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Influence of KCl salt on the germination and early seedling characteristics of *Aegilops biuncialis* Vis.

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Abstract: The aim of the study was to determine the effect of KCl salinity stress on germination characteristics and early seedling growth of 5 populations of Aegilops biuncialis Vis. maintained in the ex situ collection of the National Genebank of Bulgaria. Six different concentrations of KCl (50, 100, 150, 200, 250 and 300 mM) were used as treatments and deionised water as control. To determine the salinity tolerance, the following germination characteristics were studied: germination percentage (%), germination velocity coefficient, germination rate index (% day^{-1}) and mean germination time (day). Shoot and root length (cm), shoot and root fresh weight (mg plant^{-1}) and shoot and root dry weight (mg plant^{-1}) were measured eight days after germination. Relative injury coefficient, root and shoot height reduction and salinity tolerance index were also calculated. The results showed that salinity prolonged the mean germination time and significantly decreased the studied germination and seedling growth characteristics with increasing concentration of KCl. The inhibitory effect of increasing KCl concentrations was stronger on root height reduction compared to shoot height reduction in all populations studied except the population of BGR44617. The highest relative injury coefficient was recorded for BGR44617, BGR44900 and BGR43660 when treated with 300 mM KCl solution, respectively, these accessions were classified as susceptible for germination and seedling growth. BGR43667 was relatively the most tolerant to salinisation with KCl at the seedling growth stage.

Key words: *Aegilops biuncialis* Vis.; germination; salinity; seedling growth; tolerance

INTRODUCTION

Soil salinity is a condition in which the soil solution has too high content of water-soluble salts, including sodium (Na+), chlorine (Cl-), potassium (K+), sulphates (SO42-) and others. While ions such as potassium and sulphate also act as nutrients for plants, sodium and chloride ions are not considered nutrients and so when talking about salinity we usually focus only on their concentration. The factors that lead to soil salinization may be part of natural processes related to the soil composition, its formation, the climate and the topography of an area. Equally important are factors related to human activity and, in particular, certain poor agricultural practices over many years. Salinity leads to accelerated soil degradation and declining soil fertility, which seriously threatens food production security and ecology. Today soil salinity represents one of the most significant global threats to agricultural production, with approximately 20% of arable land and 33% of irrigated land experiencing salinity-induced damage. Such conditions result in considerable reductions in crop yield and quality (Chourasia et al., 2021; Jamshidi et al., 2023). It has been documented that approximately 1,128 million hectares of land globally are impacted by salinity and soil sodicity. Based on existing research, the extent of saline-affected soil on Earth is rising over time.

This implies that by 2050, the current area experiencing salinity stress is likely to be approximately triple its current size (FAO, 2021). In Bulgaria, about 35 500 ha of arable land are registered as affected by salinization processes, 252 ha of which are salinized with normal soda and chlorides. The processes mainly affect the districts of Burgas, Varna, Veliko Tarnovo, Pleven, Plovdiv, Sliven, Stara Zagora and Yambol.

Developing salt-tolerant cultivars is the preferred breeding strategy for improving wheat adaptation to salinity stress. The wild relatives of cultivated wheat, namely *Aegilops* species, are postulated as a source of genetic resources that could enhance the abiotic stress tolerance of cultivated wheat species (Ahmadi et al., 2018). These germplasm resources, having evolved to thrive in a range of environmental contexts over an extended period, offer promising avenues for improving wheat (Darko et al., 2020; Kiani et al., 2021).

Seed germination represents the initial phase of a plant's life cycle, and the capacity of plants to tolerate salt at this stage is of paramount importance for the successful growth of plants under saline conditions. In the presence of salt stress, seed germination may be impeded due to a reduction in seed viability or a delay in germination. The former is indicated by a decline in germination percentage (G, %), while the latter is evidenced by a reduction in shoot and root length and weight, respectively (Darko et al., 2020).

The aim of the study was to determine the effect of KCl salinity stress on germination characteristics and early seedling growth of 5 populations of *Aegilops biuncialis* Vis. maintained in the *ex situ* collection of the National Genebank of Bulgaria.

