

Effect of Azelaic Acid Priming on Some Germination and Seedling Parameter in Barley (*Hordeum vulgare* L.) Seeds under Salt Stress

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Abstract

This study was conducted to evaluate the effects of azelaic acid (AzA) priming on germination and some seedling parameters for seeds of barley (*Hordeum vulgare* L.) under salt stress conditions. In current research, four different salinity levels (0, 25 mM, 50 mM, 100 mM NaCl) and 2 different doses of AzA (0 mM, 0.5 mM) with 3 replicates were examined according to a complete randomized design. The variety of Larende barley was used as seed material. Analysis of variance showed that the effect of AzA priming on shoot length, root fresh weight and shoot fresh weight of barley was significant ($p \leq 0.01$), while the effect on other parameters was found insignificant ($p \geq 0.05$). NaCl levels significantly affected all parameters ($p \leq 0.01$). In the AzA \times NaCl interaction, root fresh weight, shoot fresh weight, salt tolerance index and relative water content were affected significantly ($p \leq 0.01$). In examine the effects of salinity levels on barley plant, especially on germination parameters, the values obtained from 0, 25 and 50 mM applications were in the same significance group. According to this result, when evaluated in terms of germination, it was concluded that water having 50 mM or less salt content can be used in barley cultivation. It is thought that it would be appropriate to try AzA at different doses and different salt levels in order to understand the effects of AzA treatment more clearly.

Keywords: Barley, Germination, Azelaic acid, NaCl, Pre-treatment.

Azelaik Asit Ön Uygulamasının Tuz Stresi Altındaki Arpa (*Hordeum vulgare* L.) Tohumlarında Bazı Çimlenme ve Fide Parametreleri Üzerine Etkisi

Öz

Bu çalışma, azelaik asit (AzA) ön uygulamasının tuz stresi koşullarındaki arpa (*Hordeum vulgare* L.) tohumlarının çimlenme ve bazı fide parametreleri üzerindeki etkilerini değerlendirmek amacıyla yürütülmüştür. Araştırma, tesadüf parselleri deneme desenine göre, dört farklı tuzluluk düzeyinde (0, 25 mM, 50 mM, 100 mM NaCl) ve iki farklı dozda AzA (0 mM, 0.5 mM) uygulaması, üç tekerrürlü olarak yürütülmüştür. Tohum materyali olarak arpa çeşidi Larende kullanılmıştır. Çalışmada çimlenme ve bazı fide gelişim parametreleri (çimlenme oranı, ortalama çimlenme zamanı, çimlenme gücü indeksi, kök uzunluğu, sürgün uzunluğu, kök yaş ağırlığı, sürgün yaş ağırlığı, tuz tolerans indeksi, su kullanım etkinliği, bağıl su içeriği) saptanmıştır. Varyans analiz sonuçlarına göre arpada AzA ön uygulaması; sürgün uzunluğu, kök yaş ağırlığı ve sürgün yaş ağırlığı üzerine önemli düzeyde ($p \leq 0.01$) etki etmiş olup, diğer parametreler üzerine etkisi önemsiz bulunmuştur ($p \geq 0.05$). NaCl seviyeleri tüm parametreleri önemli düzeyde etkilemiştir ($p \leq 0.01$). AzA \times NaCl interaksyonunda ise kök yaş ağırlığı, sürgün yaş ağırlığı, tuz tolerans indeksi ve bağıl su içeriği önemli düzeyde etkilenmiştir ($p \leq 0.01$). Arpa bitkisinde tuzluluk seviyelerinin özellikle çimlenme parametreleri üzerindeki etkilerine bakıldığında, 0, 25 ve 50 mM uygulamalarından elde edilen değerler aynı önemlilik grubunda yer almıştır. Bu sonuca göre çimlenme açısından değerlendirildiğinde arpa yetiştiriciliğinde 50 mM ve daha az tuz içeren suların kullanılabileceği sonucuna varılmıştır. AzA uygulamasının etkilerinin daha net anlaşılabilmesi için farklı dozlarda ve farklı tuz seviyelerinde denenmesinin uygun olacağı düşünülmektedir.

Anahtar Kelimeler: Arpa, Çimlenme, Azelaik asit, NaCl, Ön uygulama.

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1. Introduction

Barley (*Hordeum vulgare* L.) is a member of the Poaceae family and one of the first field crops to be cultivated [1]. This crop, one of the most cultivated cereals in the world, ranks fourth following by wheat, maize and paddy [2] and is an important source of animal feed, rich in fibre and bioactive compounds and highly nutritious, as well as an important component of the malt industry [3]. Compared to other cereals, barley has advantages such as earliness, drought tolerance and adaptability to different soils [4, 5].

