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Determination of Salinity Effects on Seed Germination in Different Red Fescue (*Festuca rubra* L.) Varieties

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Abstract

Seeds of 12 red fescue cultivars, which were included creeping red fescue (*Festuca rubra L. subsp. rubra*), chewings fescue (*Festuca rubra* L. *subsp. commutata*), and slender red fescue (*Festuca rubra L. subsp.* trichophylla) (Barustic, Corail, Livision, Lustrous, Redskin, Rufi, Garnet, Wilma, Casanova, 07-Seas, and Libano) subspecies, 1 of which were newly introduced (J-5) in 2014, were used to investigate the effects of different NaCl concentrates (0, 50, 100, 150 and 200 mM) on their germination rate, shoot and root length, shoot and root fresh weight, shoot/root rate, and salt tolerance index. The experiment was designed as a completely randomized with 4 replicates at the laboratory of Kizilirmak Vocational High School, Cankiri Karatekin University, in 2014. The results indicated that different treatments of salinity had statistically remarkable effects on all of the properties, studied. These parameters reduced with raising salt concentrate. Important decreases evaluated by 100 mM concentrate in all parameters and the lowest values assessed at 200 mM. According to results, remarkable variations were determined under salinity conditions among the red fescue cultivars. The overall findings suggest that the Lustrous and Rufi varieties of creeping red fescue (*Festuca rubra* L. *subsp. rubra*) were more tolerant than the other ones.

Key Words: Salt stress, salinity, turfgrass, tolerance, seedling

Farklı Kırmızı Yumak (*Festuca rubra* L.) Çeşitlerinde Tohum Çimlenmesi Üzerine Tuzluluğun Etkilerinin Belirlenmesi

Abstract

Çeşitlerden 1 tanesi (J-5) 2014 yılında tescil edilen, rizomlu kırmızı yumak (*Festuca rubra L. subsp. rubra*), rizomsuz kırmızı yumak (*Festuca rubra* L. *subsp. commutata*) ve narin kırmızı yumak (*Festuca rubra L. subsp.* trichophylla) alt türlerine ait 12 farklı kırmızı yumak (*Festuca rubra* L.) çeşidinde (Barustic, Corail, Livision, Lustrous, Redskin, Rufi, Garnet, Wilma, Casanova, 07-Seas ve Libano) farklı tuz konsantrasyonlarının (0, 50, 100, 150 ve 200 mM) çeşitlerin çimlenme oranı, sürgün ve kök uzunluğu, sürgün ve kök yaş ağırlığı, sürgün/kök oranı ve tuza tolerans indeksleri üzerine etkileri araştırılmıştır. Araştırma, Çankırı Karatekin Üniversitesi, Kızılırmak Meslek Yüksekokulu Laboratuvarı'nda, 4 tekrarlamalı olarak, tesadüf parselleri deneme desenine göre, 2014 yılında yürütülmüştür. Araştırma sonuçları, farklı tuz uygulamalarının çimlenme oranı, sürgün ve kök uzunluğu, sürgün ve kök yaş ağırlığı, sürgün/kök oranı ve tuza tolerans indeksi üzerinde istatistiksel olarak önemli düzeyde etkili olduğunu göstermiştir. İncelenen parametrelerin değerleri artan tuz konsantrasyonu ile azalmıştır. Değerlerde 100 mM tuz konsantrasyonu ile önemli azalmalar meydana gelmiş ve en düşük değerler 200 mM'da elde edilmiştir. Araştırma bulguları göz önüne alındığında rizomlu kırmızı yumakın (*Festuca rubra L. subsp. rubra*) Lustrous ve Rufi çeşitlerinin diğerlerine kıyasla daha tolerant oldukları sonucuna varılmıştır.

