

STUDY OF SENSITIVITY TO DIFFERENT CONCENTRATIONS OF KCl IN SAMPLES OF THE SPECIES *Aegilops geniculata* Roth

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Abstract

The study investigated the sensitivity of *Aegilops geniculata* Roth to salinity stress from varying concentrations of potassium chloride (KCl) during seed germination and early seedling development. The research utilized five accessions of *Aegilops geniculata* Roth sourced from the Genebank of Bulgaria's working collection. A completely randomized experimental design was employed, incorporating seven KCl concentrations (0 mM - control, 50, 100, 150, 200, 250, and 300 mM), with two replicates per treatment. Results revealed significant adverse effects on germination rates and seedling traits, except for an extended germination period under saline conditions. Notably, shoot growth was more hindered by high salinity than root development, particularly at 300 mM KCl. At this concentration, all genotypes except BGR43677 failed to germinate or sustain shoot and root growth. In contrast, the BGR43677 genotype exhibited exceptional salt tolerance, maintaining viable germination and promoting both shoot and root growth under extreme salinity. These findings underscore the variability in salt tolerance among *A. geniculata* accessions and highlight BGR43677 as a promising candidate for further study in saline environments.

Key words: *Aegilops geniculata* Roth, salinity, KCl, sensitivity, germination, seedling growth.

INTRODUCTION

Salinity is a major environmental stress factor affecting plant growth and productivity worldwide. It is estimated that over 20% of irrigated land globally is affected by salinity, resulting in significant economic losses (Machado et al., 2017; Singh et al., 2024). Salinization arises primarily from natural processes such as weathering of rocks and deposition of salts by wind and rain, but is often exacerbated by human activities like irrigation with saline water, inappropriate land management practices, and fertilizer overuse (Tarolli et al., 2024). The accumulation of salts in the soil disrupts plant water uptake, ionic balance, and nutrient acquisition, leading to physiological and biochemical imbalances that ultimately limit growth and survival (Balasubramaniam et al., 2023; Atta et al., 2023; Zhou et al., 2024).

Aegilops geniculata Roth, commonly known as ovate goatgrass, is an annual grass native to the Mediterranean region, now naturalized in many

parts of the world, including North America. It is often found in disturbed habitats, agricultural fields, and rangelands (Winston et al., 2014). Its adaptability to a wide range of environmental conditions suggests some level of tolerance to stress, including salinity (Guo et al., 2022). Understanding the salinity tolerance of *Aegilops geniculata* Roth is important for several reasons:

- ✓ Ecological Implications: Salinity levels can influence the distribution and abundance of *Aegilops geniculata* Roth in different environments. Knowledge of its tolerance limits can help predict how this species will respond to increasing salinization in various habitats;
- ✓ Agricultural Management: *Aegilops geniculata* Roth can be a problematic weed in wheat fields. Understanding its salinity tolerance relative to wheat can inform management strategies aimed at minimizing its impact;
- ✓ Genetic Resources: *Aegilops* species are closely related to wheat and represent a valuable source of genetic diversity for improving wheat's tolerance to abiotic

stresses, including salinity (Darko et al., 2020). Studying the salinity response of *Aegilops geniculata* Roth may provide insights into potential genes or mechanisms that can be transferred to wheat.

Salinity stress is complex, involving osmotic effects, ionic toxicity, and nutritional imbalances (Farooq et al., 2017; Zhao et al., 2020; Maryum et al., 2022). Sodium chloride (NaCl) is the most abundant salt in saline soils, but other salts, such as potassium chloride (KCl), magnesium sulfate (MgSO₄), and calcium chloride (CaCl₂), can also contribute significantly to the overall salinity (Waisel, 1972). While NaCl is often the focus of salinity studies, the specific effects of other salts, such as KCl, on plant growth and development are less well understood. Potassium (K⁺) is an essential macronutrient for plants, playing crucial roles in enzyme activation, osmoregulation, and protein synthesis (Hasanuzzaman et al., 2018; Papadakis et al., 2023; Islam et al., 2025). However, high concentrations of K⁺ can be toxic, disrupting nutrient uptake and cellular functions (Pantha et al., 2023).

This study aims to examine how varying concentrations of potassium chloride (KCl) influence the germination and early growth stages of *Aegilops geniculata* Roth, assessing the species' sensitivity to salinity stress under controlled conditions.