Plants can be exposed to various stresses known as biotic and abiotic. Stress factors significantly affect plant development processes, cause oxidative damage and lead to significant agricultural production losses. Drought and salinity are the most common abiotic stress factors [6]. Due to water shortage problem low quality water in arid and semi-arid regions has to be used intensively in agricultural production. For this reason, the problem of salinisation and alkalinisation in agricultural lands is increasing dramatically. This situation increases the osmotic pressure in the rhizosphere zone and prevents the plant's water uptake through the roots or causes ion toxicity [7, 8]. Therefore, it is very important to understand the response mechanisms of plants to be able to evaluate stress tolerances and crop resilience in different crops. One of the primary physiological responses to salinity stress is a reduction in photosynthetic activity (performance). Salinity stress causes stomatal closure in exposed plants and reduces the amount of photosynthesis by limiting CO₂ uptake [9]. In general, salt stress is characterised by disruption in the homeostasis of Na⁺ and Cl⁻ ions starting with specific ion toxicity, resulting in stomatal closure and increased ROS production in chloroplasts, and causes inhibition of plant growth [10, 11]. Therefore, amelioration of stress tolerance through various strategies is critical for plant survival and productivity.

Germination and early seedling phases of plants are the most sensitive cycles to stress factors. For this reason, adaptation possibilities during germination and different techniques to increase the tolerance of seeds to salinity have been investigated for many years. One of the most frequently used techniques is seed or seedling priming. Seed priming is based on pre-exposure of seeds to chemicals or stress factors (salinity, drought, etc.). This increases the plant's resistance to subsequent stresses and its ability to rapidly detect secondary signals [12]. Many effective compounds are used to increase the practicability of this technique and are the subject of research.

Azelaic acid (AZA), a compound whose effect on plants has been tested under normal conditions or biotic stress (pathogen) conditions, has been used in medicine, cosmetics and pharmacological fields since it was first discovered [13, 14]. In a study, it was reported that azelaic acid compounds, exist in the biochemical cycle of plants, can survive biotic stress conditions [15]. Recently, this molecule, which is known to accumulate in the root system, [16] has attracted attention as a preparatory molecule in the systemic acquired resistance (SAR) mechanism of plants. In addition, azelaic acid, one of the structures that constitute the signal transduction components of the induced systemic resistance (ISR) mechanism, is reported to provide a faster and stronger accumulation of salicylic acid (SA) in response to infection or oxidation [17]. However, studies on its effects against abiotic stress factors are quite limited. In

a study, it was reported that overexpression of AZI (Azelaic Acid Induced) gene in Arabidopsis plants is sensitive to drought stress, notwithstanding increases cold and salt tolerance via abscisic acid (ABA) [18]. Haghighi and Sheibanirad [19]; applied 0, 8, 10 and 24 mg/L azelaic acid exogenously to tomato plants under 0, 100, 150 and 200 mM salinity conditions. They reported that especially 8 mg/L AzA dose can maintain the gas exchange capacity at optimum level, induce osmotic balance and reduce the effects of salinity at salinity levels up to 100 mM.

In the investigation of tolerance mechanisms with different seed preparation methods, the effect of salicylic acid (SA), jasmonic acid (JA) and similar signalling molecules have been investigated, but it has been stated that AzA is also involved in tolerance mechanisms [20] Although barley is one of the plants with high salt tolerance, it is a plant that is severely affected by salt stress during germination and early seedling periods [21, 22]. The aim of this study was to determine the agronomic responses of barley to different doses of salt (NaCl) with azelaic acid priming during germination and early seedling stage.

2. Material and Methods

In this study, two different doses of AzA (0 mM, 0.5 mM) and four different salt (NaCl) levels (0; control, 25, 50, 50 and 100 mM) were applied to seeds of the barley Larende cultivar. Barley seeds were kept in different concentrations of AzA solutions at 4 °C for 12 hours. Those seeds were then dried at +25 °C for 5 hours. The seeds primed with pure water and AzA were sown in pots of 9 cm in diameter and 14 cm in height, using a mixture of sand and topsoil (1:2), with 15 seeds per pot. The pots were designed according to a completely randomised experimental design with three replications. Samples of the soil mixture placed in the pots were analysed such as field capacity and permanent wilting point in the laboratory. Irrigation water was applied at 2-day irrigation intervals according to the weighting method to fill the moisture deficit to the field capacity. During irrigation applications, special care was taken to avoid direct contact of irrigation water with plant leaves. For 10 days, the germinated seeds in the pots were counted daily at the same time and the data were recorded. A seedling was considered to have emerged when a hypocotyl appeared on the soil surface [23]. At the end of this period, the seedlings were harvested and physiological measurements were made using a metric ruler and a precision scale.

