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ORIGINAL PAPER

The effect of salinity (NaCl) on germination and early seedling growth of rye seeds

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Abstract

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The aim of study is to determine the effect of NaCl salinity stress on germination characteristics and seedling growth of 3 Bulgarian rye accessions - Milenium, B7E0131 and B7E0055 maintained in the *ex situ* collection of the National genebank of Bulgaria. Six different concentrations of NaCl (25, 50, 75, 100, 125 and 150 mM) are used as treatments and deionized water is used as control. To determine the salinity tolerance, the following germination characteristics - germination energy (%), germination percentage (%), coefficient of velocity of germination (% day^{-1}), germination rate index and mean germination time (day), water uptake are studied. The data for the shoot and root length (cm), fresh weight (mg $plant^{-1}$) of shoot and root and dry weight (mg $plant^{-1}$) of shoot and root are measured seven days after germination. Vigor index and solt tolerance index are also calculated. The effect of increasing NaCl levels on germination energy and final germination percentages are not recorded for B7E0131 and B7E0055. Increasing salinity concentration from 25 to 150 mM NaCl prolonged mean germination time and had an inhibitory effect on the initial seedling growth. B7E0055 is the most tolerant at seedling growth stage.

Key words: Rye, *Secale cereale* L., Germination, Salinity, Seedling growth, Correlation.

Introduction

Seed germination and early seedling growth are the major factors limiting the establishment of plants under saline conditions and most critical phase in plant life under extreme conditions (Gebreslassie, 2013). Two major stresses affecting plants under salinity are osmotic and ionic stresses. Osmotic stress, occurring immediately in the root medium on exposure to salts, can result in inhibition of water uptake, cell expansion and lateral bud development (Munns and Tester, 2008). Ionic stress develops when toxic ions (e.g. Na+) accumulate in cells causing increase in leaf mortality, chlorosis, necrosis and decrease in the activity of cellular metabolism including photosynthesis (Glenn and Brown, 1999). Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves due to high Na+ and Cl_2 which affect plants by disrupting protein synthesis and by interfering with enzyme activity (Munns, 2002; Panuccio et al, 2014).

Seed germination is an essential process in plant development to obtain optimal seedling numbers that result in higher seed yield (Houle et al., 2001; Ashraf and Foolad, 2005; Sanchez et al., 2014; Tabatabaei and Naghibalghora, 2014). High concentration of salt has detrimental effects on germination of seeds (Rahman *et al*., 2000; Khajeh-Hosseini et al., 2003; Sharma *et al*., 2004; Saboora and Kiarostami, 2006; Mohammadi, 2009; Shila et al., 2016; Ahmed et al., 2017). Plant growth is ultimately reduced by salinity stress (Almansouri et al., 2001; Datta et al., 2009; Haque et al., 2014; Sikder et al., 2016; Ahmed et al., 2017). Bybordi (2010) noted that germination and plantlet growth indices (e.g., percentage and vigor) are two of the most important criteria for selection of tolerant cultivars, while Ahmed et al. (2017) point that information about tolerance of crop variety at germination and early seedling growth stage will help to identify salt tolerant varieties and/or genotypes and to develop saline soil management strategies.

The aim of study is to determine the effect of salinity stress on germination characteristics and early seedling growth of 3 rye accessions maintained in the National collection of Bulgaria.

Materials and methods

Seeds of three Bulgarian rye accessions (two landraces –B7E0131 and B7E0055 and one modern variety-Milenium) maintained in the National collection of Bulgaria in the Institute of Plant Genetic Resources “Konstantin Malkov” are used. The seeds are surface sterilized by dipping the seeds in 30% etanol solution for 3 minutes and rinsed thoroughly with distilled water and air-dried before being used in the germination tests to avoid any fungal attacks. Six different concentrations of NaCl (25, 50, 75, 100, 125 and 150 mM) are used as treatments and deionized water is used as the control. For each variant of the experiment, two replicates of 25 seeds

