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RESEARCH PAPER

# Does azelaic acid priming increase the germination ability of cucumber (*Cucumis sativus* L.) seeds under salt stress?

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## Abstract

The aim of this study was to evaluate the effects of Azelaic Acid (AzA) pretreatment on germination of cucumber seeds under salt stress conditions. The experiment was conducted according to a complete randomized experimental design with five different salinity levels (0 mM NaCl; (Control: distilled water), 20 mM, 40 mM, 60 mM and 80 mM NaCl) and 3 different doses of AzA (0 mM, 0.25 mM, 0.5 mM) with 3 replicates. Cucumber variety Beith Alpha was used as plant material. Germination and growth parameters (germination rate, radicle length, wet weight, dry weight, salt tolerance index, root length, shoot length) were determined. According to the results of analysis of variance, the effect of AzA pretreatment on germination rate, salt tolerance index and shoot length of cucumber was found to be insignificant, while it affected radicle length, wet weight, dry weight and root length ($p \leq 0.01$, $\leq 0.05$). NaCl levels significantly affected all parameters except germination rate and dry weight. When AzA × NaCl effects were evaluated together, salt tolerance index was affected at different levels of significance, while there was no significant effect on other parameters. AzA pretreatment at the dose of 0.25 mM was significant for many parameters. Consequently, under salt stress conditions, we can say that AzA, when used at appropriate doses, has a positive effect on germination ability. For various plants grown in areas experiencing salinity problems, priming or foliar application is recommended to determine the role of AzA in stress physiology.

## Introduction

Salinity stress is one of the most significant abiotic stress factors affecting agricultural land, and it is reported to negatively impact approximately 932 million hectares of agricultural land (Er et al., 2021). It is estimated that approximately 50% of the world's arable land will be affected by this negative stress factor by 2050 (Bartels and Sunkar, 2005). Salt stress limits plant growth by increasing soil osmotic pressure, specific ion toxicity, and irregularity in nutrient uptake (Läuchli and Epstein, 1990). Increasing salt concentration in the rhizosphere creates osmotic stress and inhibits water uptake by plants. This condition, referred to as "physiological drought," threatens agricultural sustainability (Yavuz et al., 2022). Salt stress affects plant physiology and biochemistry, causing a significant decrease in crop yield (Ostaci, 2024). In salinity conditions, the osmotic stress observed is caused by an imbalance in the availability of water around plant roots, high concentrations of soluble substances, which significantly limits plant growth and biomass yield (Slabu, 2009). In plants under salt stress, reduced cell extension and stomatal closure are among the first signs of stress (Lindberg and Premkumar, 2023). The second stage of salt stress is when the ion concentrations accumulated in plants increase to toxic levels. Accumulated sodium ($Na^+$) and chloride ($Cl^-$) cause harmful effects on metabolism, depending on the type of plant and its stage of development (Munns, 2011). Salt tolerance varies among cucurbits on a scale ranging from sensitive tolerance to moderate tolerance (Naseer et al., 2022) and it has been reported that the effect of salinity during the germination stage is more harmful than during other stages of product growth (Irik and Bikmaz, 2024). Melons, which belong to the Cucurbitaceae family, are considered to be moderately tolerant to salinity, but it has been reported that they are significantly affected by the salt factor during germination, shooting, and early seedling stages (Huang et al., 2012; Pinheiro et al., 2019).

Cucumber (*Cucumis sativus* L.) is one of the most important vegetables worldwide and belongs to the Cucurbitaceae family. Its shallow roots are highly sensitive to salt stress, which causes a decrease in growth and yield (Wang et al., 2021). Like other salt-sensitive plants, salt stress in cucumbers can cause reduced germination, root growth, and water uptake, and in extreme conditions, plant death (Liu et al., 2021). The assessment of salinity effects can be an important marker in researching the salt tolerance of cucumber varieties.

Various techniques have been researched for years to improve the adaptability of seeds during germination and increase their tolerance to salinity stress conditions. One of these is the seed priming technique. Seed priming involves exposing seeds to chemicals or stress factors in advance. This process increases the plant's resistance and ability to detect stress signals (Borges et al., 2014). Many compounds are being studied for their efficiency in increasing the applicability of these and similar anti-stress techniques.

Azelaic acid (AzA), which is commonly used in medicine, cosmetics, and pharmacology, is a compound that has been tested in plants under normal conditions and biotic stress (pathogen) conditions (Yu et al., 2013; Cecchini et al., 2019). In a study, it has been reported that AzA is already in the plant's biochemical cycle (Jung et al., 2009). This molecule, known to accumulate in the root system (Mukhtarova et al., 2011), is a preparatory molecule in the resistance mechanism of plants. In addition, AzA, one of the components that make up the signal transduction pathway, is reported to contribute to the rapid accumulation of salicylic acid (SA) in cases of infection or oxidation. (Dinler and Cetinkaya, 2024). Haghighi and Sheibanirad (2018); applied 0, 8, 10, and 24 mg $l^{-1}$ azelaic acid exogenously to tomato plants under salinity conditions of 0, 100, 150, and 200 mM, and found that, particularly at 8 mg $l^{-1}$ AzA dose-maintained gas exchange capacity at an optimal level up to 100 mM salinity, induced osmotic balance, and reduced the effects of salinity. In a different study, the effect of priming corn seeds with AzA under salt stress conditions on germination and early seedling development was investigated, and it was concluded that 1 mM AzA applied to seeds could regulate water uptake, particularly under low and medium salinity conditions, and increase total biomass (Güleç et al., 2025). Despite these noteworthy results, studies on the effects of AzA against abiotic stress factors are rare.