Anahtar Kelimeler: Çim bitkileri, çimlenme, tuzluluk, tuz stresi, tolerans

INTRODUCTION

Salinity is one of the main factors limiting the growth and production of agricultural products. Many species of higher plants, including most crops, are subjected to growth inhibition under high NaCl conditions [1-2]. High soil salinity, a common problem in turfgrass management, is caused by various activities, such as deficient precipitation, water percolation from high water tables, low quality irrigation water, and salts from fertilizers and deicer [3]. The necessity of salinity tolerant turfgrasses is increasing because of the increased use of effluent or other low quality waters for turfgrass irrigation [4]. The identification and development of turfgrasses with improved salinity tolerance is necessary to maintain adequate turf quality when utilizing nonpotable water for irrigation [5]. Consequently, newer varieties which are tolerant to salinity must be improved to maintain turf quality.

The salt stress-induced inhibition of plant growth is caused not only by osmotic effects on the water uptake but also by variable effects on plant cell metabolism under salt stress. Zhang et al. [3] indicated that salinity adversely affects plant growth and development, resulting in reduced aesthetical and playable functions in turfgrass. Salt can have adverse effects on turfgrass growth, including physiological drought, ion toxicity, and ion imbalances.

Most cool-season turfgrass species are particularly susceptible to salinity stress during seed germination, with the possible exception of perennial ryegrass (*Lolium perenne* L.). As salt problems become one of the most complex management challenges, screening and breeding of salt-tolerant cool-season turfgrass cultivars during both seed germination and vegetative growth becomes important

[6]. Seed germination is the most important part of the life cycle of plants and fast and uniform germination are the intended characteristics, as in many plant types. However, germination and seedling growing phases are very sensitive to salt stress. In general, the highest germination rate occurs under saltless conditions and it decreases depending on the ascending salt concentrates [7].

The effect of salinity on plant growth is related to the stage of plant development at which salinity is imposed [8]. This stress affects seed germination either through osmotic effects, by preventing or delaying germination, or through ion toxicity, which can render the seeds unviable [9]. Many studies have been done to determine the effects of salinity on the germination of many cultivars. In a study about the effects of different salt concentrates (from 2 to 48 dSm^{-1}) on germination of turfgrass [10]. Dai et al. [6] stated that increasing the salt concentrate decreased considerably the germination rate and increased the germination time. In another study, the effect of 0.5% and 1.5% salt (NaCl) on the germination and growth of wheat cultivars was studied [2].

In this study, the seeds, belonging to 12 different red fescue subspecies to which different salt concentrates were applied, were examined for germination ability under salt stress conditions and for their salt tolerance during germination. For this purpose, salt concentrates of 0 (control), 50, 100, 150, and 200 mM were applied to the seeds, and germination rate, shoot and root length, shoot and root fresh weight, shoot/root rate, and salt tolerance index were evaluated at the end of the study.

MATERIALS and METHODS

Seeds of 12 red fescue cultivars, which were included creeping red fescue (*Festuca rubra L. subsp. rubra*), chewings fescue (*Festuca rubra* L. *subsp. commutata*), and slender red fescue (*Festuca rubra L. subsp.* trichophylla) (Barustic, Corail, Livision, Lustrous, Redskin, Rufi, Garnet, Wilma, Casanova, 07-Seas, and Libano) subspecies, 1 of which were newly introduced (J-5) in 2014, were used for this study. Seeds of each turfgrass varieties used in the experiment were surface-sterilized. Twenty five representative seeds per cultivar were placed on a filter paper in 10 cm petri dishes containing two sheets of Watman No. 1 filter paper moistened initially with 5 ml of distilled water (control), or with different concentrates solutions 50, 100, 150, and 200 mM NaCl (saline conditions). Germination chamber was at 20 °C and with dark conditions. The seeds were considered germinated when there was radicle protrusion through the seed coat.

Germination rate (%)

Of each turfgrass varieties, 25 seeds were placed in petri dishes for germination. The germinated seeds were counted after 15 days in the petri dishes and the germination rate was calculated. Seedlings were discarded from the petri dishes until only 20 remained for further study of their characteristics.