In order to determine the effect of the treatments on germination parameters, germination rate (GR) was calculated by Equation 1, germination vigour index (GVI) was calculated by Equation 2 in accordance of Türkoğlu, et al. [23] and mean germination time (MGT) was calculated by Equation 3 suggested by Süheri, et al. [24].

$$GR (\%) = (Total\ number\ of\ germinated\ seeds / total\ number\ of\ seeds) \times 100 \text{ (Eq.1)}$$

$$GVI = (mean\ shoot\ length + mean\ root\ length) \times total\ germination\ rate \text{ (Eq.2)}$$

$$MGT = \Sigma(fx) / \Sigma f \text{ (Eq. 3)}$$

Where f is the number of germinating seeds on the counting day and x is the number of days of counting.

To determine the effect on early seedling performance; root fresh weight (RFW), shoot fresh weight (SFW) values were measured and salt tolerance index (STI) was calculated using Equation 4 according to Kusvuran, et al. [25], water use efficiency (WUE) was calculated using Equation 5 according to Liu, et al. [26] and relative water content (RWC) was calculated using Equation 6 according to Yavuz, et al. [27];

$$STI (\%) = (Total\ fresh\ weight\ at\ salt\ concentration / total\ fresh\ weight\ in\ control) \times 100 \text{ (Eq. 4)}$$

$$WUE = Dry\ biomass / water\ consumption\ in\ the\ experiment \text{ (Eq. 5)}$$

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100 \text{ (Eq. 6)}$$

In this equation, FW stands for fresh weight, DW for dry weight and TW for turgor weight.

The effects of AzA priming and NaCl doses on the parameters examined in the experiment were subjected to analysis of variance according to 2×4 factorial and a completely randomised trial design with 3 replications (SPSS 27 software, SPSS Inc., IBM). The differences between the means of these parameters were determined by Duncan test at 5% significance level.

3. Results and Discussion

3.1. Results

Barley seeds germinated in about 2 days and their development was observed for 10 days (Figure 1). AzA applied to the seed did not contribute positively to the germination parameters under salt stress conditions, while it exhibited ameliorative effects on some seedling parameters. The mean values and significance groups of germination parameters obtained from the treatments are given in Table 1.

The applied NaCl levels had a significant effect ($p \leq 0.01$) on germination rate (GR), while the effect of AzA and AzA \times NaCl interaction was found insignificant ($p \geq 0.05$) (Table 1). Considering the means of NaCl levels, the highest germination rate (GR) was obtained from 0, 25 and 50 mM NaCl stress treatments as 96%, 94% and 93%, respectively, and the lowest GR was obtained from 100 mM NaCl application as 74% due to the increase in stress (Table 1).

Mean germination time (MGT) is defined as the time taken for a seed to germinate. The effect of salt stresses on MGT was significant ($P \leq 0.01$) (Table 1). The effects of AzA and AzA \times NaCl stresses interactions on MGT were found insignificant ($p \geq 0.05$) (Table 1).



Figure 1. Some agronomic differences in barley seedlings primed with water (0 mM) and azelaic acid (0.5 mM) before sowing.

In terms of salinity, the mean germination time was 4.60 days in the control (0 mM NaCl), followed by 25 mM NaCl application with 4.70 days, and the latest MGT value was realised in 100 mM NaCl application with 5.77 days. As the salt content increased, germination time was delayed as expected. When AzA treatments were analysed based on mean s, the lowest mean germination time was obtained from 0.5 mM AzA treatment (5.04 days) compared to the control treatment (5.15 days) (Table 1). AzA and salt stress interactions were not separated into significance groups and the lowest MGT was obtained as 4.42 days from the group with 0.5 AzA priming and no salt stress (0.5-0) with 8% difference compared to the control group (0-0).

Germination vigour index (GVI) is an important parameter to determine rapid and uniform emergence and seedling formation under various conditions [29]. NaCl stresses had a statistically significant ($P \leq 0.01$) effect on GVI values, whereas the interactions of AzA and AzA x NaCl stresses were insignificant ($p \geq 0.05$) as seen in Table 1. When the mean s were analysed in terms of NaCl stresses, the highest GVI was found in the control group with 2449.58, while 100 mM NaCl treatment ranked last with 1332.69 (Table 1). Although AzA x NaCl interaction values were statistically insignificant, the highest GVI value was obtained as 2490.44 in 0.5 mM AzA priming and 25 mM salt level (Table 1).

