, to better elucidate the behavior of Goldex and Grand Prix hybrids and changes in plant metabolism of this species under salt stress, biochemical analyses were performed.

**Organic solutes**

In Goldex melon seedlings, the accumulation of total amino acids and soluble sugars occurred as a function of the water used, with the highest concentrations of these substances obtained under conditions of greater salinity (Figure 8). Generally, to carry out osmoregulation or osmotic adjustment, the seedling accumulates biomolecules of sugars and proteins (Khan, Fatma, Per, Anjum, & Khan, 2015).

The greater accumulation of sugars and amino acids in seedlings grown under higher salinity is due to the metabolic function of these substances in the cellular osmotic adjustment, which accumulate in the cytosol to reduce the water potential of the cell. Thus, the difference in water potential allows the cell to maintain turgor pressure and tissue hydration, even under osmotic stress conditions. In addition, sugars may have a signaling function in the osmotic adjustment process (Singh & Gautam, 2013).
In the present study, no significant differences in proline content were observed, although this metabolite is considered one of the main agents acting in osmotic adjustment, membrane protection, and protein stabilizers (Iqbal, Umar, Khan, & Iqbal, 2014). Such finding indicates that proline in Goldex hybrid melon seedlings did not act as the main osmoprotectant, as there are other molecules responsible for this process, such as glycine, betaine, mannitol, and trehalose sugar, with citrulline as the main osmoprotectant (Song, Joshi, Dipiazza, & Joshi, 2020).

Grand Prix melon seedlings treated with gibberellic acid and irrigated with W3 water had greater accumulation of total amino acids (Figure 9a). This result may be related to the role of gibberellic acid in the metabolism of total amino acids, and its subsequent role in the metabolism of osmotic protection under salinity conditions. Herein, the use of groundwater, regardless of dilution, was found to promote seedlings with a higher concentration of proline (Figure 9b).

Figure 9. Total amino acids (a) and proline (b) content of the melon seedling, Grand Prix hybrid, irrigated with well water dilutions and subjected to different seed treatments. Seed treatment: W1 = urban supply water; W2 = 50% urban supply water + 50% well water; W3 = well water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. ¹Means followed by the same lowercase letter do not differ in the water factor by the Scott-Knott test (p ≤ 0.05). ²Means followed by the same uppercase letter do not differ in the seed treatment factor by the Scott-Knott test (p ≤ 0.05).

The highest values of proline were found in Grand Prix melon seedlings under higher salt concentrations (W2 and W3), indicating that under these conditions, this metabolite is used in cellular osmotic adjustment. However, proline accumulation may not be an indication that this metabolite is used by cells for osmotic adjustment. This increase may be the product of metabolic disorders caused by stress, as its synthesis still depends on the gene expression of the material studied and the integrity of the glutamate pathway (Nelson & Cox, 2019).

The use of saline groundwater can harm agricultural production, especially during the initial development of plants (Torres et al., 2014). In this study, salinity was found to affect the emergence and growth of melon seedlings in both hybrids. However, the use of regulators in seed treatment can mitigate the effects of stress during the initial stages of seedling development. In the Goldex hybrid, this effect was smaller according to the results obtained. However, the use of gibberellic acid increased the emergence speed index, while salicylic acid and thiamethoxam promoted greater root length. The treatment of Grand Prix melon seeds with salicylic acid and thiamethoxam mitigated the effects of salt stress caused by irrigation water.

Conclusion

The use of saline well water (W3 = 5.3 dS·m⁻¹) affects the initial development of melon seedlings of Goldex and Grand Prix hybrids. Further, pre-germination treatment of Goldex melon seeds with gibberellic acid is inefficient at mitigating salt stress. The effects of irrigation water salinity on Grand Prix melon seeds pretreated with salicylic acid and thiamethoxam were attenuated. Thus, salicylic acid and thiamethoxam can be used to treat Grand Prix melon seeds to obtain seedlings in saline water.

References


