https://doi.org/10.61308/PNIQ9348

Tolerance of *Aegilops cylindrica* Host to sodium chloride salinity

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Citation: Desheva, G., Valchinova, E., Pencheva, A., Kyosev, B. & Deshev, M. (2025). Tolerance of *Aegilops cylindrica* Host to sodium chloride salinity. *BulgarianJournal of Crop Science, 62*(3) 61-69

Abstract: The aim of this study was to investigate the effect of six different concentrations of sodium chloride (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM) on seed germination and early seedling growth of *Aegilops cylindrica* Host. The experiment was conducted, with seeds from five accessions taken from an trait conducted at IPGR-Sadovo, Bulgaria. For each variant of the experiment, two replicates of 25 seeds were set for germination between rolls of filter paper (Grade FT 55) with 20 ml of the respective solutions tested. Deionized water was used as control. Data were analyzed by analysis of variance (ANOVA) and Duncan's multiple test. The concentrations of sodium chloride studied had different effects on germination and seedling characteristics. The mean germination time was prolonged, and the germination characteristics (germination (%), coefficient of velocity of germination, germination rate index (% day^{-1}), and germination index) and seedling growth characteristics (shoot and root length (cm), fresh and dry weight per shoot and root (mg/plant)) were suppressed with increasing salt concentration. The highest relative injury coefficient was recorded when treated with 250 and 300 mM NaCl solution. The reduction in shoot length ranged widely from 14% (at 50 mM NaCl) to 94% (at 300 mM NaCl). *Aegilops cylindrica* Host were classified as having very high to high germination tolerance when treated with 50-150 mM NaCl solution, medium tolerance at 200 mM NaCl solution, very low tolerance at 250 mM NaCl solution and susceptible to germination at 300 mM NaCl solution. At salinity levels of 200-300 mM NaCl solution, the analyzed specimens of *Aegilops cylindrica* Host exhibited low to very low tolerance to seegling growth.

Keywords: *Aegilops cylindrica* Host; salinity; NaCl; tolerance; germination; seedling growth

INTRODUCTION

Soil salinity is a major abiotic constraint affecting agricultural productivity worldwide. It can increase due to human activities, climate changes, natural mineral weathering, and secondary salinization. By 2050, when the world population is projected to be 9.1 billion, arable land loss is estimated to increase to 30% due to salinization (Arabbeigi et al., 2018). Soil salinity usually hinders plant growth and reproduction. This occurs due to an initial osmotic-stress phase, followed by ionic toxicity caused by the accumulation of sodium (Na^+) and chloride (Cl^-) in the cell cytosol. This ultimately leads to nutritional deprivation and oxidative stress (Arzani and Ashraf, 2016; Kiani et al., 2021; Singh et al., 2021). Salinity affects plant physiological, morphological, and biochemical processes, as well as seed germination, growth, and water and nutrient uptake. As a result, high salinity level causes a delay in germination, even inhibition of seed germination depending on salt tolerance of plants.

The limited variation in existing genomes restricts the improvement of modern wheat's tolerance to salinity stress. Many authors (Kiani et al., 2015; Arzani and Ashraf 2016; Saha et al. 2016; Arabbeigi et al., 2018; Kotula et al., 2024) noted that the wild relatives of cultivated plants, which include their ancestors and more or less distantly related species, perform superior under adverse environments, and hence constitute a valued gene pool for tolerance to abiotic stress. *Aegilops* spp. is the closest genus to wheat (Triticum spp.) and is considered as a potential source of genetic variation for wheat breeding programs (Shavrukov et al., 2009; Kiani et al., 2021a). *Aegilops cylindrica* Host is a wild species, sharing a common genome (D) with common wheat (*Triticum aestivum* L.) (Kiani et al., 2021a). It is commonly known as Jointed goat grass and is an amphidiploid (2n = 4x = 28; CCDD) originated from the ancestors of *Aegilops markgrafii* (Greuter) Hammer (2n = 2x= 14; CC) and *Aegilops tauschii* Coss. (2n = 2x = 14; DD) species. Arabbeigi et al. (2018) noted that this species represents a valuable source of salt tolerance genes.