Root and shoot length are important indicators in determining the level of stress tolerance. The effect of NaCl treatments on root length (RL) was significant ($P < 0.01$), while the effect of AzA and AzA x NaCl stresses interaction was insignificant (Table 1).

In terms of salinity, the highest root length (RL) values were obtained from 0 mM NaCl (14.95 cm), 25 mM NaCl (14.45 cm) and 50 mM NaCl (14.01 cm) treatments and the lowest value was obtained from 100 mM NaCl (10.91) treatment. According to the mean s of AzA treatments; the highest root length was obtained from 0.5 mM AzA treatment with 14.06 cm and the lowest value was obtained from 0 mM AzA treatment with 13.09 cm.

Table 1. Germination parameters of barley against salinity stresses and azelaic acid priming.

Treatments	GR (%)	MGT (Day)	GVI	RL (cm)	SL (cm)
AzA (mM)					
0	92	5.15	2100.76	13.09	9.54 ^B
0.5	87	5.04	2160.10	14.06	10.33 ^A
NaCl (mM)					
0	96 ^A	4.60 ^B	2449.58 ^A	14.95 ^A	11.35 ^A
25	94 ^A	4.70 ^B	2392.39 ^A	14.45 ^A	10.80 ^{AB}
50	93 ^A	5.29 ^{AB}	2347.06 ^A	14.01 ^A	10.49 ^B
100	74 ^B	5.77 ^A	1332.69 ^B	10.91 ^B	7.11 ^C
Aza × NaCl					
0 mM × 0 mM	98	4.79	2467.72	14.28	10.98
0 mM × 25 mM	93	4.52	2294.33	13.88	10.56
0 mM × 50 mM	96	5.55	2285.67	13.57	10.28
0 mM × 100 mM	80	5.73	1355.33	10.65	6.34
0.5 mM × 0 mM	89	4.42	2431.44	15.63	11.73
0.5 mM × 25 mM	96	4.89	2490.44	15.01	11.03
0.5 mM × 50 mM	96	5.02	2408.44	14.45	10.70
0.5 mM × 100 mM	69	5.82	1310.06	11.17	7.88
Sources of Variation					
F value (AzA)	3,6 ^{ns}	0,3 ^{ns}	0,3 ^{ns}	3,3 ^{ns}	11,7 ^{**}
F value (NaCl)	18,1 ^{**}	6,2 ^{**}	27,2 ^{**}	11,7 ^{**}	68,6 ^{**}
F value (AzA × NaCl)	1,9 ^{ns}	0,9 ^{ns}	0,3 ^{ns}	0,1 ^{ns}	1,2 ^{ns}

***P*<0.01; **P*<0.05; ns: not significant. Uppercase and lowercase letters indicate Duncan groups for Azelaic acid and salinity (NaCl) stress respectively. GR, germination rate; MGT, mean germination time; GVI, germination vigor index; RL, root length; SL, shoot length.

According to these data, 0.5 mM AzA treatment increased root length more by comparison to the control (Table 1). While no significant difference was observed in the AzA x NaCl interaction, the highest root length of 15.63 cm was obtained from 0.5 mM AzA x 0 mM NaCl treatment. This value represents 9% longer root length compared to the control group (0-0) (Table 1).

The effect of AzA priming and NaCl treatments on shoot length (SL) was highly significant (*P*<0.01) and the effect of AzA x NaCl stresses interaction was insignificant (*p* ≥ 0.05) (Table 1). In examined the AzA means, 8% more shoot length was obtained from the group treated with 0.5 mM AzA priming (10.33 cm) compared to the untreated group (9.54 cm). According to this assessment, AzA application positively affected the shoot length parameter. According to the means of NaCl treatments, the highest shoot length was obtained from 0 mM NaCl (11.35 cm) and the lowest value was obtained from 100 mM NaCl (7.11 cm) due to the increase in salt stress (Table 1). When the interaction values between AzA and salt stress were examined, the highest shoot length was observed as 11.73 cm in 0.5 mM AzA - 0 mM NaCl treatment with a 6% difference compared to the control (0-0) (Table 1).