MATERIAL AND METHODS

The effect of potassium chloride (KCl) at six different salt concentrations (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM) on seed germination and early seedling growth parameters was studied in five Bulgarian populations of *Aegilops biuncialis* Vis. (Table 1.). Deionised water was used as a control. Two replicates of 25 seeds from every variant were germinated on filter paper with 20 ml of test solutions. The papers were replaced every two days to prevent salt build-up. The paper with seeds was put in plastic bags to keep it moist. The seeds were left to germinate in the dark at 20±1°C for 8 days. Seeds were considered germinated when the radicle was at least 2 mm long. The number of germinated seeds was recorded daily until it stayed the same. We studied several germination characteristics, including germination energy (GE, %), germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day^{-1}), mean germination time (MGT), and relative injury rate (RIR). CVG was calculated using Kader and Jutzi (2004), GRI and MGT using Kader (2005), and RIR using Li (2008). On day 8, 10 seedlings were randomly selected from each treatment to measure shoot and root length and fresh weight. We also recorded the dry weight of the shoot and root after drying in an oven at 80°C for 24 hours. Seedling height reduction was determined using the method described by Islam &

Table 1. List of accessions used in the study

Accession number	Species	Geographical coordinate of the habitat	Altitude, m
BGR43667	*Aegilops biuncialis* Vis.	41°33'50"N, 26°06'32"E	282
BGR44900	*Aegilops biuncialis* Vis.	42°07'27"N, 27°36'07"E	275
BGR43701	*Aegilops biuncialis* Vis.	41°38'45"N, 25°40'48"E	166
BGR44617	*Aegilops biuncialis* Vis.	**42°8'15.791"N, 24°15'57.683"E**	**167**
BGR43660	*Aegilops biuncialis* Vis.	41°37'15.31"N, 25°40'30"E	166

Karim (2010), and salt tolerance was estimated according to Mujeeb-ur-Rahman et al. (2008).

The analysis of variance (ANOVA) and Duncan's multiple test were conducted using the statistical software package SPSS 19.0.

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RESULTS AND DISCUSSION

Currently, the literature is unavailable on the individual effect of salinity on the seed germination of populations of wild wheat (*Aegilops*). In the present study, the effect of potassium chloride (KCl) at six different salt concentrations (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM) on seed germination and early seedling growth parameters was studied.

Figure 1 illustrates the impact of salinity (KCl concentration) on germination characteristics. The application of increasing salt stress had a substantial negative impact on the germination (G, %), coefficient of velocity of germination (CVG) and germination rate index (GRI, % day[-1]) and significantly prolonged the mean germination time (MGT, day). Significant genotypic differences were observed in response to KCl concentrations between 50 and 150 mM for investigated germination characteristics. CVG ranged from 16.78 for BGR44900 at 150 mM KCl to 45.83 for BGR43660 at 50 mM KCl. GRI was 3.50% day-1 for BGR43660 and 18.50% day-1 for BGR43667, respectively. MGT(day) varied between 1.44 days for BGR43660 (control variant) to 8 days for BGR43701 (variant with 300 mM KCl) (Figure 1.).

At 200 mM KCl, only BGR43701 and BGR43667 differed from the other two samples, with the highest germination rate of only 20% reported for BGR43667. Application of the highest KCl concentration (300 mM) resulted in partial or complete suppression of seed germination in all populations (Fig.1). Yildiz et al. (2006) studied the effect of salinity on the seed germination of populations of wild wheat species (*Aegilops*)