In this context, this study aimed to evaluate the effects of AzA priming on the germination of cucumber seeds under salinity stress conditions, which is an important abiotic stress factor.

## Materials and Methods

In the study, three different AzA doses (0 mM, 0.25 mM, 0.5 mM) and five different salt (NaCl) levels (0; control, 20, 40, 60, and 80 mM) were used for the cucumber variety Beith Alpha. Cucumber seeds were soaked in AzA solutions of different concentrations using 0 mM, 0.25 mM, 0.5 mM doses. The seeds were then dried at +25 °C for 12 hours. After pretreatment, the seeds were sterilized in a 0.5% (v/v) sodium hypochlorite solution for 10 minutes and then rinsed three times with distilled water. Sterilized seeds were placed on double-layered Whatman filter paper in petri dishes (90 mm x 15 mm). Ten seeds were placed in each Petri dish. NaCl solutions prepared in five different concentrations were applied to each Petri dish at a rate of 10 ml. In the study, the number of germinated seeds was counted at the same time every day. Until the end of the study ($10^{th}$ day), NaCl solutions were added to the seeds in the petri dishes every 48 hours (depending on the humidity conditions). At the end of the $24^{th}$ hour of the experiment, the germination rate (GR) on plants randomly selected from each treatment (Pour et al., 2021), and radicle length (RL), fresh weight (FW), dry weight (DW), and salt tolerance index (STI) (Kusvuran et al., 2015) values were determined at the end of the $48^{th}$ hour. Afterwards, the root lengths (RL) and shoot lengths (SL) of the plants grown until the $10^{th}$ day were measured at the end of the $10^{th}$ day. The effect of three different AzA priming doses on cucumber seeds under five different NaCl concentration conditions was analyzed according to a randomized design. Differences between the means of variation sources in terms of germination and seedling growth characteristics were determined using Duncan's multiple comparison test at the 5% significance level.

## Results

According to the variance analysis results in Table 1, AzA and NaCl treatments significantly affected the mean germination rate (GR) at 24 hours, while the effect of the AzA X NaCl interaction was insignificant. In terms of AzA doses, the highest mean germination rate (82.67%) was obtained with the 0.25 mM AzA priming treatment, which was 35.5% higher than the control at 24 hours. This was followed by 0.5 mM AzA priming with 68.67%, and the lowest mean germination rate was obtained from the control group with 53.33% (Table 1). The improving effect of the 0.25 mM AzA dose on the mean germination rates is shown in Figure 1a. When examined in terms of NaCl treatments, a decrease in germination rates occurred due to the increase in salt stress (Figure 1b). The highest mean germination rate was obtained from the control group (0 mM NaCl) at 86.67%, while the lowest value was obtained from the 80 mM NaCl stress level at 37.78%, representing a 56.4% decrease (Table 1).

Although the interaction between AzA and NaCl didn't show a statistically significant effect on the germination rate, significant differences were observed when the treatments were evaluated separately. Accordingly, the highest germination rate was 100% in the 0.25 mM AzA and 0 mM NaCl interaction, while the lowest mean was 30% in the 0 mM AzA (Control) and 80 mM NaCl interaction (Table 1).

According to measurements taken at the end of 48 hours, the effect of AzA priming and NaCl stress treatments on radicle length (RL) means was significant ($p \leq 0.001$), while the effect of AzA X NaCl interaction was found to be insignificant (Table 1). In terms of AzA priming doses, the mean radicle lengths were found to be 22.48% and 16.99% higher in the 0.25 mM AzA (3.78 mm) and 0.5 mM AzA (3.53 mm) treatments, respectively, compared to the control (2.93 mm) (Table 1; Figure 2a). Increases in stress (NaCl) levels pressured mean radicle lengths (Figure 2b). The highest mean radicle length was 4.29 mm in the control group without salt treatment, while the lowest mean radicle length was 2.67 mm in the 80 mM NaCl treatment (Table 1).