The effects of AzA, NaCl stress treatments and AzA x NaCl stress interactions on root fresh weight (RFW) were statistically significant (*P*<0.01) (Table 2). According to AzA means; the highest RFW was obtained from 0.5 mM AzA priming treatment with 0.190 g and the lowest value was obtained from 0 mM AzA treatment with 0.140 g.

According to the means of salinity doses, the highest root fresh weight was 0.212 g at 50 mM NaCl dose, followed by 0 mM NaCl (0.188 g) and 25 mM NaCl (0.181 g), respectively. The lowest root fresh weight was obtained from 100 mM NaCl (0.079 g) stress treatment (Table 2). When the means of AzA x NaCl interactions in Figure 1 prepared according to Table 2 data are analysed, the highest root fresh weight was obtained from 0.5 mM AzA priming and 0 mM NaCl interaction with 0.246 g, followed by 0.5 mM AzA priming and 25 mM NaCl interaction with 0.235 g. The lowest root fresh weight values were obtained from the 100 mM NaCl stress levels of 0 and 0.5 mM AzA priming treatments with 0.080 g and 0.077 g, respectively (Fig.1).

The effect of AzA, NaCl stress applications and AzA x NaCl interaction on salt tolerance index (STI) was statistically significant ($P < 0.01$) (Table 2). According to the means of AzA priming treatments; the highest STI value was obtained from 0.5 mM AzA priming (125.9) with 43% difference and the lowest STI value was obtained from 0 mM AzA priming groups (72.1). According to the means of salinity stresses, the highest salt tolerance index value was 131.7 in 50 mM NaCl stress conditions and the lowest value was 57.7 in 100 mM NaCl stress treatments (Table 2). When the means of AzA x NaCl interaction were analysed, the highest salt tolerance index values were 156.56 and 156.18 in 0 mM and 25 mM NaCl stress conditions with 0.5 mM AzA priming, and the lowest STI values were 51.1 and 64.26 in 100 mM salt level of both priming treatments (Fig. 3).

Table 2. Early seedling performance parameters of barley against salinity stresses and azelaic acid priming.

Treatments	RFW (g)	SFW (g)	STI (%)	WUE (g/L)	RWC (%)
AzA (mM)					
0	0.14 ^B	0.15 ^B	72.11 ^B	0.114 ^B	86.33
0.5	0.19 ^A	0.18 ^A	125.91 ^A	0.132 ^A	84.37
NaCl (mM)					
0	0.19 ^A	0.189 ^{AB}	78.28 ^B	0.145 ^A	88.57 ^A
25	0.18 ^A	0.195 ^A	128.40 ^A	0.139 ^A	88.71 ^A
50	0.21 ^A	0.175 ^B	131.68 ^A	0.125 ^A	84.11 ^B
100	0.08 ^B	0.091 ^C	57.68 ^B	0.081 ^B	80.01 ^C
Azelaic acid x NaCl					
0 mM x 0 mM	0.130 ^b	0.167 ^b	0.00 ^d	0.13	94.70 ^a
0 mM x 25 mM	0.126 ^b	0.176 ^b	100.61 ^b	0.13	85.62 ^b
0 mM x 50 mM	0.225 ^a	0.177 ^b	136.72 ^a	0.12	84.26 ^{bc}
0 mM x 100 mM	0.080 ^b	0.074 ^d	51.10 ^c	0.07	80.75 ^{bc}
0.5 mM x 0 mM	0.246 ^a	0.211 ^a	156.56 ^a	0.16	82.44 ^{bc}
0.5 mM x 25 mM	0.235 ^a	0.213 ^a	156.18 ^a	0.15	91.81 ^a
0.5 mM x 50 mM	0.198 ^a	0.172 ^b	126.63 ^{ab}	0.13	83.95 ^{bc}
0.5 mM x 100 mM	0.077 ^b	0.108 ^c	64.26 ^c	0.09	79.26 ^c
Sources of Variation					
F value (AzA)	9,9 ^{**}	20,6 ^{**}	44,1 ^{**}	6,4 [*]	2,9 ^{ns}
F value (NaCl)	13,4 ^{**}	63,6 ^{**}	20,7 ^{**}	16,4 ^{**}	12,9 ^{**}
F value (AzA x NaCl)	5,2 ^{**}	3,3 [*]	20,7 ^{**}	0,09 ^{ns}	10,9 ^{**}

** $P < 0.01$; * $P < 0.05$; ns: not significant. Uppercase and lowercase letters indicate Duncan groups for Azelaic acid and salinity (NaCl) stress respectively. RFW, root fresh weight; SFW, shoot fresh weight; STI, salt tolerance index; WUE, water use efficient; RWC, relative water content.