MATERIALS AND METHODS

The study was performed on five Bulgarian populations of *Aegilops geniculata* Roth collected in 2024. A fully randomized layout was adopted, comprising seven KCl treatments, each replicated twice. The KCl concentrations applied were 0 mM (control), 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM, prepared by dissolving analytical grade potassium chloride in distilled water.

Germination Test

For each treatment, two replicates of 50 seeds were surface sterilized (1 % NaOCl for 5 min, followed by rinses with distilled water) and placed on a double layer of filter paper moistened with 20 mL of the corresponding KCl solution. Petri dishes were sealed in plastic bags

to minimise evaporation and incubated in a growth chamber set at 20°C, 16 h light/8 h dark. Germination was recorded daily for 14 days; a seed was scored as germinated when the radicle extended ≥ 2 mm. The following germination parameters were calculated: Final Germination Percentage (GP), Germination Rate Index (GRI) and Mean Germination Time (MGT) (Kader, 2005).

Seedling Growth Assessment

After 14 day germination period, ten seedlings were randomly chosen from each replicate to evaluate the following growth traits: Root length (measured from the radicle emergence point to the tip of the longest root (mm) with a ruler), Shoot length (measured from the coleoptile emergence point to the tip of the longest leaf - mm), Fresh biomass (fresh weight of shoots and roots recorded immediately after harvest using an analytical balance - mg), Dry Biomass (shoots and roots were oven dried at 80°C for 24 h until constant weight, then weighed - mg). Relative dry weight and seedling height reduction was quantified by Kandil et al. (2012).

Statistical Treatment

All data were subjected to analysis of variance (ANOVA) with SPSS v.22. Treatment means were separated using Duncan's multiple range test at a 5 % significance level.

To elucidate genotype performance across the salinity gradient and to identify the most tolerant accession, the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI) were computed as described by Pour Aboughadareh et al. (2019).

RESULTS AND DISCUSSIONS

The germination of *A. geniculata* Roth seeds was profoundly inhibited by increasing concentrations of KCl, demonstrating a clear dose-dependent response that was statistically significant ($p < 0.05$) (Table 1). As KCl concentration rose, both the percentage and rate of germination declined markedly across all tested seed accessions, while the mean germination time (MGT) increased, indicating not only reduced germination success but also a substantial delay in the initiation and progression of germination under salt stress.

Table 1. Influences of KCl salinization on germination characteristics in five populations of *Aegilops geniculata* Roth

Accession number	Concentration of KCl, mM	GRI, % day ⁻¹	MGT, day	GE, %	G, %
BGR43663	0	35.83b	2.31a	80.00c	80.00b
BGR43677	0	38.50b	2.21a	80.00c	80.00b
BGR43704	0	35.00b	2.37a	80.00c	80.00b
BGR43496	0	43.06b	3.75b	60.00b	85.00b
BGR43682	0	20.83a	2.12a	40.00a	40.00a
Average		34.65	2.56	68.00	73.00
BGR43663	50	30.88b	3.19c	70.00b	80.00bc
BGR43677	50	24.17b	2.85bc	65.00b	65.00b
BGR43704	50	32.50b	2.62ab	80.00b	80.00bc
BGR43496	50	33.08b	3.29c	75.00b	85.00c
BGR43682	50	13.33a	2.33a	30.00a	30.00a
Average		26.79	2.86	64.00	68.00
BGR43663	100	18.33b	4.27b	70.00b	75.00c
BGR43677	100	20.12b	3.36a	50.00b	55.00b
BGR43704	100	31.67c	3.12a	60.00b	80.00cd
BGR43496	100	23.76b	4.54b	60.00b	85.00d
BGR43682	100	2.00a	5.00b	10.00a	10.00a
Average		19.18	4.06	50.00	61.00
BGR43663	150	14.91c	4.78b	60.00d	70.00d
BGR43677	150	8.84b	5.00b	15.00b	35.00b
BGR43704	150	20.45d	4.75b	50.00d	80.00e
BGR43496	150	10.66bc	4.91b	35.00c	50.00c
BGR43682	150	0.00a	0.00a	0.00a	0.00a
Average		10.97	3.89	32.00	47.00
BGR43663	200	4.00ab	7.66c	0.00a	30.00b
BGR43677	200	5.05ab	5.25b	10.00a	25.00b
BGR43704	200	17.68c	4.87b	30.00b	60.00c
BGR43496	200	9.82b	7.50c	10.00a	70.00c
BGR43682	200	0.00a	0.00a	0.00a	0.00a
Average		7.31	5.06	10.00	37.00
BGR43663	250	2.36b	8.50c	0.00	20.00b
BGR43677	250	2.80b	6.83b	0.00	20.00b
BGR43704	250	6.43c	6.25b	0.00	40.00c
BGR43496	250	0.00a	0.00a	0.00	0.00a
BGR43682	250	0.00a	0.00a	0.00	0.00a
Average		2.32	4.32	0.00	16.00
BGR43663	300	0.00	0.00	0.00	0.00
BGR43677	300	1.43	9.47	0.00	10.00
BGR43704	300	0.00	0.00	0.00	0.00