**Table 1.** Mean values and importance groups for the parameters examined

| **Treatments** | **GR**[1] (24 h) | **RL** (48 h) | **FW** (48 h) | **DW** | **STI** | **RL** | **SL** |
|---|---|---|---|---|---|---|---|
| **Priming (P)** | | | | | | | |
| 0 mM AzA (Control) | 53.33$^{c}$ | 2.93$^{b}$ | 0.077$^{b}$ | 0.026$^{b}$ | 64.51 | 6.37$^{b}$ | 5.85 |
| 0.25 mM AzA | 82.67$^{a}$ | 3.78$^{a}$ | 0.094$^{a}$ | 0.029$^{a}$ | 70.98 | 7.67$^{a}$ | 6.58 |
| 0.5 mM AzA | 68.67$^{b}$ | 3.53$^{a}$ | 0.081$^{b}$ | 0.026$^{b}$ | 70.61 | 5.96$^{b}$ | 5.53 |
| **NaCl (S)** | | | | | | | |
| 0 mM (Control) | 86.67$^{a}$ | 4.29$^{a}$ | 0.095$^{a}$ | 0.028 | 0.00 | 9.63$^{a}$ | 8.39$^{a}$ |
| 20 mM | 78.89$^{ab}$ | 3.80$^{b}$ | 0.088$^{b}$ | 0.028 | 93.27$^{a}$ | 7.22$^{b}$ | 6.30$^{b}$ |
| 40 mM | 72.22$^{bc}$ | 3.40$^{c}$ | 0.088$^{b}$ | 0.027 | 92.51$^{b}$ | 6.31$^{bc}$ | 6.92$^{b}$ |
| 60 mM | 65.56$^{c}$ | 2.90$^{d}$ | 0.078$^{c}$ | 0.027 | 82.67$^{c}$ | 4.99$^{d}$ | 4.53$^{c}$ |
| 80 mM | 37.78$^{d}$ | 2.67$^{d}$ | 0.071$^{d}$ | 0.025 | 75.05$^{d}$ | 5.17$^{d}$ | 3.79$^{c}$ |
| **P × S** | | | | | | | |
| 0 mM × 0 mM | 86.67 | 4.13 | 0.091 | 0.026 | 0.00 | 8.20 | 8.10 |
| 0 mM × 20 mM | 60.00 | 2.97 | 0.078 | 0.028 | 86.27$^{e}$ | 7.47 | 6.20 |
| 0 mM × 40 mM | 50.00 | 2.81 | 0.076 | 0.028 | 83.82$^{g}$ | 5.57 | 7.30 |
| 0 mM × 60 mM | 40.00 | 2.48 | 0.075 | 0.026 | 82.97$^{h}$ | 5.40 | 4.17 |
| 0 mM × 80 mM | 30.00 | 2.24 | 0.063 | 0.021 | 69.49$^{k}$ | 5.20 | 3.47 |
| 0.25 mM × 0 mM | 100.00 | 4.54 | 0.104 | 0.031 | 0.00 | 9.90 | 8.30 |
| 0.25 mM × 20 mM | 93.33 | 4.29 | 0.100 | 0.030 | 96.14$^{c}$ | 7.57 | 6.37 |
| 0.25 mM × 40 mM | 90.00 | 3.74 | 0.103 | 0.029 | 99.79$^{a}$ | 8.83 | 7.73 |
| 0.25 mM × 60 mM | 83.33 | 3.28 | 0.084 | 0.026 | 80.81$^{l}$ | 5.80 | 5.77 |
| 0.25 mM × 80 mM | 46.67 | 3.03 | 0.081 | 0.028 | 78.14$^{i}$ | 6.23 | 4.73 |
| 0.5 mM × 0 mM | 73.33 | 4.18 | 0.089 | 0.026 | 0.00 | 10.80 | 8.77 |
| 0.5 mM × 20 mM | 83.33 | 4.16 | 0.087 | 0.025 | 97.39$^{b}$ | 6.63 | 6.33 |
| 0.5 mM × 40 mM | 76.67 | 3.63 | 0.084 | 0.025 | 93.91$^{d}$ | 4.53 | 5.73 |
| 0.5 mM × 60 mM | 73.33 | 2.94 | 0.075 | 0.027 | 84.22$^{f}$ | 3.77 | 3.67 |
| 0.5 mM × 80 mM | 36.67 | 2.73 | 0.069 | 0.025 | 77.52$^{j}$ | 4.07 | 3.17 |
| **Sources of Variance** | | | | | | | |
| F value (P) | 23.063$^{**}$[2] | 22.660$^{**}$ | 33.988$^{**}$ | 5.948$^{**}$ | 2.826 | 5.031$^{**}$ | 2.987 |
| F value (S) | 22.563$^{**}$ | 30.676$^{**}$ | 20.836$^{**}$ | 1.676 | 45.199$^{**}$ | 13.581$^{**}$ | 21.251$^{**}$ |
| F value (P × S) | 2.111 | 1.203 | 1.227 | 1.359 | 3.639$^{**}$ | 1.711 | 0.871 |

[1]: GR (%): germination rate (24$^{th}$ hours); RL (cm): radicle length (48$^{th}$ hours); FW (g): fresh weight (48$^{th}$ hours); DW (g): dry weight; STI (%): salt tolerance index; RL (cm): root length (10 days); SL (cm): shoot length (10$^{th}$ days).

[2]: $^{*}p < 0.05$, $^{**}p < 0.01$. Different lowercase letters within a column or row indicate significant differences at the 0.05 level according to Duncan's multiple range test.