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are germinated between rolled filter paper (Grade FT 55) with 20 ml of respective test solutions. The papers are replaced every 2 days to prevent accumulation of salts (Rehman et al., 1996). The rolled paper with seeds is put into sealed plastic bags to avoid moisture loss. Seeds are allowed to germinate at 20±1 ºC in the dark for 7 days after cooling at 6 ºC for twenty-four-hour period. Seeds are considered germinated when radicle had extended at least 1 mm. The number of germinated seeds is recorded daily until a constant count is achieved. From the germination counts several germination characteristics are studied including germination energy (%) as first count after 4 days (GE), germination percentage (%) as final count after 7 days (G, %), coefficient of velocity of germination (CVG, % day-1), germination rate index (GRI), and mean germination time (MGT, day). Coefficient of velocity of germination (CVG, % day-1) is calculated according to Kader and Jutzi (2004). Germination rate index (GRI, % day-1) and mean germination time (MGT, day) are calculated according to the formula of Kader (2005).

The data for the shoot and root length (cm) (LSh and LR), fresh weigh (mg) of shoot and root (FWSh and FWR) and dry weight (mg) of shoot and root (DWSh and DWR) are measured seven days after germination. Dry weights are measured after drying at 80°C for 24 h into an oven.

In order to determine the seed vigor index (VI), equation from Florez et al. (2007) is used.

Water uptake of seeds is determined after soak the seeds for 24 h in the different concentrations of NaCl. It is calculated by the formula given from Nizam (2011).

The coefficient of depression was calculated according to the Blum et al. (1989):

Coefficient of depression, % = [(A-B)/A] x 100, where

A – average length of the roots /shoots in the control variant, cm

B – average length of the roots/shoots in the salt stress variant, cm.

Salt tolerance is calculated by the formula given from Mujeeb-ur-Rahman et al. (2008).

Data are analyzed by analysis of variance (ANOVA), LSD test and Duncan‘s multiple range test (Duncan, 1955). The analysis of variance is calculated according to randomized complete block design with two factors: genotype and treatment (salinity). To estimate the degree of genotype and treatment (salinity) influence on different germination and seedling characteristics is applied method described by Plochinskii (1970).

Statistical analyses are performed using the statistical program SPSS 19.0.

Results and discussion

Germination characteristics

Table 1. Effects of different salinity levels on germination characteristics of three rye genotypes