germination (G, %), germination rate index (GRI, % day⁻¹), mean germination time (MGT, day)

The mean values in the same column followed by the same letters do not differ significantly at $p \leq 0.05$ according to Duncan's test.

Under optimal, non-stressful control conditions (0 mM KCl), germination performance was strongest, with the BGR43496 accession achieving the highest germination percentage at 85% and a germination rate index of 43.06% per day - values significantly superior to those of the poorest-performing accession, BGR43682. However, as salinity intensified, this advantage diminished progressively. At 200 mM KCl, germination was severely suppressed, with BGR43682 exhibiting complete inhibition (0% germination), whereas BGR43496 still maintained a relatively resilient 70% germination rate. The inhibitory effects escalated at 250 mM KCl and approached near-

total suppression at 300 mM, where only a single accession, BGR43677, managed to germinate, and even then only at a minimal level of 10%. Concomitantly, salt stress drastically slowed the germination dynamics: MGT extended from a rapid 2.12 days in the control to a markedly delayed 9.47 days at 300 mM KCl, underscoring the physiological constraints imposed by high salinity. These findings highlight the sensitivity of *Aegilops geniculata* seeds to potassium chloride-induced osmotic and ionic stress and reveal notable inter-accession variability in salt tolerance, suggesting potential for selecting more resilient genotypes in saline environments (Prasad et al., 2018; Solangi et al., 2018; Tebini et al., 2022; Bibi et al., 2025).

Elevated concentrations of potassium chloride (KCl) exert a pronounced inhibitory effect on seedling development, operating in a dose-dependent manner ($p < 0.05$), with escalating salinity progressively impairing both root and shoot elongation. Shoot growth is consistently more vulnerable to KCl stress than root growth across all genotypes evaluated, reflecting a differential response to osmotic and ionic imbalances (Shoukat et al., 2020; Gul et al., 2024; Raneenga et al., 2024). As outlined in Table 2, varying KCl concentrations elicit substantial divergence in root height reduction (RHR), shoot height reduction (ShHR), and overall seedling height reduction (SHR), underscoring the complex interplay between salinity and genotype-specific resilience. At 50 mM KCl, a moderate stress threshold, genotype BGR43704 exhibits the smallest reductions in RHR, ShHR, and SHR, suggesting a relatively robust adaptation to early salinity stress. However, as KCl concentration escalates to 100 mM, root growth inhibition surges to an average of 42%, while shoot elongation plummets by 49%, with BGR43677 registering the highest reduction- albeit not significantly distinct from BGR43704. This dynamic shifts further under 150 mM KCl, where root growth inhibition spans 32% to 79% (mean: 59%), paralleled by a starkly broader shoot inhibition range of 31% to 100%. BGR43677 demonstrates the least suppression, signalling its emergent resilience, whereas BGR43682 emerges as the most sensitive genotype.

Table 2. Variation in the degree of reduction in the length of roots, shoots and seedlings at different levels of KCl salinity in populations of the species *Aegilops geniculata* Roth