Improving salinity tolerance in wheat plant breeding programs requires crucial characterization and screening of germplasm (Kiani et al., 2015). The identification of ideal genotypes for salinity tolerance involves screening numerous genotypes and isolating the best-adapted ones. Evaluation of genetic plasma under field conditions is associated with a number of difficulties (Arzani, 2008), which is why it is preferred to screen germplasm under controlled conditions for salinity stress (Kiani et al., 2015). Therefore the aim of this study was to investigate the effect of six concentrations of sodium chloride (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM) on seed germination and seedling characteristics of *Aegilops cylindrica* Host. under laboratory condition.

MATERIALS AND METHODS

The effect of sodium chloride (NaCl) at six different salinity levels (50, 100, 150, 200, 250, 300 mM) on seed germination and early seedlings growth parameters was studied in *Aegilops cylindrica* Host. Deionized water was used as control variant. An experiment was conducted with seeds from five Bulgarian populations of *Aegilops cylindrica* Host (Table 1), taken from an experiment established at IPGR-Sadovo in 2023, Bulgaria. For each variant of experiment, two replicates of 25 seeds were germinated on rolled filter paper (Grade FT 55) with 20 ml of the respective test solutions. The papers were replaced every 2 days to prevent salt accumulation. The rolled paper with seeds was placed in sealed plastic bags to prevent moisture loss. The seeds were allowed to germinate in the dark at 20±1 ◦C for 8 days. Seeds were considered germinated when the radicle had extended at least 2 mm. The number of germinated seeds was recorded daily until a constant count was achieved. From the germination counts several germination characteristics were studied including germination energy (GE, %), germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day-1), mean germination time (MGT, day) and relative injury rate (RIR). CVG was calculated according to Kader and Jutzi (2004), GRI and MGT according to the formula of Kader (2005), while RIR according to Li (2008).

On the eighth day of the experiment, ten seedlings were randomly selected from each treatment for the measurement of shoot and root

Table 1. Populations of *Aegilops cylindrica* Host included in the study

№	Accession number	Species	Geographical coordinates of the habitat	Altitude, m
1	BGR43676	Aegilops cylindrica Host	42° 2′ 6.10″ N, 24° 50′ 40″ E	208
2	BGR44228	Aegilops cylindrica Host	41° 37′ 37.90″ N, 25° 41′ 8.60″ E	172
3	BGR44229	Aegilops cylindrica Host	41° 38′ 48.10″ N, 25° 41′ 39″ E	312
4	BGR44230	Aegilops cylindrica Host	41° 54′ 29″ N, 24° 46′ 44″ E	527
5	BGR44234	Aegilops cylindrica Host	41° 43′ 53.20″ N, 24° 25′ 22.20″ E	700

length (LSh and LR in cm) and fresh weight of shoot and root (FWSh and FWR in mg/plant). Additionally, data for dry weight of shoot and root (DShW and DRW in mg/plant) were recorded after drying in an oven at 80°C for 24 hours.

Seedling height reduction was determined using the method described by Islam and Karim (2010), and salt tolerance (ST) was estimated according to Mujeeb-ur-Rahman et al. (2008).

Data were analysed by analysis of variance (ANOVA) and Duncan‘s multiple test using the statistical programme SPSS 19.0.

RESULTS AND DISCUSSIONS

Salinity stress can delay or prevent the germination of high-quality seeds, resulting in crop loss. Rapid seed germination and subsequent seedling establishment are crucial factors in determining crop production and yield under salinity stress (Uçarlı, 2020).

In our study, CVG as an indicator for the rapidity of germination decreased with increasing the concentration of NaCl. The maximum reduction was found at the high salinity level of 250 mM NaCl). Mean CVG was inhibited with salinity compared with control by 54.45% at 250 mM NaCl (Table 2).

Higher concentrations of sodium chloride negatively affect the germination rate during each day of the germination period. The lower values recorded for GRI indicate lower and slower seed germination under salinity (Table 2).