Name of accessions	Нива на соленост/Salinity levels							
	0 mM NaCl	25 mM NaCl	50 mM NaCl	75 mM NaCl	100 mM NaCl	125 mM NaCl	150 mM NaCl	LSD 5%
Germination energy, %								
Milenium	100,00a	100,00a	98,00a	98,00ab	98,00a	92,00a*	90,00a*	5,92
B7E0131	96,00a	96,00a	96,00a	100,00b	98,00a	96,00a	94,00ab	5,77
B7E0055	98,00a	100,00a	97,00a	96,00a	98,00a	100,00a	98,00b	3,24
Means	98,00	95,33	94,00	98,00	98,00	96,00	94,00	
Germination, %								
Milenium	100,00a	100,00a	98,00a	98,00ab	98,00a	92,00a*	90,00a*	5,92
B7E0131	96,00a	96,00a	96,00a	100,00b	98,00a	96,00a	94,00ab	5,77
B7E0055	98,00a	100,00a	97,00a	96,00a	98,00a	100,00a	98,00b	3,24
Means	98,00	95,33	94,00	98,00	98,00	96,00	94,00	
Coefficient of velocity of germination, % day^{-1}								
Milenium	98,06a	95,91a	92,45a	92,89a	85,96b*	85,13b*	80,55a*	6,00
B7E0131	100,00a	97,93a	90,22a*	89,59a*	79,05a*	75,53a*	74,87a*	8,35
B7E0055	100,00a	100,00a	97,90b	94,14a*	90,98b*	84,90b*	75,38a*	4,71
Средно/Means	99,35	97,95	93,52	92,21	85,33	81,85	76,93	
Germination rate index								
Milenium	24,75a	22,00a*	23,50c	23,67a	22,83b*	21,38ab*	19,75a*	1,26
B7E0131	24,50a	23,75b	21,75a*	23,50a	21,42a*	20,33a*	19,67a*	2,38
B7E0055	24,00a	25,00b*	22,75b*	23,25a	23,25b	22,75b*	20,50a*	0,94
Средно/Means	24,42	23,58	22,67	23,47	22,50	21,49	19,97	
Mean germination time, day								
Milenium	1,02a	1,04a	1,08b	1,08a	1,16a*	1,17a*	1,24a*	0,074
B7E0131	1,00a	1,02a	1,11b	1,12a	1,27b*	1,33b*	1,35a*	0,136
B7E0055	1,00a	1,00a	1,02a	1,06a*	1,10a*	1,18a*	1,33a*	0,059
Means	1,01	1,02	1,07	1,09	1,18	1,23	1,30	
Water uptake of seeds, %								
Milenium	68,80b	70,31a	63,42b	62,12a	53,02a	52,61ab	47,38a	21,67
B7E0131	61,07ab	57,53a	57,52ab	62,37a	57,56a	53,17b*	49,99a*	7,65
B7E0055	56,56a	56,30a	51,76a	49,18a*	48,27a*	46,39a*	46,58a*	6,09
Means	62,14	61,38	57,57	57,89	52,95	50,72	47,98	

Means in the same column followed by the same letters are not significantly different ($p<0,05$) according to Duncan‘s test.
*The mean difference in the same row is significant at the 0,05 level

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The effect of salinity (NaCl concentration) on germination characteristics are shown in Table 1. The application of increasing salt stress had substantial negative effects on the coefficient of velocity of germination (CVG, % day-1) and germination rate index (GRI, % day-1). Significant differences are found among genotypes at 50, 100 and 125 mM NaCl for CVG and GRI. CVG varied from 74.87 % day^{-1} for B7E0131 to 80.55% day^{-1} for Milenium at 150 mM NaCl. Increasing salinity concentration from 0 to 150 mM NaCl increased mean germination time. At the highest salt concentration (150 mM NaCl), the MGT varied within close ranges, respectively from 1.24 days (for Milenium) to 1.35 days (for B7E0131) (Table 1). Shahri et al. (2012) also noted that mean germination time increased in *Secale* seeds by increasing salt concentrations. Similar results about positive influence of different NaCl concentrations on MGT are reported in several crops (Demir et al., 2003; Khajeh-Hosseini et al., 2003; Atak et al., 2006; Nizam, 2011; Panuccio et al., 2014).

Kaydan and Yagmur (2008) and Nizam (2011) reported that high NaCl concentrations caused lower water uptake by seeds and so germination decreased. In our research the effect of increasing NaCl levels from 0 to 150 mM on germination energy (GE) and final germination percentages (G) are not recorded for landraces B7E0131 and B7E0055, while for Milenium variety GE and G significantly decreased at 125 and 150 mM NaCl. Water uptake of seeds (WU) was the lowest at 150 mM salt levels, respectively 47,98%. In compare with the control variant, Milenium seeds uptaked with 21,42% lower water at the highest concentration, while B7E0131 and B7E0055 with 11,08% and 9,98%, respectively (Table 1).

The results of two-way analysis of variance showed that the strongest influence for germination energy (GE) and final germination (G) had genotype X salinity interaction, respectively 41,60%, while the lowest influence had genotype, respectively 4,17%. The treatments with NaCl had the strongest influence on the coefficient of velocity of germination (CVG, % day-1), germination rate index (GRI, % day-1), mean germination time (MGT, day) and water uptake of seeds (WU, %) respectively 76,11%, 62,36%, 72,71% and 48,36% (Table 2).