The effect of AzA, NaCl stress applications and AzA x NaCl interaction on salt tolerance index (STI) was statistically significant ($P < 0.01$) (Table 2). According to the means of AzA priming treatments; the highest STI value was obtained from 0.5 mM AzA priming (125.9) with 43% difference and the lowest STI value was obtained from 0 mM AzA priming groups (72.1). According to the means of salinity stresses, the highest salt tolerance index value was 131.7 in 50 mM NaCl stress conditions and the lowest value was 57.7 in 100 mM NaCl stress treatments (Table 2). When the means of AzA \times NaCl interaction were analysed, the highest salt tolerance index values were 156.56 and 156.18 in 0 mM and 25 mM NaCl stress conditions with 0.5 mM AzA priming, and the lowest STI values were 51.1 and 64.26 in 100 mM salt level of both priming treatments (Fig. 3).

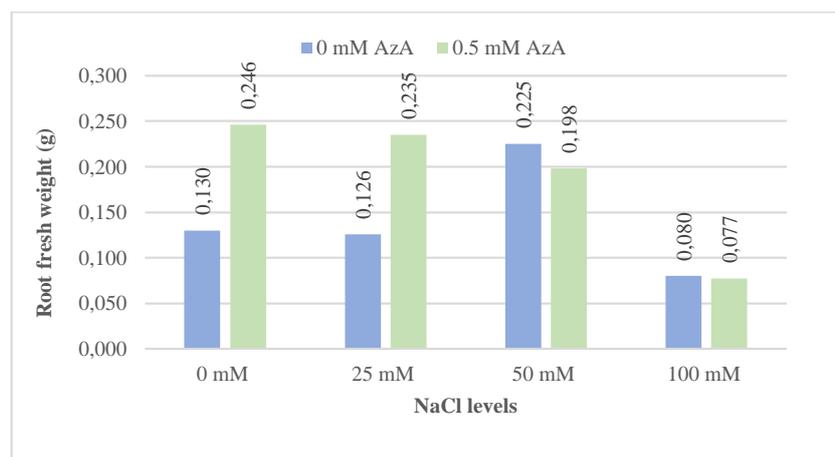


Figure 2. Changes in the root fresh weight of barley against salinity stresses and Azelaic acid priming.

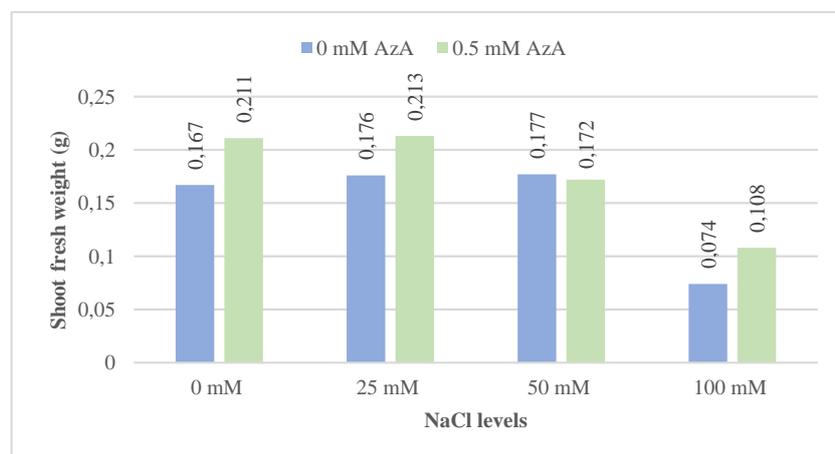


Figure 3. Changes in the shoot fresh weight of barley against salinity stresses and Azelaic acid priming

Water use efficiency (WUE) values were significantly affected by AzA priming and salt stress applications ($P < 0.01$). The effect of AzA x NaCl interaction on WUE values was statistically insignificant (Table 2). According to AzA means; the highest WUE value was obtained as 0.132

g/L from 0.5 mM azelaic acid priming groups with a difference of about 14% and the lowest WUE value was obtained as 0.114 g/L from 0 mM AzA (pure water) priming groups. According to salinity (NaCl) means, the highest WUE value (0.145 g/L was obtained from 0 mM NaCl dose and the lowest value (0.082 g/L) was obtained from 100 mM NaCl dose (Table 2). The highest WUE values were obtained from 0.5 mM AzA x 0 mM NaCl (0.157 g/L) and 0.5 mM AzA x 25 mM NaCl (0.147 g/L) interactions with 15% and 10% difference, respectively, although there was no statistically significant difference (Table 2).