Balasubramaniam et al. (2023) noted that salinity increases the external osmotic potential, which reduces water uptake during germination. Excess sodium and chloride ions can have toxic effects on embryo viability, which can affect seed germination. In our study increasing salt stress considerably reduced the germination percentage. The rate of germination loss associated with increased salinity was significantly at the high concentrations (250 mM NaCl), while an insignificant change in seed germination was recorded at the lowest concentration of 50 mM NaCl, respectively. At the highest NaCl concentration (300 mM NaCl), no normally germinated seeds were recorded in all samples included in the study. Only weak root system development was observed (Table 2). The findings of our study support the results obtained by Mustafa et al. (2006), who discovered that seed germination was partially or completely inhibited in all populations of *Aegilops biuncialis* and *Aegilops triuncialis* as salinity levels increased.

A positive relationship was found between mean seed germination time and applied doses

Table 2. Variation in germination characteristics at different NaCl salinity levels

Concentration of NaCl, mM	CVG	GRI,% day-1	MGT, day	GE, %	G,%
0	31.42d	31.05d	3.26a	73.00a	88.00c
50	29.26cd	28.36d	3.45a	64.00a	85.00c
100	27.38c	23.87cd	3.68a	52.00b	75.00bc
150	23.27b	18.11bc	4.37b	37.00bc	66.00bc
200	21.99b	14.12b	4.69b	29.00b	54.00b
250	17.14a	1.35a	6.00c	0.00a	5.00a
300	-	-	-	0.00a	0.00a
Average	25.07	19.48	4.24	36.43	53.29

CVG-coefficient of velocity of germination, GRI-germination rate index, MGT-mean germination time, GE-germination energy, G-germination

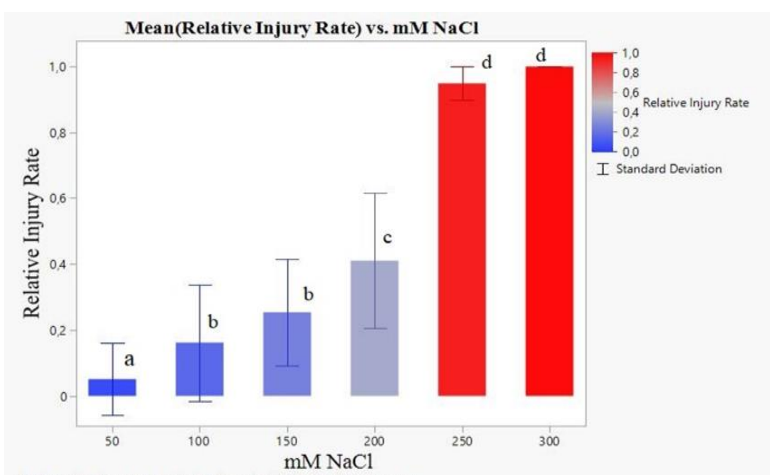
Means within a column that have different superscript letters (a-d) are significant different from each other (Duncan's multiple range test, p≤0.05) of sodium chloride, respectively increasing salt concentration from 0 to 250 mM NaCl prolonged the MGT (Table 2). There was no significant ($P \geq 0.05$) difference in mean germination time between control and lower salinities (50 mM and 100 mM). On the other hand, the increase in mean germination time was more pronounced at higher salinities (Table 2).

Recent studies confirm the above results in other cereal crops as wheat (Gholizadeh et al. 2021; Vaccarella et al., 2024), oat (Bai et al., 2018; Islam et al., 2023), sorghum (Rajabi et al., 2020), rice (Hussain et al., 2022), rye (Pascaru et al., 2015; Desheva et al., 2019).

Relative injury rate is used to determine the degree of injury on seed of any plants during germination stage which is caused by the salinity (Yohannes et al., 2020). Figure 1 depicts that *Aegilops* seedlings growing in all salts recorded significant ($p \leq 0.05$) increment in relative injury rate with the increase of salinity levels. It was found to be most severely damaged when salinity levels between 250 and 300 mM NaCl were applied, but there were no significant differences in the relative injure rates between both concentrations.