Seedling characteristics

In our study significant differences are observed between NaCl treatments and VI. Considerable decrease in VI is observed, depending on the increase in the concentration of NaCl (Table 3 and Table 4). Similar trend is observed by other authors on different plants (Khajeh-Hosseini et al., 2003; Kader and Jutzi, 2004; Basalah, 2010; Cokkizgin, 2012; Keshavarzi, 2011; Saddam Hussain et al., 2013). The highest VI is observed in the control variant for B7E0131 (1974,51), while the lowest is noted at 150 mM NaCl (720,67) for Milenium. The VI increased when the NaCl concentration decreased, which shows that increased NaCl concentration caused a harmful effect in the seed (Table 3).

The analysis of variance showed that the effect of genotype, NaCl levels and genotype x NaCl levels interaction are significant at $p \leq 0,01$ and $p \leq 0,001$ for both length of shoot and root (Table 4). The salinity had the strongest influence on the variance for the shoot and root length. Generally, increasing salinity levels inhibited the shoot and root length. Mean of shoot length varied between 3,69 and 8,88 cm at various NaCl concentrations. The longest shoot length is observed in the control variant for B7E0131 (9,71 cm). The shortest shoot length is 3,54 cm at 150 mM NaCl concentration also for B7E0131, respectively (Table 3). The rate of depression in shoot length for B7E0055 is recorded as the lowest at the highest salinity level, respectively 56,81% (Fig.1).

Table 2. Analysis of variance for the characteristics germination energy (GE, %), germination percentage (G, %), coefficient of velocity of germination (CVG, % day^{-1}), germination rate index (GRI, % day^{-1}), mean germination time (MGT, day), water uptake of seeds (WU, %).

Source of Variation	*df*	*Mean square*					
		GE	***G***	***CVG***	***GRI***	***MGT***	***WU***
Genotype	2	21,14 ns	21,14 ns	144,36***	4,70**	0,029***	444,2**
Salinity	6	30,29**	30,29**	638,70***	19,78***	0,112***	260,81**
Interaction	12	35,14**	35,14**	27,09ns	2,14*	0,0056ns	40,24ns
Degree of influence, %							
Genotype	2	4,17	4,17	5,73	4,90	6,34	26,03
Salinity	6	17,93	17,93	**76,11**	**62,36**	**72,71**	**48,36**
Interaction	12	**41,60**	**41,60**	6,46	13,50	7,31	18,75

** Significant at the 0,01 probability level according to F-test,

Roots play an important role in shoot growth under saline conditions (Kumawat et al., 2017). In the study mean root length varied between 4,53 cm and 9,36 cm for different salinity levels. As is expected, the control and the highest NaCl concentration had the longest and the shortest root length, respectively (Table 3).

Nizam (2011) noted that high salinity could be rapidly inhibit root growth and hence water and essential mineral nutrients uptake capacity of the roots from the soil is decreased. In our study the lowest reduction in root length at 150 mM NaCl is recorded for B7E0055, where the coefficient of depression was 47,04%, respectively (Figure 2). This fact indicated that this genotype is lower sensitive to this NaCl concentration in compare of the other two genotypes included in the study.

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Salt stress inhibited the seedling growth (root and shoot length). The rate of depression in shoot lengths was bigger than the rate of depression in root lengths (Figure 1 and Figure 2). Therefore the shoots were more sensitive to salinity than roots. According to Asaadi (2009) better growth of root may be due to active translocation of salt and ions from root to shoot.