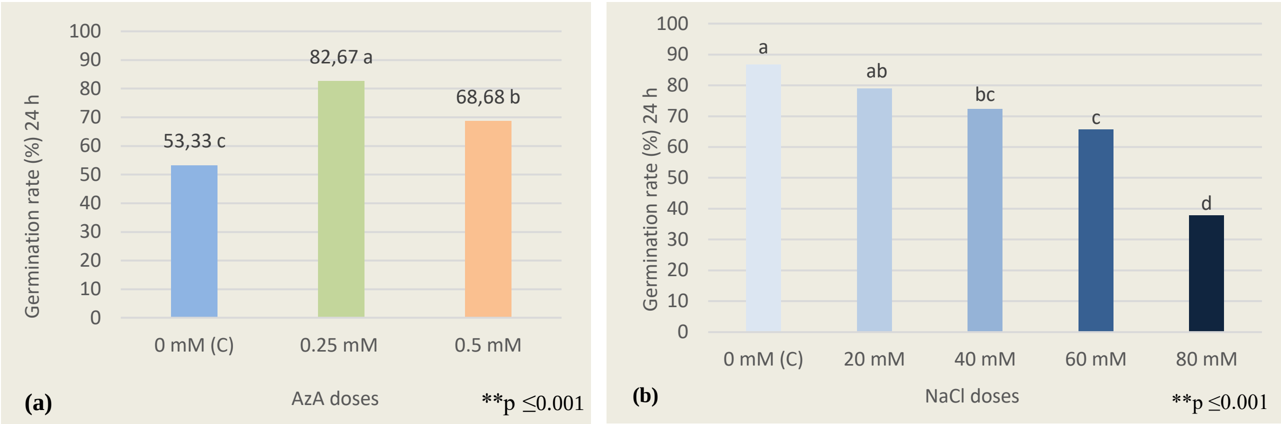


**Figure 1.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean germination rate (GR) values

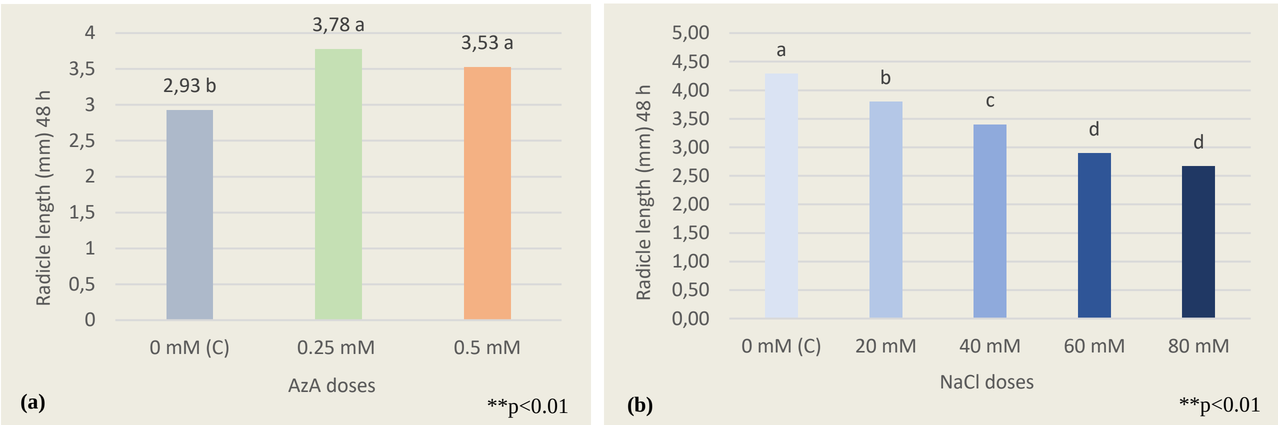


**Figure 2.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean radicle length (RL) values

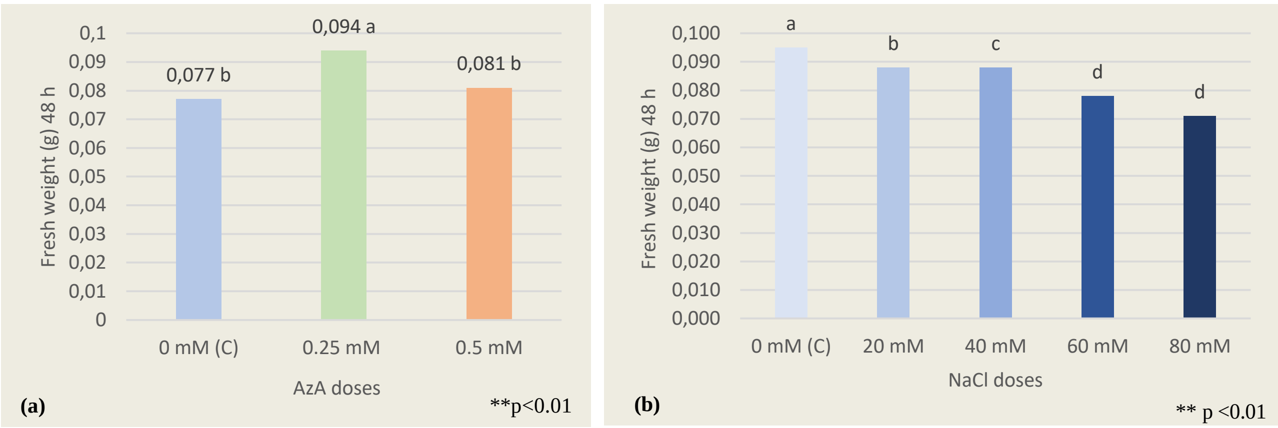


**Figure 3.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean fresh weight (FW) values

The effect of AzA and NaCl treatments on fresh weights was significant ($p < 0.01$), while the effect of the interaction between AzA and salt stress was insignificant (Table 1). The mean maximum fresh weight at 48 hours was 0.094 g from the 0.25 mM AzA dose, and the mean minimum fresh weight was 0.077 g from the control (0 mM AzA) group (Table 1; Figure 3a). When means were examined in terms of NaCl doses, it was observed that fresh weights decreased with increasing stress (Figure 3b). The highest mean fresh weight was 0.095 g in the control group treated with 0 mM NaCl, while the lowest mean fresh weight was observed at the 80 mM NaCl dose (0.071 g), which was 25.26% lower (Table 1).

Analysis of variance showed that dry weights (DW) were significantly affected by AzA priming treatments. However, NaCl doses and AzA X NaCl interaction had no significant effect on dry weights. The means for AzA treatments showed that the highest dry weight was obtained from the 0.25 mM AzA dose (0.029 g), which was 10.34% higher than the control, while the lowest dry weights were obtained from the control (0 mM AzA) and 0.5 mM AzA doses, which shared the same significance group (Table 1).