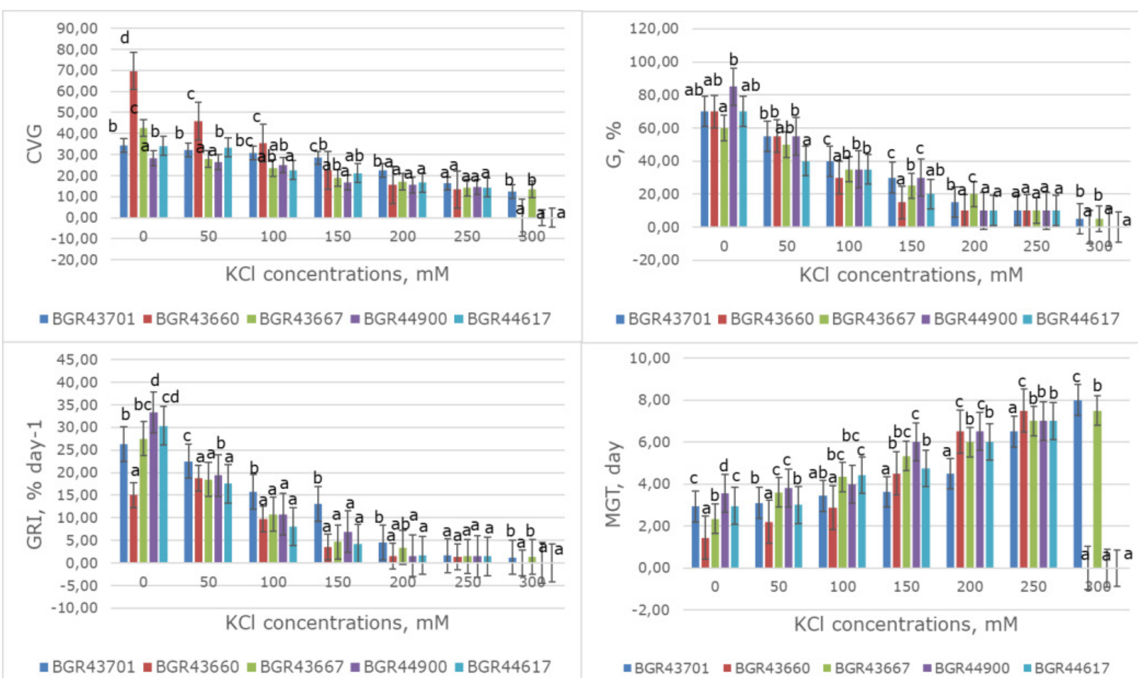

Figure 1. Effect of different KCl solutions on germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day-1), mean germination time (MGT, day) and for five *Aegilops* genotypes. Data presented are mean ± SE (Standard error). Different letters indicate a significant difference among genotypes according to Duncan´s multiple range test at 0.05 level.

and found that no seed germinated at 675 mM NaCl. In our study at 300 mM KCl seed germination was entirely suppressed in in BGR43660, BGR44900 and BGR44617.

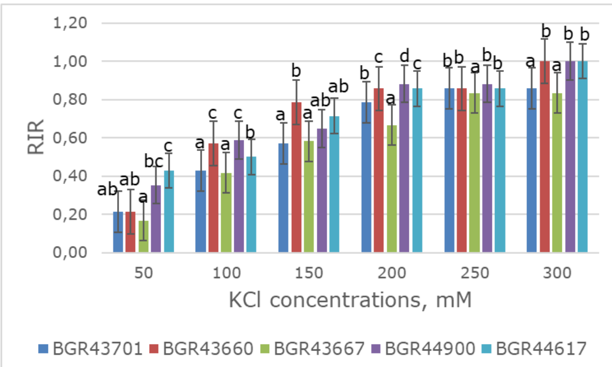

Figure 2. Effect of different concentrations of potassium chloride salt on the Relative Injury Rate in genotypes of *Aegilops biuncialis* Vis.

The coefficient of the relative injury rate was used to determine the degree of seed damage during the period of germination caused by the application of increasing concentrations of potassium chloride. In our study, this indicator increases with increasing salinity levels in all samples included in the study. The results of the study confirm toxic effect on *Aegilops biuncialis* Vis. at application levels of KCl between 250 and 300 mM. The highest relative injury coefficient was recorded for BGR44617, BGR44900 and BGR43660 when treated with 300 mM KCl solution. At the lowest concentrations of KCl (50-100 mM), the least seed damage was recorded at specimen BGR43667 (Figure 2)

The increase in the concentration of salt solutions had an inhibitory effect on shoot and root length, respectively on the seedling, such effect

Table 2. Variation in the root, shoot and seedling height reductions at different levels of KCl salinity in five populations of the species *Aegilops biuncialis* Vis.