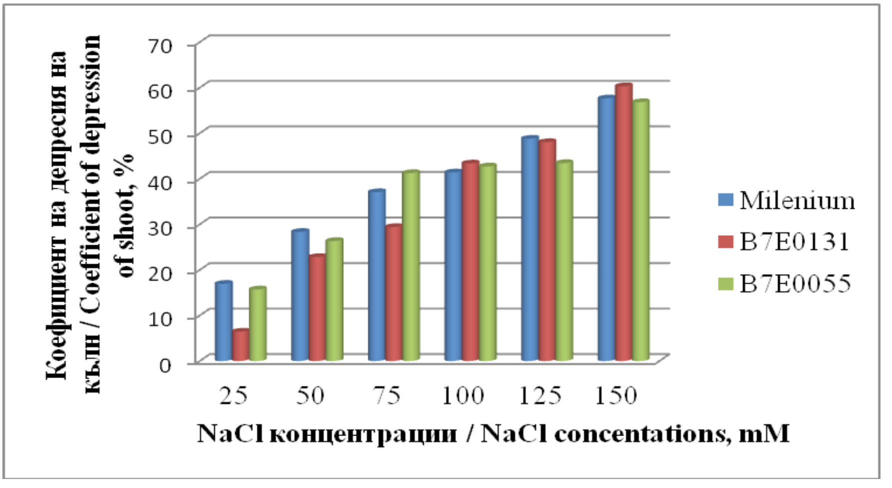

Figure 1. Variation of depression coefficient of shoot in 3 rye accessions at 6 different salinity levels

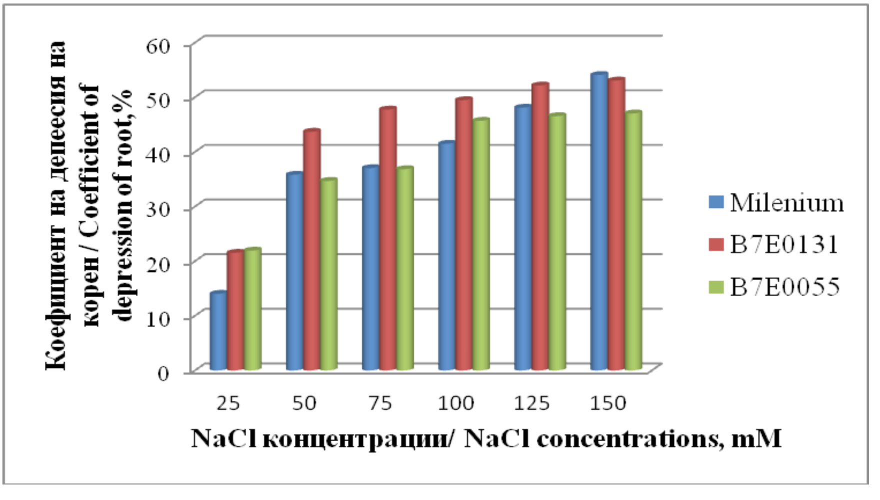

Figure 2. Variation of depression coefficient of root in 3 rye accessions at 6 different salinity levels

Salinity had significant effect on shoot and root fresh weight (Table 5). Increase in salt concentration reduced these characters (Table 3). Differences in fresh shoot

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Table 3. Effects of different salinity levels on seedling characteristics of three rye genotypes