Salinity significantly reduced all seedling characteristics of the investigated populations of *Aegilops cylindrica* Host. In general, the reduction in shoot length was greater than the reduction in root length, especially at concentrations above 200 mM NaCl (Table 3 and Figure 2). The results presented in Fig. 2 indicate that the reduction in root and shoot growth ranged from 0.11 to 0.88 for the root and from 0.16 to 1 for the shoot, respectively, in comparison to the controls. Therefore, it can be concluded that the growth of shoots was more sensitive to salt stress than roots, particularly at higher salt concentrations. Hadjadj et al. (2022, 2023) noted that, less adversely reduction of root growth relative to shoot growth under salt stress may be attributed to less accumulation of sodium ions in the roots which improves crop tolerance to salt during the initial growth stage. It is suggested that the ability of plants to develop extensive root systems can be used as a strategic approach to obtain water from deeper soil layers. This enables species to develop under conditions of limited soil moisture, thereby increasing plant productivity and drought tolerance (Lynch, 2013).

Application of increasing concentrations of NaCl had a negative effect on shoot and root fresh weight, and shoot and root dry weight. The reduction in shoot fresh and dry weight compared to root fresh and dry weight was greatest at the



Means that have different superscript letters (a-d) are significant different from each other (Duncan's multiple range test, P≤0.05)

Figure 1. Variation in Relative Injury Rate of *Aegilops cylindrica* seeds at different NaCl salinity levels

highest salinities (Table 3). It has been reported that the plants exhibited a reduction in their fresh weights in proportion to the increase in Na+ concentration. This could be indicative of an ionic effect being manifested (Jamil et al., 2006).

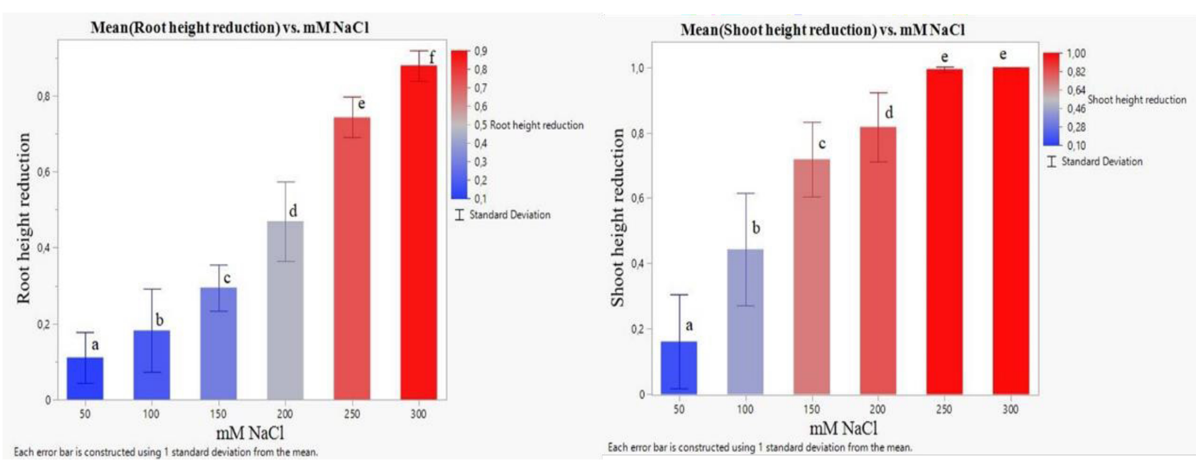
The results of the present study are consistent with those of Arabbeigi et al. (2014) and Kiani et al. (2015, 2021), which found that shoot fresh and dry weights as well as root fresh and dry weight of *Aegilops cylindrical* Host are significantly affected by salinity stress. Kiani et al. (2015) also noted that these results are to be expected, given that, the impairment of crop productivity resulting from excess salinity is often associated with nutritional imbalance caused by the excess of Na+ and Cl-, which results in either a lower uptake of potassium or impaired internal distribution of one or more of these ions.