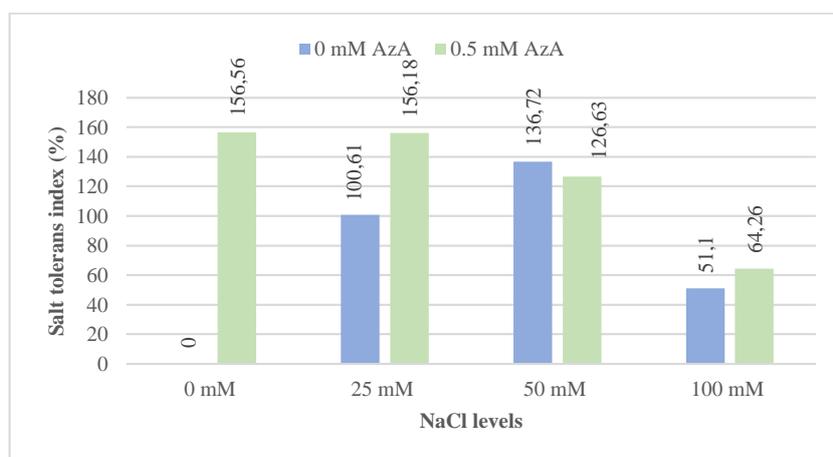


Figure 4. Changes in the salt tolerance index of barley against salinity stresses and Azelaic acid priming.

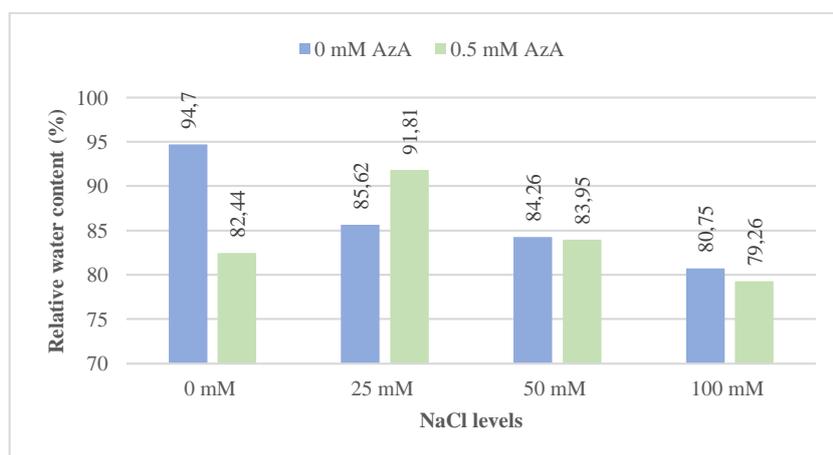


Figure 5. Changes in the relative water content of barley against salinity stresses and Azelaic acid priming.

The effect of NaCl stress applications and AzA x NaCl interactions on relative water content (RWC) was significant ($P < 0.01$), while the effect of AzA treatment was statistically insignificant (Table 2). According to salt stress means, the highest RWC value was 88.7% in 25 mM NaCl applications and the lowest value was 80.0% in 100 mM NaCl applications (Table

2). When the means of AzA x NaCl interaction were analysed, the highest RWC value was obtained as 94.7% from the control group, followed by 0.5 mM AzA x 25 mM NaCl interaction with 91.8% (Fig. 4).

3.2. Discussion

Stress conditions in germination and early seedling processes of cultivated plants limit the potential yield as well as quality [28].

Germination rate was in the same group with the control (0 mM NaCl) at NaCl doses up to 50 mM. On the contrary, the highest decrease in germination rate occurred at 100 mM NaCl dose. In many studies, it was reported that the increase in salt levels caused a decrease in germination rates [29-31]. The longest mean germination time was obtained in 100 mM NaCl application and germination took 20.4% longer than the control (0 mM NaCl) application. In similar studies, it was reported that germination time increased with increasing salt doses [5, 32].

Germination vigour index is one of the traits most affected by increasing stress conditions as a component of germination rate, root length and shoot length parameters [33]. According to the results, germination strength index values decreased by 2 to 46% compared to the non-salt (NaCl) group due to the increase in salt stress. The decrease in germination strength with increasing NaCl levels has been reported in studies on different plants [30, 34, 35]. Although there was no statistically significant difference in terms of the azelaic acid treatment agent used and its interaction with NaCl, it was observed that the germination vigour was higher in seeds primed with azelaic acid, especially when the same salinity groups at 25 mM and 50 mM NaCl levels were compared. It has been reported that priming applications may have different effects on the germination strength index depending on the genotype and stress factors. [36-38].