Name of accessions	Salinity levels								
	0 mM NaCl	**25 mM NaCl**	**50 mM NaCl**	**75 mM NaCl**	**100 mM NaCl**	**125 mM NaCl**	**150 mM NaCl**	**LSD 5%**	**Средни Means**
Vigor index									
Milenium	1810,00b	1378,39a*	1201,40b*	1113,97a*	1040,24a*	855,91a*	720,67a*	173,81	1181,56
B7E0131	1974,51b	1658,71b*	1230,96b*	1231,50a*	1056,71a*	963,32b*	826,00a*	234,58	1285,03
B7E0055	1580,53a	1329,00a*	1046,50a*	958,08a*	895,44a*	900,50ab*	772,15a*	129,96	1077,08
Means	1788,35	1455,37	1159,62	1101,18	997,46	906,58	772,94		
Length of shoot, cm									
Milenium	8,65ab	7,19a*	6,20a*	5,45a*	5,07b*	4,43a*	3,67a*	0,85	5,61
B7E0131	9,71b	9,09b	7,50b*	6,86b*	5,50c*	5,05a*	3,54a*	0,84	6,79
B7E0055	8,29a	6,90a*	6,03a*	4,81a*	4,69a*	4,63a*	3,86a*	0,61	5,55
Means	8,88	7,72	6,58	5,70	5,09	4,70	3,69		
Length of root, cm									
Milenium	9,45b	8,13b*	6,07a*	5,95a*	5,53b*	4,91b*	4,34a*	1,10	6,34
B7E0131	10,45c	8,20b*	5,89a*	5,46a*	5,28ab*	5,00b*	4,91b*	0,64	6,45
B7E0055	8,19a	6,39a*	5,35a*	5,17a*	4,45a*	4,38a*	4,34a*	0,88	5,46
Means	9,36	7,57	5,77	5,53	5,09	4,76	4,53		
Fresh weight of shoot (mg plant^{-1})									
Milenium	74,8b	66,8a*	55,7a*	53,5b*	45,6a*	40,4a*	38,1a*	6,78	53,56
B7E0131	85,8c	82,7b	71,2b*	62,7c*	57,6b*	46,4a*	31,2a*	5,69	63,79
B7E0055	61,7a	60,8a	49,4a*	43,9a*	43,7a*	43,45a*	40,0a*	7,22	47,75
Means	74,12	70,13	58,80	53,40	48,98	43,42	36,38		

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Fresh weight of root (mg plant^{-1})									
Milenium	51,8a	44,3a	41,7a*	35,4a*	33,6ab*	33,0a*	28,2a*	9,82	38,26
B7E0131	53,0a	45,3a	40,3a*	35,4a*	34,7b*	33,0a*	28,4a*	8,24	39,14
B7E0055	40,1a	35,9a	37,3a	33,1a	31,5a*	29,6a*	32,4a*	7,71	33,68
Means	48,27	41,78	39,73	34,60	33,27	31,87	29,65		
Dry weight of shoot (mg plant^{-1})									
Milenium	6,95a	7,50b	6,40ab	6,00a*	5,80a*	5,40b*	5,10a*	0,83	6,16
B7E0131	8,50b	7,50b*	7,25b*	7,15b*	6,65b*	6,65c*	5,15a*	0,47	7,01
B7E0055	6,95a	6,50a	6,10a	6,10a	5,80a*	5,15a*	5,40a*	0,92	5,96
Means	7,47	7,17	6,58	6,42	6,08	5,73	5,22		
Dry weight of root (mg plant^{-1})									
Milenium	6,65b	6,25b	5,20a*	5,05a*	5,05a*	5,00a*	4,60b*	1,24	5,40
B7E0131	5,7ab	5,60ab	5,05a	5,05a	5,20a	5,00a	3,55a*	0,82	5,16
B7E0055	5,25a	5,25a	5,10a	5,00a	4,80a	4,15a*	4,55b*	0,91	4,73
Means	5,87	5,70	5,12	5,03	5,02	4,72	4,23		

Means in the same column followed by the same letters are not significantly different ($p<0,05$) according to Duncan‘s test.

*The mean difference in the same row is significant at the 0,05 level and root weight are significant in different accessions under most of salinity levels (P ≤0,05) (Table 3). B7E0131 had the highest value of fresh shoot weight under control variant (0,0 mM NaCl), while B7E0055 showed the highest values of both shoot and root fresh weigth under 150 mM NaCl treatment (Table 3).

The increase in NaCl concentrations decreased also the shoot and root dry weight of all the rye plants (Table 3 and Table 5). The shoot and root dry weight values changed by increasing salt concentration. The highest shoot and root dry weights are obtained from control treatments. On the other hand a decrease is observed on shoot and root dry weight values in accordance with increasement on salt concentration. The highest shoot and root dry weight reductions at 150 mM NaCl level in compare with control variants are obtained for B7E0131, respectively with 3,35 mg plant^{-1} and 2,15 mg plant^{-1}, while the lowest are noted for B7E005 (with 1,55 mg plant^{-1} for shoot and 0,70 mg plant^{-1} for root) (Table 3).