The calculated mean salt tolerance indices (STI) at different salinity levels are presented

Table 3. Variation in seedling characteristics at different NaCl salinity levels

Concentration of NaCl, mM	Length of root, cm	Length of shoot, cm	Seedling length, cm	Fresh weight per root, mg/plant	Fresh weight per shoot, mg/plant	Dry weight per root, mg/plant	Dry weight per shoot, mg/plant
0	10.51g	9.85f	20.68g	23.53e	47.60f	2.49b	5.63f
50	9.49f	8.42e	18.47f	20.27d	41.37e	2.29b	4.69e
100	8.36e	6.05d	14.86e	16.23c	31.29d	2.19b	3.53d
150	6.95d	3.60c	11.26d	13.89c	18.10c	2.49b	2.29c
200	4.51c	1.64b	6.80c	8.28b	7.59b	1.09a	1.18b
250	2.51b	0.39a	3.57b	3.93a	1.70a	0.51a	0.34a
300	1.14a	0.00a	1.47a	1.77a	0.00a	0.28a	0.00a
Average	6.21	4.29	11.01	12.56	21.10	1.62	2.54

Means within a column that have different superscript letters (a-f) are significant different from each other (Duncan's multiple range test, p≤0.05)



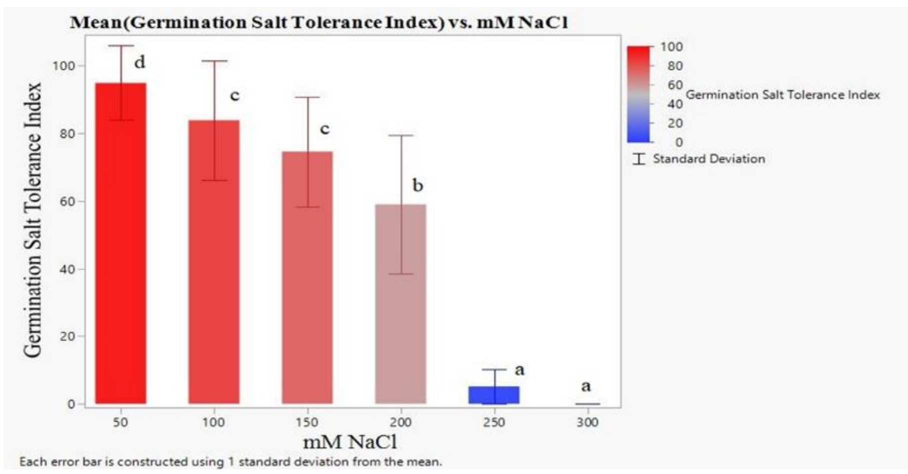
Means that have different superscript letters (a-f) are significant different from each other (Duncan's multiple range test, p≤0.05)

Figure 2. Variation in root and shoot height reduction of *Aegilops cylindrica* seeds at different NaCl salinity levels

in Figures 3-5. The STI values indicated a large difference in salt tolerance of *Aegilops cylindrica* Host depending on salinity levels. Their values decreased respectively with the increasing salinity level. At salinity levels in the range of 50-100 mM NaCl, *Aegilops cylindrica* seeds showed very high germination tolerance, at 150-200 mM NaCl - between high and medium tolerance, and at 250 mM NaCl - low tolerance. Seeds were classified as susceptible to germination when applied at 300 mM NaCl.

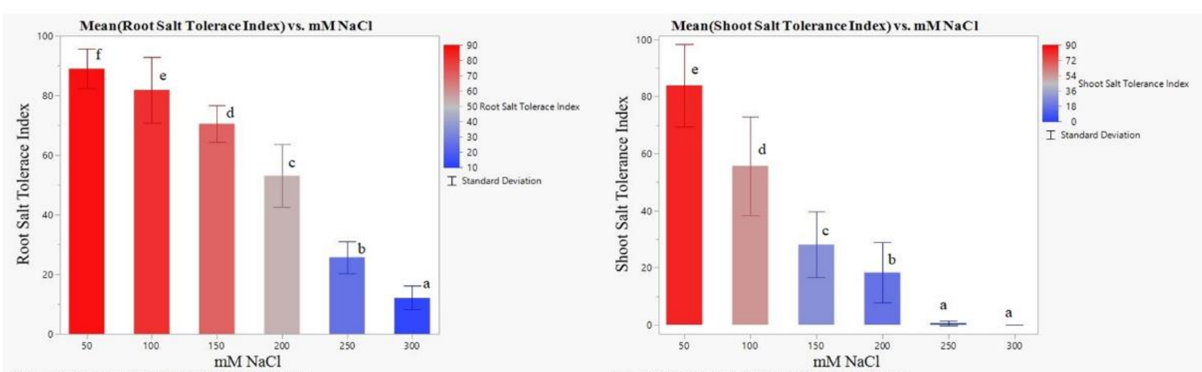
Salt tolerance indicates varied with a range of 12.08 to 88.92 for the root and a range of 0 to 83.87 for the shoot (Figure 4). The results of the study indicate that *Aegilops cylindrica* exhibited low to very low tolerance with respect to root growth and, correspondingly, very low tolerance and sensitivity to shoot growth at salinity levels of 250-300 mM NaCl.