Genotype	Root height reduction	Shoot height reduction	Seedling height reduction	Root height reduction	Shoot height reduction	Seedling height reduction
	50 mM KCl			**100 mM KCl**		
BGR43667	0.15a	0.12b	0.11a	0.35ab	0.20b	0.25a
BGR44900	0.18a	0.06a	0.11a	0.33ab	0.37c	0.36b
BGR43701	0.25b	0.21c	0.22c	0.48c	0.40c	0.43c
BGR44617	0.18a	0.27d	0.24c	0.37b	0.47d	0.43c
BGR43660	0.26b	0.09ab	0.16b	0.32a	0.15a	0.22a
Average	0.20	0.15	0.17	0.37	0.32	0.34
	150 mM KCl			**200 mM KCl**		
BGR43667	0.50d	0.40b	0.44b	0.61c	0.61b	0.60b
BGR44900	0.47c	0.48c	0.47c	0.61bc	0.68c	0.65c
BGR43701	0.56e	0.58d	0.58e	0.65d	0.62b	0.63bc
BGR44617	0.44b	0.56d	0.51d	0.53a	0.67c	0.61bc
BGR43660	0.40a	0.28a	0.34a	0.58b	0.42a	0.50a
Average	0.48	0.46	0.47	0.60	0.60	0.60
	250 mM KCl			**300 mM KCl**		
BGR43667	0.70a	0.67b	0.67a	0.77a	0.69a	0.72a
BGR44900	0.81c	0.78c	0.79b	1.00c	1.00c	1.00c
BGR43701	0.79c	0.79c	0.79b	0.93b	0.88b	0.90b
BGR44617	0.75b	0.80c	0.78b	1.00c	1.00c	1.00c
BGR43660	0.74b	0.59a	0.66a	1.00c	1.00c	1.00c
Average	0.76	0.73	0.74	0.94	0.92	0.93

Different letters in the column indicate a significant difference among genotypes according to Duncan's multiple range test at 0.05 level.

was more pronounced on roots. At the lowest salinity level (50 mM KCl), the inhibition of shoot growth was between 6% and 21% relative to the control, and on the root between 18% and 26%. The lowest values were recorded at BGR44900 (RHR=0.18, ShHR=0.06). At 100 mM KCl the average root height reduction was 0.37 and that of the shoot was 0.32. At the highest salinity level (300 mM KCl), RHR varied between 0.77 for BGR43667 to 1 for BGR44617, BGR43660 and BGR44900, while ShHR was significantly the lowest for BGR43667, respectively 0.72 (Table 2).

The influence of different salts of NaCl, KCl, $MgCl_2$ and $CaCl_2$ and their concentrations on MGT has been shown to have a similar positive effect in different crops (Panuccio et al., 2014; Tabatabaei et al., 2014; Kalhori et al., 2018). However, these salts have also been shown to have a negative effect on germination and seedling characteristics at the early growth stage (Panuccio et al., 2014; Tabatabaei et al., 2014; Kalhori et al., 2018). The studies by Liu et al. (2018), Anshori et al. (2020), Vishnu et al. (2020), Yohannes et al. (2020), Kadri et al. (2021), Chakma (2021), Nikolić et al. (2023) and Irik et al. (2024) are also relevant to this topic.

Table 3 shows the indices of salt tolerance at different levels of salt concentrations in the five studied samples of *Aegilops*. At 50 mM KCl, all accessions, except BGR44617 showed very high seed germination tolerance between 78.57% and 83.33%. At 100 mM KCl, all population had medium tolerance to germination. At levels of 150 mM BGR43667 and BGR43701 were with medium and the others with low tolerance, but the significant differences were recorded only between

Table 3. The mean salt tolerance indices of *Aegilops biuncialis* Vis. at germination and early seedling stage