Shoot and root length (cm)

The 20 remaining seedlings in each petri dish were used for measuring the shoot and root characteristics. After 15 days, in the petri dishes, the seedlings were separated into roots and shoots. The distances from the crown to leaf tip and root tip were measured as the shoot length and root length, respectively.

Shoot and root fresh weight (g)

The shoot and root fresh weights of each seedling were measured. The average fresh weights of shoot and root of each plant seedling were measured by dividing the total weight by the total number of seedlings.

Shoot/root ratio

This was calculated for weight by dividing shoot values by root values.

Salt tolerance index

The salt tolerance index (STI) is the ratio of shoot fresh weight of the control treatment and fresh weight of the salt concentration. The STI was calculated by the following formula: STI = (TFW at Sx / TFW at S) × 100], where STI is the salt tolerance index, TFW is the mean of the total fresh weight, S refers to the control treatment, and Sx is the x treatment.

Experimental design and statistical analysis

The experiment was designed as a completely randomized plot with 4 replicates. Data were analyzed statistically and the means of each treatment were analyzed using Duncan’s multiple range test and SAS (9.0) packet program.

RESULTS and DISCUSSIONS

In general, salt stress significantly decreased the germination rate of the turfgrass varieties in comparison to the control (Table 1). With the 150 and 200 mM NaCl concentrates, significant differences were observed among the varieties. 07-Seas, Lustrous and Rufi varieties had germination rates (GRs) higher than 90%, even with the 150 mM NaCl treatment, while the Garnet, Barustic, and Libano varieties had lower germination rates with the same NaCl treatment (33-39%). Salt stress caused 32.8% reductions in the germination rate in the turfgrass varieties with the 200 mM NaCl treatment. The highest GR was observed in the Lustrous (88.3%), Casanova (84.8%), and Rufi (83.8%) varieties. However, the Garnet, Barustic, and Libano (59-79%) varieties showed the lowest germination rate with the 200 mM NaCl treatment. Cokkizgin [11] indicated that an increase in salinity induces both a reduction in the rate of germinating seeds and a delay in the initiation of the germination process.

The present results are in agreement with those reported by Kokten et al. [12], who observed a significant difference in the salt tolerance of lentil genotypes and their different responses to increasing salt concentrate. The present study identified and compared the salinity tolerance levels of 12 varieties of turfgrass during germination, where the Lustrous and Rufi varieties showed better performance than the others. In addition, because of the depressing effect of NaCl, seeds saturated with 150 and 200 mM NaCl had lower germination rates. Increasing salt concentrates reduces the water potential of the soil water. Salts decrease the osmotic potential of the media solution, making water less available to the plant. Under salt stress, turfgrasses can experience indirect water stress, resulting in a seed germination block [13]. Similarly, Cokkizgin [11] and Katembe et al. [14] reported that the imbibition of water following NaCl treatments decreased with the decrease in water potential of the solutions. An increasing concentrate of NaCl is probably caused by the decrease in water potential gradient between the seeds and their surrounding media. Salinity stress negatively affects seed germination, either osmotically through reduced water absorption or ionically through the accumulation of Na^+ and Cl^-, causing an imbalance in the nutrient uptake and toxicity effect [11,15].

There were significant differences among the genotypes in terms of the shoot and root lengths (Table 2 and Table 3).

Increasing the NaCl treatment resulted in a significant decrease in shoot elongation. While the highest shoot length were founded in Redskin and Rufi varieties (5.97 and 5.57 cm $plant^{-1}$, respectively), the highest root length were observed in Corail and 07-Seas (3.29 and 3.11 cm $plant^{-1}$, respectively) under control treatment. On the other hand, the lowest values were determined in Barustic and Corail varieties with 200 mM NaCl. Compared to the control plants, the shoot and root lengths decrease determined as 62.4% and 83.8% with 200 mM NaCl, respectively. The lowest reductions of shoot and root lengths were detected in the 07-Seas and Casanova varieties as 45.67% and 50.22%, respectively. However considerable shoot and root reductions were observed in Barustic, Corail, Redskin and J-5 varieties up to 78-93%.