Number of accession	Concentration of KCl, mM	RHR	ShHR	SHR
BGR43663	50	0.35c	0.38c	0.37c
BGR43677	50	0.14ab	0.04a	0.09a
BGR43704	50	0.09a	0.01a	0.05a
BGR43496	50	0.24bc	0.25b	0.25b
BGR43682	50	0.22c	0.44c	0.38c
Average		0.23	0.23	0.23
BGR43663	100	0.45b	0.49b	0.47b
BGR43677	100	0.27a	0.17a	0.22a
BGR43704	100	0.29a	0.32a	0.30a
BGR43496	100	0.40b	0.58b	0.50b
BGR43682	100	0.66c	0.88c	0.79c
Average		0.42	0.49	0.46
BGR43663	150	0.51b	0.54b	0.53b
BGR43677	150	0.32a	0.31a	0.32a
BGR43704	150	0.62c	0.48b	0.55b
BGR43496	150	0.67d	0.94c	0.82c
BGR43682	150	0.79e	1.00c	0.91d
Average		0.59	0.66	0.63
BGR43663	200	0.74a	0.64b	0.68b
BGR43677	200	0.72a	0.35a	0.52a
BGR43704	200	0.78a	0.75b	0.77c
BGR43496	200	0.72a	0.95c	0.85c
BGR43682	200	1.00b	1.00c	1.00d
Average		0.80	0.74	0.77
BGR43663	250	0.82ab	0.79b	0.80b
BGR43677	250	0.74a	0.75a	0.75a
BGR43704	250	0.85b	0.92c	0.89c
BGR43496	250	0.83ab	1.00d	0.92c
BGR43682	250	1.00c	1.00d	1.00d
Average		0.85	0.90	0.88
BGR43663	300	0.90b	1.00b	0.96b
BGR43677	300	0.76a	0.79a	0.78a
BGR43704	300	0.90b	1.00b	0.95b
BGR43496	300	1.00c	1.00b	1.00c
BGR43682	300	1.00c	1.00b	1.00c
Average		0.92	0.96	0.94

RHR-root length reduction rate, ShHR-shoot length reduction rate, SHR-shoot length reduction rate
Mean values in the same column followed by the same letters are not significantly different at $p \leq 0.05$, according to Duncan's test.

Under extreme stress at 200 mM KCl, both BGR43677 and BGR43496 experience a 72% root length reduction, while BGR43682 undergoes complete root growth cessation, reflecting its inability to withstand high salinity. Shoot growth inhibition at this concentration varies markedly: BGR43677 experiences a modest 35% decline, in contrast to total inhibition in BGR43682. At the highest tested concentrations (250-300 mM KCl), BGR43677 exhibits 75% shoot suppression at 250 mM, yet

retains marginal viability, whereas BGR43496 and BGR43682 face total growth collapse. Across all salinity thresholds and measured parameters, BGR43677 consistently displays minimal growth reduction, cementing its status as the most salt-tolerant genotype under KCl-induced stress (Table 2). These findings highlight the critical role of genotype-specific mechanisms in mitigating salinity effects (Sadiq et al., 2024), with BGR43677 serving as a potential model for enhancing crop resilience in high-salinity environments.

KCl-induced salinity exerts a pronounced detrimental effect on the biomass accumulation of *Aegilops geniculata* seedlings, markedly decreasing the relative dry weight of shoots, roots, and the whole plant (Figure 1 and Figure 2). This decline can be attributed primarily to the combined action of ionic toxicity - resulting from excessive accumulation of potassium and chloride ions - and the accompanying osmotic stress that impairs water uptake and cellular metabolism. Among the genotypes examined, BGR 43677 displayed the greatest resilience under severe KCl salinity, maintaining the highest relative dry weights for roots, shoots, and whole seedlings relative to the other genotypes. These findings are consistent with previous reports documenting the inhibitory influence of elevated KCl concentrations on dry matter production across a range of species (Solang et al., 2014; Gebremedhn et al., 2020; Valchinova et al., 2025).

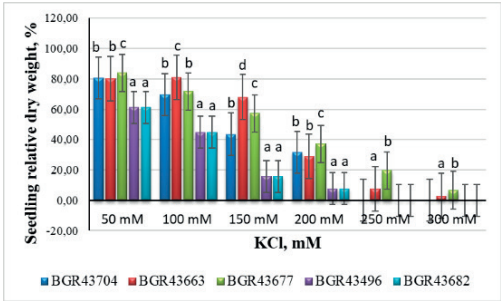


Figure 1. Variation of seedling relative dry weight in five accessions from *Aegilops geniculata* Roth at different levels of salinity stress