At salinity levels of 200-300 mM NaCl solution, the *Aegilops cylindrica* exhibited low to very low tolerance to seedling growth (Figure 5).



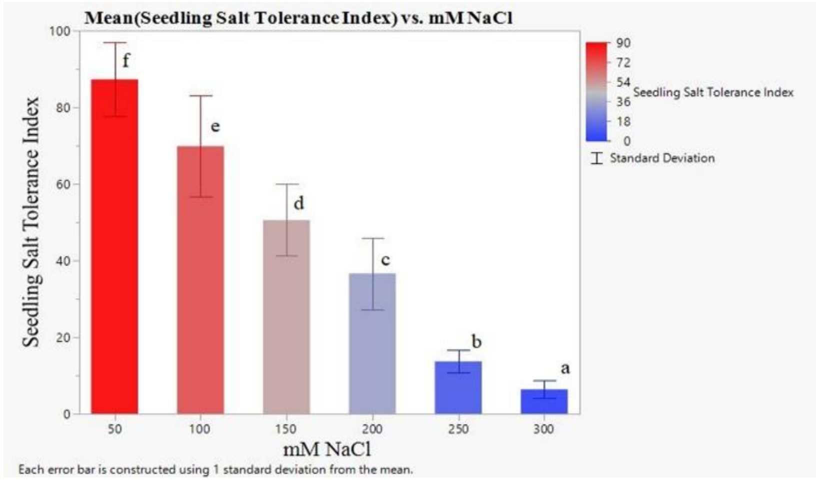
Means that have different superscript letters (a-d) are significant different from each other (Duncan's multiple range test, P≤0.05)

Figure 3. Variation in Germination Salt Tolerance Index of *Aegilops cylindrica* seeds at different NaCl salinity levels



Means that have different superscript letters (a-f) are significant different from each other (Duncan's multiple range test, p≤0.05)

Figure 4. Variation in Root and Shoot Tolerance Index of *Aegilops cylindrica* seeds at different NaCl salinity levels



Means that have different superscript letters (a-f) are significant different from each other (Duncan's multiple range test, p≤0.05)

Figure 5. Variation in Seedling Tolerance Index of *Aegilops cylindrica* seeds at different NaCl salinity levels

CONCLUSION

Application of increasing concentrations of sodium chloride (50-300 mM NaCl) on the *Aegilops cylindrica* seeds prolonged mean germination time and suppressed germination characteristics (germination rate, coefficient of velocity of germination, germination rate index, germination index and relative injury rate) and seedling growth characteristics (shoot and root length, shoot and root fresh and dry weight). The growth of shoots was more sensitive to salt stress than roots, particularly at salt concentrations above 200 mM NaCl.

Aegilops cylindrica Host showed very high to high germination tolerance when treated with 50-150 mM NaCl solution, medium tolerance at 200 mM NaCl solution, very low tolerance at 250 mM NaCl solution and susceptible to germination at 300 mM NaCl solution. At salinity levels of 200-300 mM NaCl solution, the *Aegilops cylindrica* exhibited low to very low tolerance to seedling growth.

Acknowledgments

This work was financially supported by the Bulgarian National Science Fund, Ministry of Education and Science, project КП-06-ПН76/3 „Study of the genetic diversity of *Aegilops* species in the flora of Bulgaria"

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Received: January, 21, 2024; Approved: April, 26, 2025; Published: June, 2025