Table 1. Effects of different salt concentration on germination rate

	Germination rate (%)				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	97.8 d	95.5 d	84.8 f	66.8 ı	33.3 j
Corail	98.8 b	90.0 f	83.8 g	80.8 g	71.8 f
Livision	98.5 bc	97.3 ab	91.8 b	79.0 h	70.8 g
Lustrous	99.8 a	95.8 cd	95.0 a	91.0 c	88.3 a
Redskin	99.0 b	97.0 b	94.8 a	86.8 e	79.3 d
Rufi	99.8 a	97.8 a	94.8 a	92.8 b	83.8 c
Garnet	96.8 e	91.8 e	87.3 e	78.8 h	32.8 j
J-5	97.8 d	90.0 f	87.8 e	82.8 f	76.8 e
Wilma	99.8 a	89.8 f	88.8 d	89.0 d	64.8 h
Casanova	98.5 bc	96.3 c	90.8 c	87.3 e	84.8 b
07-Seas	98.0 cd	97.0 b	95.3 a	96.0 a	71.5 f
Libano	98.0 cd	86.5 g	74.0 h	58.8 j	38.5 ı
Mean	98.5**	93.7**	89.0**	82.5**	66.3**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Table 2. Effects of different salt concentration on shoot length

	Shoot length (cm $plant^{-1}$)				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	4.16 j	3.22 ı	2.42 j	1.98 k	0.87 k
Corail	5.25 c	3.85 c	3.21 e	2.58 f	2.53 a
Livision	4.73 g	3.75 d	2.83 h	2.72 d	2.30 b
Lustrous	5.13 d	2.82 k	2.67 ı	2.20 ı	1.67 f
Redskin	5.97 a	3.64 e	3.51 b	2.57 f	1.29 j
Rufi	5.57 b	4.03 b	3.82 a	2.30 h	2.22 c
Garnet	5.06 e	3.45 f	3.15 f	2.48 g	1.50 h
J-5	4.50 h	3.21 ı	3.23 e	2.69 e	1.64 g
Wilma	4.71 g	3.12 j	2.35 k	2.16 j	1.41 ı
Casanova	4.40 ı	3.31 g	3.31 d	2.90 c	2.19 d
07-Seas	4.05 k	3.26 h	3.10 g	3.06 a	2.20 d
Libano	4.86 f	4.75 a	3.45 c	3.01 b	1.92 e
Mean	4.87**	3.54**	3.09**	2.55**	1.81**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Table 3. Effects of different salt concentration on root length