Genotype	GSTI	RSTI	ShSTI	SSTI	GSTI	RSTI	ShSTI	SSTI
	50 mM KCl				100 mM KCl			
43667	83.33c	85.20b	91.13c	88.44c	58.33b	64.50bc	82.26c	74.19c
44900	64.71ab	82.44b	93.85c	89.03c	41.17a	66.41cd	62.57b	64.19b
43701	78.57bc	74.64a	79.02b	77.23a	57.14b	51.41a	59.51b	56.20a
44617	57.14a	81.90b	72.04a	75.93a	50.00ab	62.85b	52.17a	56.39a
43660	78.57bc	74.27a	90.36c	83.07b	42.85a	67.87d	84.92c	77.19c
Average	72.46	79.69	85.29	82.74	49.9	62.61	68.29	65.64
	150 mM KCl				200 mM KCl			
43667	41.67b	49.11b	61.57c	55.91d	33.33c	38.46b	39.90b	39.24b
44900	35.29ab	52.67c	51.95b	52.26c	11.76a	38.93bc	31.84a	34.84a
43701	42.86b	43.66a	41.46a	42.36a	21.43b	34.50a	37.56b	36.31ab
44617	28.57ab	55.23d	43.47a	48.12b	14.29a	46.66d	32.91a	38.34ab
43660	21.43a	59.39e	71.35d	65.93e	14.29a	41.21c	57.28c	50.00c
Average	33.96	52.02	53.97	52.92	19.02	39.96	39.90	39.75
	250 mM KCl				300 mM KCl			
43667	16.67a	29.59c	34.48b	32.26b	16.66b	22.48c	32.02c	27.69c
44900	11.76a	19.08a	21.79a	20.65a	0.00a	0.00a	0.00a	0.00a
43701	14.29a	20.42a	20.97a	20.75a	14.28b	6.69b	11.22b	9.36b
44617	14.28a	24.76b	19.87a	21.80a	0.00a	0.00a	0.00a	0.00a
43660	14.29a	25.09b	40.60c	33.57b	0.00a	0.00a	0.00a	0.00a
Average	14.26	23.79	27.55	25.81	6.19	5.84	8.65	7.41

GSTI-Germination Salt tolerance Index, RSTI- Root salt tolerance index, ShSTI-Shoot salt tolerance index, SSTI-Seedling salt tolerance index, Different letters in the column indicate a significant difference among genotypes according to Duncan's multiple range test at 0.05 level.

BGR43667 and BGR 43660. At 200 mM KCl BGR43667 and BGR43701 had low tolerance, while the others three accessions had very low tolerance. At levels of 250 mM KCl, all samples demonstrated very low tolerance to germination of seeds. BGR44900, BGR44617 and BGR 43660 were sensitivity to germination of their seeds at 300 mM KCl.

At low salinity levels of 50-100 mM KCl, the root salt tolerance index exhibited a range of variation, from 51.41% (BGR43701, 100 mM) to 85.20% (BGR4366, 50 mM). The shoot tolerance index ranged from 52.17% (BGR44617, 100 mM) to 93.85% (BGR44900, 50 mM). At medium levels (150-200 mM KCl), the studied populations of *Aegilops biuncialis* Vis. exhibited from medium to low tolerance for the growth of their shoot and root at the early growth stage. At the high level of salinization with 250 mM KCl, the seedling tolerance indices of all accessions were low, with values ranging from 20.65% to 33.57%. The average value for the group was 25.81%. At the highest level of salinization (300 mM KCl), the following samples were classified as sensitive to seedling growth: BGR44900, BGR44617 and BGR43660, while BGR43667 with low tolerance and BGR43701 with very low tolerance (Table 3).

CONCLUSION

The results of the study showed that increasing the KCl concentration from 50 to 300 mM in the studied five populations of *Aegilops biuncialis* Vis. prolonged the mean seed germination time and significantly reduced the germination and seedling growth characteristics. The inhibitory effect of increasing concentrations of KCl was more pronounced in terms of root height reduction compared to shoot height reduction in all populations studied except BGR44617. The highest relative injury coefficient was recorded for BGR44617, BGR44900 and BGR43660 when treated with 300 mM KCl solution. These cultivars were classified as sensitive with respect to germination and seedling growth. In contrast, BGR43667 was identified as relatively the most tolerant to KCl salinity in the early seedling growth phase.

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