Root and shoot length are the most important parameters for salt stress. The root is in direct contact with the soil and absorbs soil water, while the shoot transmits the water to the rest of the plant [39]. Thus, the responses of root and shoot length values to salt stress are very important. In our study, it was observed that root length values decreased as NaCl dose increased. In different studies supporting our results, it has been reported that root development was decreased in relation to the optimum water intake and the dose of salt factor during the germination period [40, 41]. There is no significant effect on root lengths in terms of interactions with AzA and NaCl, but when the interactions of salinity groups with AzA priming and pure water priming were compared, it was observed that root lengths were 9% (0 mM NaCl), 8% (25 mM NaCl), 6% (50 mM NaCl) and 5% (100 mM NaCl) higher in seeds primed with 0.5 mM azelaic acid. Altuner, et al. [42] and Başaran and Doğrusöz [43] reported that osmopriming treatment applied to seeds increased root length. However, depending on the type and intended use of the osmopriming agent, growth and development inhibition may be caused by increases in dose [44]. According to the results obtained from in this study, AzA had a significant effect on shoot length and increased it by 8%. It can be said that 0.5 mM AzA priming application encouraged shoot development. Haghghi and Sheibanirad [19] reported that azelaic acid improved growth under salinity conditions. In contrast, decreases in shoot length values were observed with increasing salinity stress.

According to the results obtained from the barley plant, root fresh weights did not response a significant difference at salinity levels up to 50 mM, and a root fresh weight reduction of approximately 58% was observed at 100 mM NaCl dose compared to the control. On the other hand, it was observed that azelaic acid application had a positive effect in 0 and 25 mM NaCl conditions and increased root fresh weights by 47% and 44%, respectively, compared to the control (0 mM AzA × 0 mM NaCl) group. In different studies, it has been reported that salinity problems in the growing environment of the plant are effective on root fresh weight and increases in salt dose cause root fresh weights to decrease [45, 46].

According to the current study results, no significant difference was observed in the water use efficiency values of salt treatments up to 50 mM, while the lowest WUE value was observed at 100 mM NaCl dose. In a lot of studies, a steady increase in water use efficiency was observed under salt stress due to a reduction in transpiration due to a decrease in photosynthetic activity. This regulation contributes to the survival and development of the stressed plant under stress conditions by enabling it to optimise water use within its tolerance limits. [47-49]. Boussora, et al. [50] reported that salt stress had a significant effect on water use efficiency in their study on the tolerance of barley to salt stress. Contrary to this, in this study, it was observed that 0.5 mM AzA treatment of barley seeds increased the WUE values by about 14% compared to the control (0 mM AzA) group.

Relative water content is a measure of plant water status, reflecting the balance between water supply and water loss. In this study, decreases in RWC compared to the control were observed due to increasing salt stress conditions. As stated in previous studies, decreases in relative water content occur under salt stress conditions [51, 52]. Alsamadany, et al. [53] reported that there was a general tendency of decrease in RWC values compared to the control under salt stress conditions in their study with barley. On the other hand, 0.5 mM AzA treatment to seed resulted in 7% higher RWC under low (25 mM NaCl) salt stress conditions compared to the same salinity group without azelaic acid treatment.

4. Conclusion

In this research, although azelaic acid treatment of barley seeds did not have a significant effect on germination parameters against increasing salt doses, it was concluded that it positively supported the germination vigour index under low salinity conditions. However, it was determined that azelaic acid alleviated the negative effect of increasing salt doses especially in some seedling parameters. Germination and development processes were adversely affected due to the disruption of water uptake at high salt concentration. When the data are evaluated in terms of germination; it is predicted that water containing salt with values of 50 mM and below can be used in barley production. However, it was observed that azelaic acid reduced the negative effects of salt up to a certain point and contributed positively in terms of biomass. It is considered that this study may contribute to the utilisation of marginal land and water resources in agriculture. Accordingly, in order to better understand the osmoregulatory effect of azelaic acid that increases biomass values, different doses and priming durations under different salt stress conditions should be investigated by comprehensively.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Güleç A.: Writing, Materials, Investigation, Formal analysis.

Yavuz N.: Writing – review & editing, Methodology, Formal analysis, Validation.

Türkoğlu A.: Methodology, Formal analysis, Validation.

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