	Root length (cm $plant^{-1}$)				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	1.90 j	1.49 ı	1.35 ı	1.06 e	0.34 f
Corail	3.29 a	2.74 a	2.05 b	1.05 e	0.20 h
Livision	2.83 e	2.73 a	1.63 f	1.56 a	0.37 e
Lustrous	2.77 f	1.69 f	1.73 d	1.20 d	0.34 f
Redskin	3.07 c	1.71 f	1.67 e	1.25 c	0.51 c
Rufi	2.36 g	2.25 c	2.20 a	1.45 b	0.35 f
Garnet	2.09 h	2.06 d	2.02 c	1.04 e	0.44 d
J-5	2.11 h	1.63 g	1.28 k	0.67 g	0.24 g
Wilma	2.92 d	1.90 e	1.40 h	0.78 f	0.45 d
Casanova	2.76 f	1.69 f	1.36 ı	0.67 g	0.34 f
07-Seas	3.11 b	1.53 h	1.31 j	0.66 g	0.53 b
Libano	1.97 ı	2.48 b	1.57 g	1.55 a	0.73 a
Mean	2.60**	1.99**	1.63**	1.08**	0.40**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Zabihi-e-Mahmoodabad [2] indicated that salt stress at the germination stage is a reliable test in evaluating the tolerance of many species, because salinity reduces the root and shoot growth. Some varieties are affected less and grow equally with the control plants and no inhibition effects are caused under saline growth. This is in accordance with the previous reports in melon, eggplant, bean, and tomato [16-19]. The general effects of salinity on plant growth reported a reduction in plant growth with shorter stature and sometimes fewer leaves, and roots are also reduced in length and mass [20]. The salinity treatments had a significant effect on the fresh weights of the shoots and roots (Table 4 and Table 5). Increasing the salinity levels reduced shoot and root fresh weights from 10 and 70% compared to control group. When the salinity increased from 50 mM to 200 mM, the shoot and root fresh weights were markedly decreased in the varieties by 54.9% and 26.2%, respectively. Salt-tolerant and salt-sensitive varieties showed very different growth patterns. While the sensitive varieties Redskin, Garnet, Casanova, and Libano, had high reductions in the shoot and root fresh weights (72.74% and 87.45% decrease, respectively), in the tolerant varieties, Lustrous, Rufi and Wilma, the shoot and root fresh weights decreased less in comparison to the control (0.86% and 61.37%, respectively). The average shoot and root fresh weights of the varieties was 4.63 mg plant^{-1}and 1.37 mg plant^{-1} under control conditions, and this value gradually decreased throughout the increasing salt concentrates, and reaching 1.37 mg plant^{-1} and 0.77 mg plant^{-1} with 200 mM NaCl, respectively. The shoot/root rate of the salt tolerance varieties was 1.07-6.09 with 200 mM NaCl (Table 6). Since salt stress involves both osmotic and ionic stresses, and growth suppression is directly related to the total concentrate of soluble salts and osmotic potential of the soil solution. The detrimental effects of salinity can be occurred at the whole-plant level as the death of plants or a decrease in productivity [21]. Plant growth of turfgrass varieties was significantly reduced by 200 mM NaCl. Zabihi-e-Mahmoodabad et al. [2] reported that the shoot and root fresh and dry weights decreased with increasing salinity and many other studies also reported this trait as the main indicator of salinity tolerance. Moreover, Hussein et al. [22] and Carpici et al. [23] reported that a negative relationship was detected between the vegetative growth parameters and increasing salinity. The STI showed a large variation among the varieties at different salt concentrates (Table 7). The STI varied between 11.8% and 53.6% with 200 mM NaCl. The Rufi (53.6%) and Livision (50.7%) varieties performed best with the 200 mM NaCl. On the other hand, Casanova (11.8%) and Redskin (15.6%) showed the lowest STI. The other varieties were moderately affected by the salt treatments. Carpici et al. [23] reported that the effects of different salt concentrates on the salt tolerance indices of cultivars were of importance. As the salt concentrates increased, the salt tolerance indices of the cultivars decreased. Kokten et al. [12] determined that tolerant genotypes showed higher salt tolerance indices than sensitive ones.

Table 4. Effects of different salt concentration on shoot fresh weight

	Shoot fresh weight (mg plant^{-1})				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	4.98 d	4.73 b	3.19 e	2.05 g	1.86 c
Corail	4.32 h	3.24 j	3.42 d	1.80 ı	0.92 g
Livision	4.52 f	4.42 d	4.10 a	4.13 a	2.29 b
Lustrous	5.03 c	3.76 g	3.63 c	2.50 f	1.53 e
Redskin	5.91 a	4.18 f	3.78 b	1.55 j	0.93 g
Rufi	5.52 b	5.39 a	4.10 a	3.88 b	2.96 a
Garnet	4.42 g	3.59 ı	2.03 j	1.55 j	0.74 ı
J-5	4.64 e	4.39 e	2.41 h	2.48 f	0.82 h
Wilma	3.21 k	2.76 k	2.82 g	2.59 e	1.24 f
Casanova	3.98 j	2.16 l	2.14 ı	1.92 h	0.47 j
07-Seas	4.09 ı	3.73 h	2.85 f	2.64 d	0.93 g
Libano	4.98 d	4.61 c	4.11 a	2.89 c	1.77 d
Mean	4.63**	3.91**	3.21**	2.50**	1.37**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Table 5. Effects of different salt concentration on root fresh weight