The salt tolerance index (STI) was significantly affected by NaCl doses and AzA X NaCl interaction, while the effect of AzA doses on STI was insignificant (Table 1). Although AzA priming treatments did not show a statistically significant effect, they revealed noticeable differences in salt tolerance index means. The highest STI mean was observed in the 0.25 mM AzA priming treatment, which differed by 9.12% from the control, followed by the 0.5 mM AzA priming treatment, which differed by 8.64% from the control. The lowest STI mean was obtained from the control group without AzA treatment (64.51%) (Figure 4a). Increases in NaCl stress have reduced the mean salt tolerance indices (Figure 4b). The highest tolerance index was determined to be 93.27% at 20 mM salinity stress, while the lowest tolerance index was 75.05% at 80 mM NaCl stress level (Table 1). In the interactions between AzA and salinity stress, the highest salt tolerance index was observed at the 0.25 mM AzA × 40 mM NaCl interaction (99.79%), which provided 16.00% higher tolerance compared to the control group where azelaic acid was not applied and 40 mM salinity stress was applied. The lowest tolerance to salinity stress was observed at 69.49% in the 0 mM AzA and 80 mM NaCl interaction (Table 1; Figure 5).

Root lengths were significantly affected by AzA and salt stress, while the effect of AzA and NaCl interaction on root lengths was found to be insignificant (Table 1). In terms of azelaic acid, the highest mean root length value was obtained from the 0.25 mM AzA priming treatment, which was 16.9% higher than the control. In contrast, the 0.5 mM AzA treatment shared the same significance group as the control (Figure 6a). When salt stress means were examined, the highest root length value of 9.63 cm was observed in the control group without salt treatment, while the lowest values were detected at 4.99 cm and 5.17 cm, respectively, in the 60 mM and 80 mM NaCl doses, which belonged to the same significance group (Table 1; Figure 6b).

According to the results of the variance analysis for shoot length (SL), no significant interaction was observed between AzA and AzA x NaCl (Table 1). However, the 0.25 mM AzA dose showed an 11.1% difference in shoot length compared to the control group without AzA treatment (Figure 7a). Shoot length means were significantly affected by salt stress conditions (Table 1). The highest mean shoot length was observed in the 0 mM NaCl (control) group, while the lowest mean shoot length was observed at 60 and 80 mM NaCl doses, which were approximately 50% lower than the control (Figure 7b).