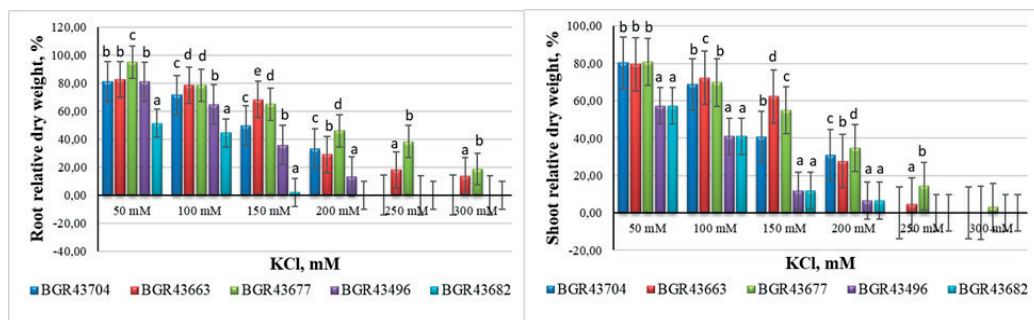


Figure 2. Variation of root and shoot relative dry weights in five accessions from *Aegilops geniculata* Roth at different levels of salinity stress

To pinpoint the genotype with the highest resilience to salinity, our analysis utilized two mathematical indicators: the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI).

Adapted from the research by Pour-Aboughadareh et al. (2019), these indices were calculated using shoot dry weight measurements obtained from both non-stress (normal) and salt-stress (saline) environments.

The SSI evaluates the degree of yield loss a genotype experiences under saline conditions, where smaller values denote superior tolerance. Conversely, the STI assesses a genotype's capacity to sustain high yields under stress, with larger values reflecting enhanced tolerance. Therefore, the genotypes demonstrating a combination of a low SSI and a high STI were identified as possessing the greatest capacity to withstand salinity.

According to the data presented in Table 3, which details the variation in these indices across different salinity levels, genotype BGR43663 emerged as particularly resilient under potassium chloride salinity concentrations ranging from 50 to 200 mM, demonstrating consistently low SSI and high STI values.

At more extreme salinity levels (250-300 mM), however, genotype BGR43677 exhibited the most favourable response, registering the lowest SSI values and relatively elevated STI values among the tested genotypes, suggesting a superior capacity to maintain physiological function and growth under intense salt stress.

Table 3. Variation in the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI) at different levels of KCl salinity in populations of the species *Aegilops geniculata* Roth

Genotype	SSI	STI	Genotype	SSI	STI
50 mM KCl					
BGR43704	0.61	0.21	BGR43704	0.67	0.18
BGR43663	0.64	1.37	BGR43663	0.60	1.25
BGR43677	0.60	0.64	BGR43677	0.65	0.55
BGR43496	1.31	1.48	BGR43496	1.28	1.05
BGR43682	1.79	0.19	BGR43682	1.83	0.07
150 mM KCl					
BGR43704	0.90	0.11	BGR43704	0.85	0.08
BGR43663	0.57	1.08	BGR43663	0.89	0.48
BGR43677	0.68	0.44	BGR43677	0.80	0.27
BGR43496	1.34	0.31	BGR43496	1.15	0.17
BGR43682	1.52	0.00	BGR43682	1.23	0.00
250 mM KCl					
BGR43704	1.04	0.00	BGR43704	1.01	0.00
BGR43663	0.99	0.08	BGR43663	1.01	0.00
BGR43677	0.89	0.11	BGR43677	0.97	0.02
BGR43496	1.04	0.00	BGR43496	1.01	0.00
BGR43682	1.04	0.00	BGR43682	1.01	0.00
300 mM KCl					
BGR43704	1.04	0.00	BGR43704	1.01	0.00
BGR43663	0.99	0.08	BGR43663	1.01	0.00
BGR43677	0.89	0.11	BGR43677	0.97	0.02
BGR43496	1.04	0.00	BGR43496	1.01	0.00
BGR43682	1.04	0.00	BGR43682	1.01	0.00

CONCLUSIONS

This study provides valuable information on the sensitivity of *Aegilops geniculata* Roth to salinity stress, specifically to different concentrations of KCl, during the processes of germination and seedling growth. The findings indicate a significant negative correlation between the concentration of KCl and key growth metrics, including germination, germination speed, root development, and shoot elongation. In order to further elucidate the underlying physiological responses, future research endeavours should prioritise unravelling the molecular mechanisms driving the observed salt sensitivity, as well as assessing the species' performance under diverse saline conditions and composite salt regimes.

Furthermore, the identification of BGR43677 as a resilient genotype emphasises its potential as a prime candidate for salt-tolerant breeding initiatives, tailored to thrive in high-salinity environments.

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