	Root fresh weight (mg plant^{-1})				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	2.47 c	1.68 d	1.46 c	0.73 ı	0.31 h
Corail	1.85 f	1.61 e	1.47 c	1.42 a	0.65 e
Livision	1.98 e	1.61 e	1.15 f	1.04 f	1.15 b
Lustrous	2.10 d	1.46 f	1.42 d	1.16 d	1.08 c
Redskin	2.54 b	2.73 a	1.35 e	1.11 e	0.76 d
Rufi	2.65 a	2.33 b	1.76 a	1.36 b	1.35 a
Garnet	1.53 h	0.78 ı	1.11 g	1.25 c	0.52 f
J-5	1.79 g	0.73 j	0.93 ı	0.80 h	0.76 d
Wilma	1.16 k	0.69 k	0.70 j	0.52 k	1.15 b
Casanova	1.18 j	1.24 g	1.06 h	0.90 g	0.31 h
07-Seas	1.51 h	1.08 h	0.66 k	0.57 j	0.76 d
Libano	1.42 ı	1.73 c	1.61 b	0.72 ı	0.39 g
Mean	1.84**	1.47**	1.22**	0.97**	0.77**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Table 6. Effects of different salt concentration on shoot/root

	shoot/root				
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	2.02 j	2.82 e	2.20 g	2.83 e	6.09 a
Corail	2.34 h	2.02 ı	2.34 f	1.27 h	1.41 e
Livision	2.29 h	2.75 e	3.58 c	3.99 c	1.99 d
Lustrous	2.40 g	2.57 g	2.56 e	2.16 f	1.42 e
Redskin	2.33 h	1.54 k	2.80 d	1.40 g	1.23 f
Rufi	2.08 ı	2.32 h	2.34 f	2.85 e	2.20 c
Garnet	2.89 c	4.62 b	1.83 ı	1.24 h	1.44 e
J-5	2.59 f	6.05 a	2.59 e	3.12 d	1.07 g
Wilma	2.78 d	4.01 c	4.05 b	5.03 a	1.08 g
Casanova	3.37 b	1.75 j	2.03 h	2.13 f	1.51 e
07-Seas	2.71 e	3.47 d	4.32 a	4.66 b	1.22 f
Libano	3.52 a	2.66 f	2.55 e	4.00 c	4.56 b
Mean	2.61**	3.05**	2.76**	2.89**	2.10**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

Table 7. Effects of different salt concentration on salt tolerance index (STI)

	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Barustic	94.97 b	64.17 h	41.25 ı	37.25 d
Corail	74.97 g	79.23 d	41.60 ı	21.32 h
Livision	97.78 a	90.70 a	91.52 a	50.72 b
Lustrous	72.69 g	72.10 f	49.67 g	30.45 f
Redskin	70.70 h	63.89 h	26.13 k	15.64 k
Rufi	97.69 a	74.34 e	70.26 c	53.62 a
Garnet	81.25 f	45.86 j	34.99 j	16.70 j
J-5	94.50 b	51.83 j	53.34 f	17.56 ı
Wilma	85.89 c	87.84 b	80.67 b	35.50 c
Casanova	54.23 ı	53.86 ı	45.21 h	11.75 l
07-Seas	91.13 d	69.66 g	64.55 d	22.75 g
Libano	92.66 c	82.56 c	55.04 e	35.47 e
Mean	84.04**	**	**	**

Means in a column followed by the same letter are not significantly differ according to the Duncan's test at the 0.01 level of significance. *: P<0.05, **: P<0.01

CONCLUSIONS

In conclusion, turfgrass varieties showed a marked variation in their sensitivity to salt tolerance in the present study. The increasing NaCl concentrate caused harmful effects on seed germination in the evaluated properties such as, shoot and root lengths and fresh weight. However, there were slightly more harmful effects in the tolerant varieties than in the sensitive ones.

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