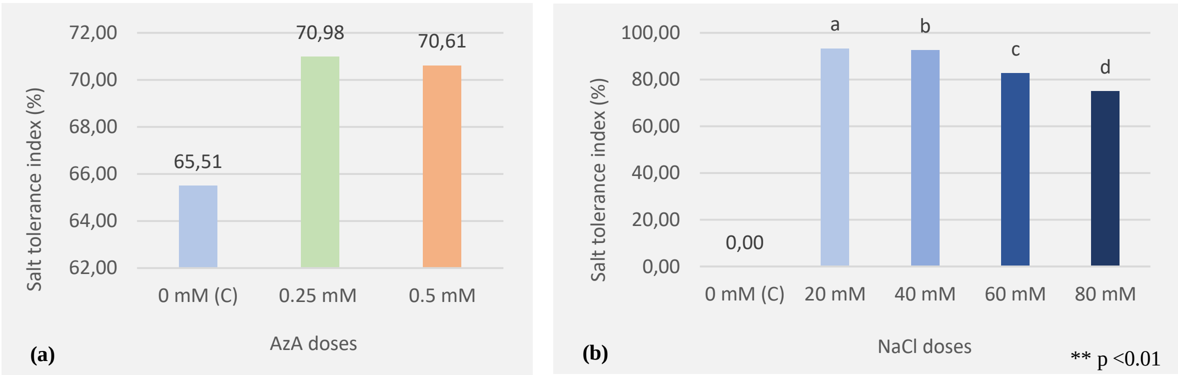


**Figure 4.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean values of the salt tolerance index (STI)

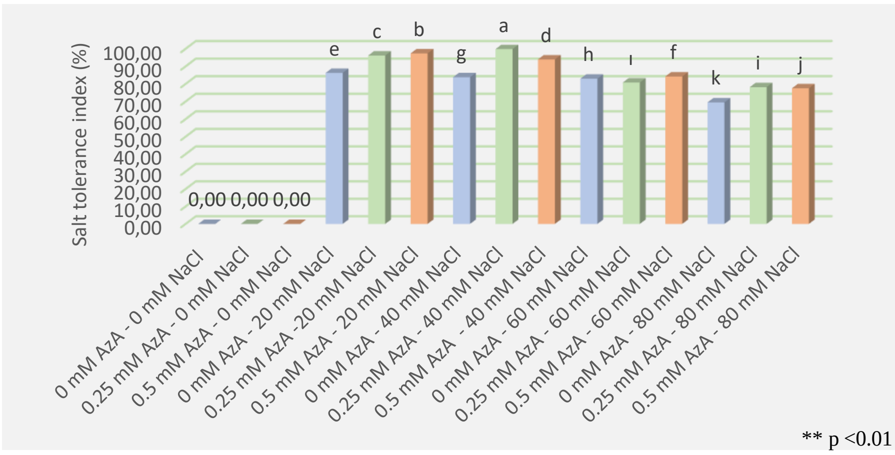


**Figure 5**. Effect of AzA priming treatments on salt tolerance index (STI) under salinity stress conditions

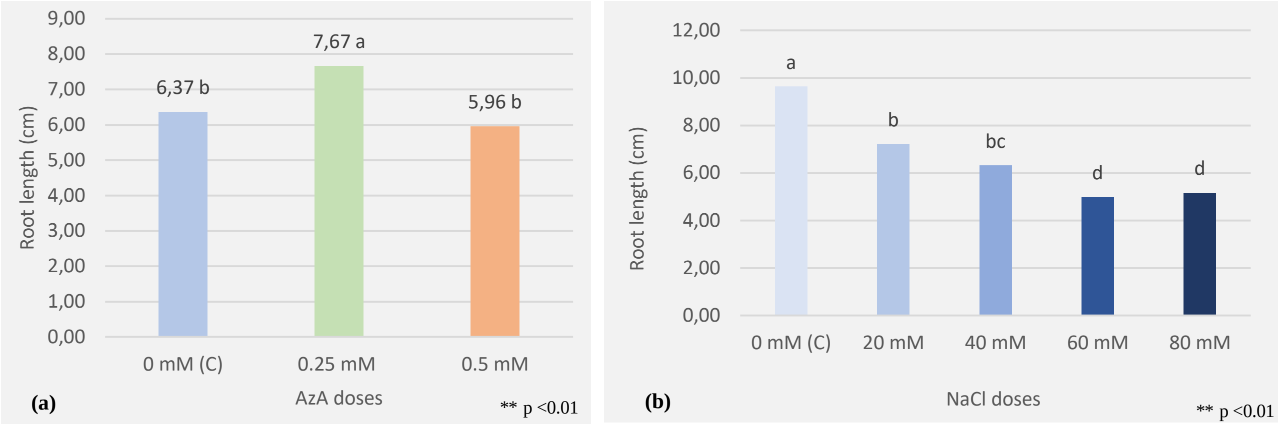


**Figure 6.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean values of root length (RL)

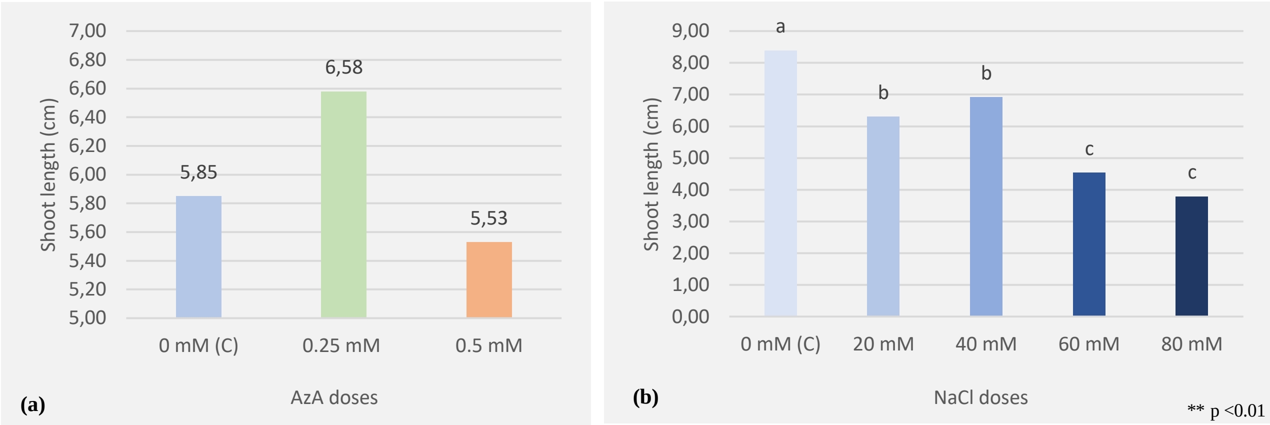


**Figure 7.** Effect of azelaic acid (a) and salinity stress (b) doses on the mean values of shoot length (SL)

## Discussion

Germination rate is a fundamental measure of seed viability. As a general typical characteristic, cucumber seeds can show a germination rate of 95% within 24 hours under optimal conditions. In our study, the highest mean germination rate was obtained in the first 24-hour period following priming with azelaic acid (AzA), particularly with the treatment of 0.25 mM AzA. In a study, it was reported that seed germination increased in cucumber seeds that underwent priming compared to seeds that did not undergo priming (Pandey et al., 2017). Salt stress caused a decrease in germination rates. Our results are consistent with similar studies conducted on cucumbers (Mahdy et al., 2020; Rezvani et al., 2025) and beans (Özkorkmaz et al., 2020).

Radicle lengths were measured at 48 hours after germination. Our results, which are consistent with those of Li et al. (2023) in cucumbers, indicate that increasing NaCl doses suppressed radicle lengths (Figure 8). Under salt-free conditions, the highest radicle lengths were observed in the 0.25 mM and 0.50 mM AzA treatments, respectively (Figure 8-A).

Fresh weights measured at 48 hours showed an inverse trend with increasing salt levels. The lowest fresh weight values were observed under 60 and 80mM stress conditions. Salt stress did not affect dry weights. According to Anwar et al. (2020), priming agents that support cucumber germination and early seedling stages positively affected fresh and dry weights. In our study, the highest fresh and dry weight values were obtained from the 0.25 mM AzA priming group without salt stress.

The salt tolerance index is a useful indicator for identifying salt-tolerant genotypes under high NaCl concentrations (Masuda et al., 2021). The increase in stress levels caused a decrease in salt tolerance index values, similar to the study by Kusvuran et al. (2015). However, the interaction between salt stress and azelaic acid positively affected salt tolerance index values. The highest salt tolerance index was observed under the interaction of 0.25 mM azelaic acid priming and 40 mM salt stress. Accordingly, it can be stated that the treatment of 0.25 mM azelaic acid priming to seeds of the *Beith Alpha* cucumber variety improves salt tolerance under 40 mM salinity stress conditions.