Effects of salinity on salt tolerance index of investigated rye seeds at seedling growth stage are presented in table 6. The genotypes had the following order at the higest salinity level (150 mM NaCl): B7E0137 < *Milenium*< B7E0055. The landraces B7E0055 is the most tolerant to salinity (47,78) at early seedling growth stage (Table 6).

Table 4. Analysis of variance for vigor index (VI), length of shoot (LSh) and length of root (LR)

Source of Variation	*Mean square*			
	df	***VI***	***LSh***	***LR***
Genotype	2	229420,7*	7,84***	4,60***
Salinity	6	1088154,8ns	29,33***	27,82***
Interaction	12	18245,21*	0,685***	0,82**
Degree of influence, %				
Genotype	2	6,13	7,53	4,68
Salinity	6	**87,17**	**84,55**	**84,80**
Interaction	12	2,92	3,95	5,01

* Significant at the 0,05 probability level according to F-test,
** Significant at the 0,01 probability level according to F-test,
*** Significant at the 0,001 probability level according to F-test,
ns Not significant according to F-test

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Table 5. Analysis of variance for fresh weigh of shoot and root (FWSh and FWR) and dry weight of shoot and root (DWSh and DWR)

Source of Variation	***Mean square***				
	df	***FWSh***	***FWR***	***DWSh***	***DWR***
Genotype	2	993,84***	121,03*	5,77***	1,59*
Salinity	6	1692,62***	386,03***	5,55***	2,79***
Interaction	12	121,96***	27,82ns	0,443*	0,444ns
Degree of influence, %					
Genotype	2	14	6,18	19,89	7,99
Salinity	6	**71,51**	**59,18**	**57,46**	**42,87**
Interaction	12	10,3	8,53	9,17	13,66

* Significant at the 0,05 probability level according to F-test,
** Significant at the 0,01 probability level according to F-test,
*** Significant at the 0,001 probability level according to F-test,
ns Not significant according to F-test

Table 6: Effects of salinity levels on salt tolerance index of three rye accessions at seedling growth stage at 150 mM NaCl (STSh-shoot salt tolerance index, STR-root salt tolerance index and STS- seedling salt tolerance index)

Salinity levels	**Milenium**			**B7E0131**			**B7E0055**		
	STSh	**STR**	**STS**	**STSh**	**STR**	**STS**	**STSh**	**STR**	**STS**
25 mM NaCl	83,06	85,98	**84,59**	93,56	78,47	**85,74**	83,28	78,07	**80,69**
50 mM NaCl	71,68	64,18	**67,76**	77,19	56,32	**66,37**	72,78	65,30	**69,06**
75 mM NaCl	62,95	62,96	**62,96**	70,60	52,25	**61,09**	58,06	63,16	**60,60**
100 mM NaCl	58,61	58,52	**58,56**	56,64	50,53	**53,47**	56,61	54,31	**55,46**
125 mM NaCl	51,21	51,90	**51,57**	51,96	47,85	**49,83**	55,88	53,45	**54,68**
150 mM NaCl	42,37	45,93	**44,23**	39,70	46,94	**43,45**	42,67	52,96	**47,78**
Средни/ Means	61,65	61,58	61,61	64,94	55,39	59,99	61,55	61,21	61,38

Conclusions

The strongest influence for germination energy and final germination had genotype X salinity interaction, while treatment with NaCl had the strongest influence on the germination characteristics as coefficient of velocity of germination, germination rate index, mean germination time and water uptake as well as on the seedling characteristics- length of shoot and root, fresh and dry shoot and root weight and vigor index. The landraces B7E0055 is the most tolerant to the investigated levels of salinity. Additional investigation are need to evaluate germination and early seedling growth under field conditions and also under higher salt stress.

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