According to measurements taken at the end of the 10th day, decreases in root length values were observed as NaCl doses increased. Salt stress has been reported in various studies to cause setbacks in root development, directly related to the plant's optimal water uptake (Topçu and Özkan, 2017; Yavuz et al., 2023; Güleç et al., 2025). In our study, while the mean

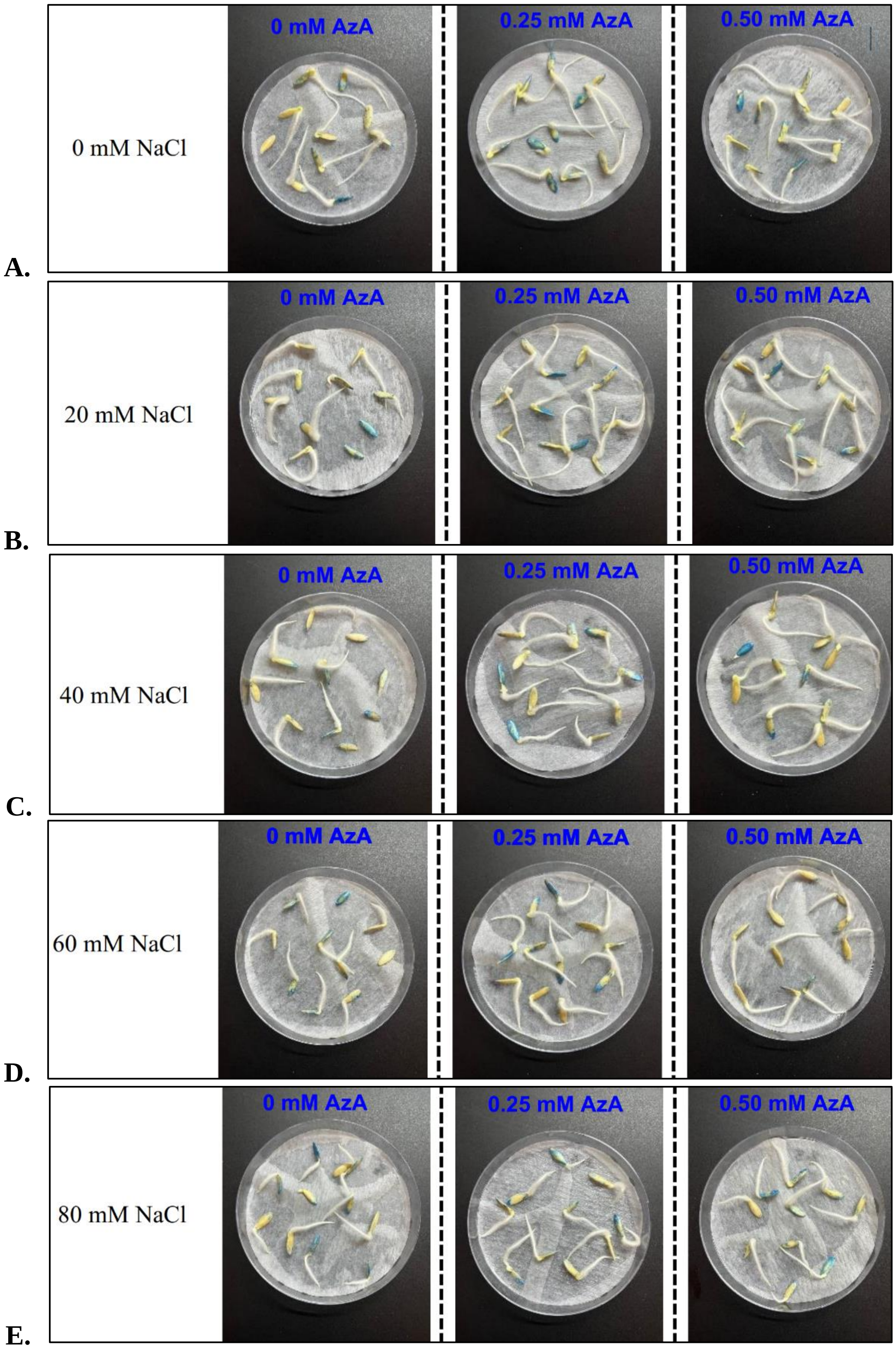


**Figure 8.** Cucumber seeds exposed to 0, 20, 40, 60, and 80 mM NaCl stress after 0, 0.25, and 0.50 mM AzA priming treatments at 48th hours.

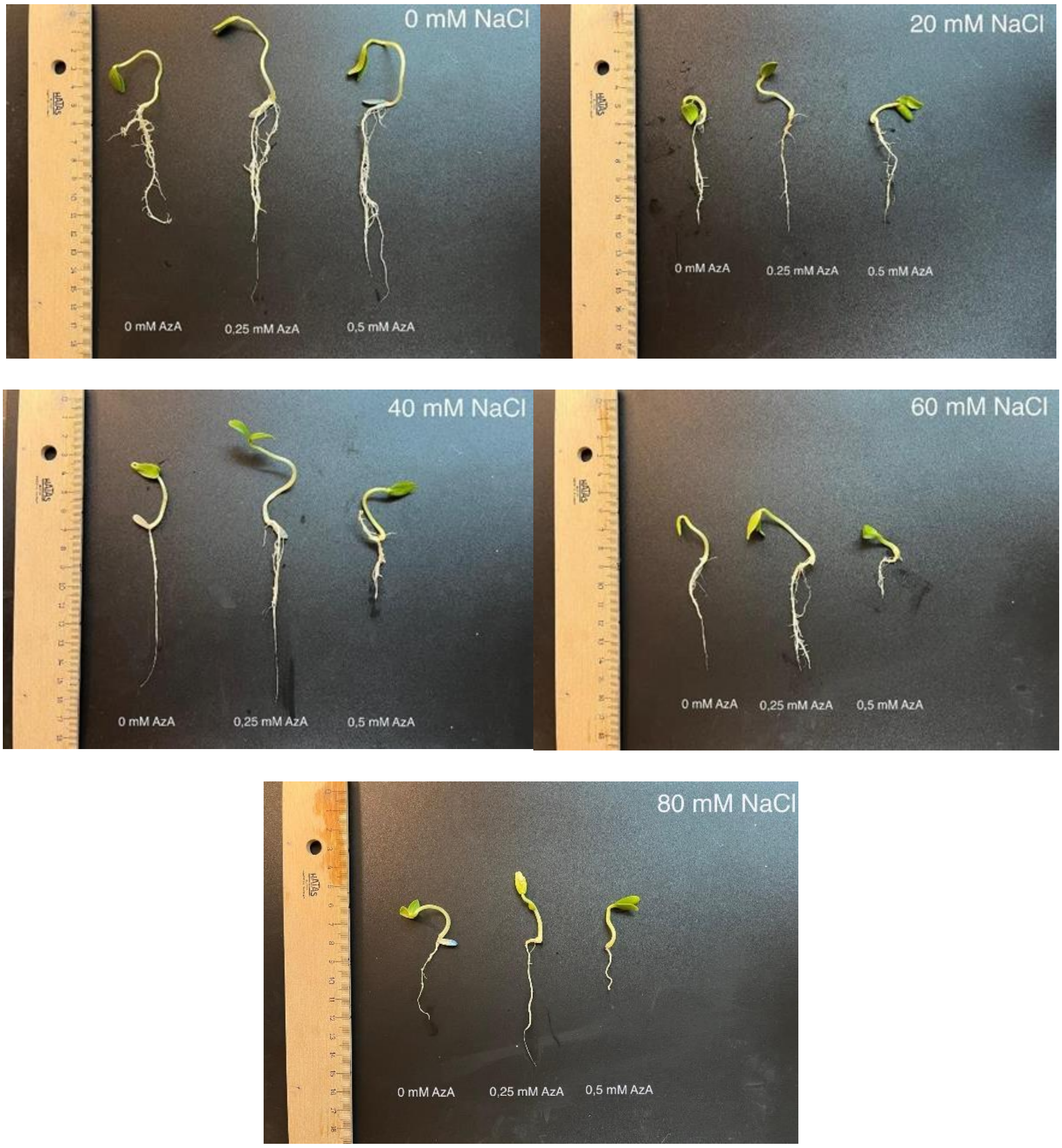


**Figure 9.** Early seedling stage appearance (10$^{th}$ day) of cucumber seeds exposed to 0, 20, 40, 60, and 80 mM NaCl stress after 0, 0.25, and 0.50 mM AzA priming treatments.

root lengths at 20 and 40 mM salinity levels were affected to the same degree of significance, the lowest mean root lengths were obtained at 60 and 80 mM salinity levels. The root length parameter was significantly affected in terms of azelaic acid means, with the highest mean value observed in the 0.25 mM priming treatment (Figure 9). Different studies have reported that root lengths increase or decrease depending on the dose of the priming agent (Süheri et al., 2019; Altuner et al., 2020).

At the end of the 10th day, the measured shoot length values were negatively affected by salt stress conditions. The lowest mean shoot lengths were observed in 60 and 80 mM salinity stresses, which share the same significance group. Although azelaic acid means did not separate into any significance group, the highest mean shoot length was again obtained from the 0.25 mM AzA dose, similar to root length means (Figure 9). Haghighi and Sheibanirad (2018) also reported that azelaic acid improved growth in tomatoes under salinity conditions.

## Conclusions

The study investigated the effects of salinity stress on cucumber germination ability and aimed to determine whether AzA, used as a bio agent, could contribute to the tolerance mechanism. When the data obtained from the study were evaluated together, it was determined that salinity stress was statistically effective in all parameters (germination rate, radicle length, fresh weight, salt tolerance index, root length, and shoot length), except for dry weight values. Generally, as the severity of salt stress increases, the obtained values tend to decrease. It can be said that the effect of salinity levels above 40 mM was particularly devastating for some parameters. AzA applications showed significant effects on germination rate, radicle length, fresh weight, dry weight, and root length. We can say that AzA, which is already active in plant metabolic processes, has positive effects on plant physiology when applied at appropriate doses. In this study, a dose of 0.25 mM AzA supported the germination ability of stressed cucumber seeds.

Based on the results obtained from the study, proper management of salinity is crucial for sustainable agriculture in arid and semi-arid regions. Given the current state of salinity worldwide, strategies such as improving drainage systems, regular leaching practices, selecting salt-tolerant varieties, and using bio agents effective in stress tolerance should be prioritized. Ultimately, it is believed that functionalizing the use of such substances naturally found in plants will contribute to agricultural production.

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The authors declare that this study received no financial support.

## Author Contribution

**A.G.:** Data curation, Investigation, Writing-Original draft. **N.Y.:** Methodology, Investigation, Formal analysis, Data curation, Validation. **D.Y.:** Writing-Original draft, Investigation.

## Conflict of Interest

No potential conflict of interest was reported by the authors.

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