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Full Length Research Paper

Effects of salinity stress on water uptake, germination and early seedling growth of perennial ryegrass

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This research was conducted for the determination of effects of salinity stress on water uptake of seed, germination and early seedling development of perennial ryegrass (*Lolium perenne* L. cv. Ovation) in the seed laboratory of Field Crops, Department of Agricultural Faculty of Namik Kemal University, Tekirdag, Turkey, 2009. In this study, eight different sodium chloride (NaCl) levels, including electrical conductivities of 0, 2, 4, 8, 12, 16, 20 and 24 dS m^{-1} were used as salinity treatments. Seeds were germinated at 25 ± 1°C for 14 days in a growth chamber. Water uptake of seeds during germination period were measured at 3, 6, 12, 24, 36, 48 and 72 h. Germination rate and some morphological and physiological characters were determinated on the 14th day after germination. Dry weights were measured after samples had been dried at 70°C for 48 h in an oven. The results showed that water uptake of seed, germination rate, and mean germination time of perennial ryegrass were affected by different salinity levels during germination period. Likewise, root and shoot length, coleoptiles length, leaf number, fresh and dry weights of root and shoot of perennial ryegrass seedling were also affected from different salinity application, but root number did not change statictically in different NaCl concentrations. While mean germination time increased with increasing salinity, germination rate decreased. Increasing NaCl concentration caused a significant reduction in root length, coleoptiles length, shoot length, and leaf number. Fresh and dry weight of root and shoot decreased significantly over 8 dS m^{-1} NaCl concentrations during the germination period. In conclusion, it can be said that perennial ryegrass has tolerance up to 8 dS m^{-1} salinity level at the germination and seedling growth stages.

Key words: Ryegrass, *Lolium perenne* L., salinity stress, water uptake, seedling growth.

INTRODUCTION

In the dry areas of the world, there is an increasing pressure to apply low quality water for plant irrigation (Schleiff, 2008). Use of low quality water is increasing salinity of soil. The osmotic pressure of soil solution is increased by increasing salt concentration. Increase of soil osmotic pressure causes difficulties in the water uptake of plants (Ergene, 1987; Kacar, 1989). Soil salinity is one of the major abiotic stresses responsible for reduced persistence, yield and biomass accumulation in many crops, including forage crops. High salinity can rapidly inhibit root growth and hence water and essential mineral nutrients uptake capacity of the roots from the soil is decreased. At the same time, it can cause an increase in mortality of plants. The most common is that salt stress is caused by high Na^+ and Cl^- concentrations in the soil solution (Gulzar et al., 2003). Mohammadi et al. (2008) reported that total shoot nitrogen absorption is mostly decreased under salinity stress because of the antagonistic effects of Na^+, NH_4^+ or NO_3^- and Cl^-.

Increasing soil osmotic pressure correlates with the amount of salt rather than type of salt. Many plants are affected adversely by the presence of relatively low levels of salt; some plants can survive at high levels (salt-tolerant plants). Consequently, it is important to develop a better understanding of salt tolerance in forage and turfgrass species that are generally grown in adverse conditions (Neuman, 1997; Taiz and Zeiger, 2002; Gulzar et al., 2003; Dombrowskia et al., 2008; Altın et al., 2009).

Perennial ryegrass cultivated with higher salt tolerance is one of the important grassland, turfgrass, and forage grass (Avcioglu, 1997; Wu et al., 2005; Baytekin et al., 2009). Acikgoz (1994) stated that perennial ryegrass has

a medium tolerance for salinity conditions. Especially, it will be useful for us to know the salinity tolerance level of perennial ryegrass varieties due to the application of low quality water. The aim of this research was to determine the performance of perennial ryegrass under various salinity stress levels, at the germination and seedling growth stages.

MATERIALS AND METHODS

This research was conducted in the seed laboratory of Field Crops, Department of Agricultural Faculty of Namik Kemal University, Tekirdag, Turkey, in 2009. In this study, seeds of perennial ryegrass (*Lolium perenne* L. cv. Ovation) were used as the plant material.

The experimental design was a randomized plot with four replications. The solutions with eight different salt levels (0, 2, 4, 8, 12, 16, 20, 24 dS m^{-1} electrical conductivity) for salinity stress were adjusted using NaCl. Distilled water was used as the control solution (0 dS m^{-1}). Seeds were initially treated with a 1.5% solution of sodium hypochlorite for 3 min for surface sterilization. Residual chlorine was eliminated by thorough washing of seeds with distilled water. 50 seeds for each of the NaCl concentration were quickly dried with a paper tissue after surface sterilization and weighed. Then, 50 seeds were placed on two rolled Whatman's No.1 filter papers in 9 cm-diameters Petri dishes with 10 ml each of NaCl solutions. The papers were replaced every two days to prevent accumulation of salts (Rehman et al., 1996). In order to prevent evaporation, Petri dishes were put into locked transparent plastic bags (Kaya et al., 2005). Seeds were allowed to germinate at 25 ± 1°C for 14 days in a growth chamber (Sehirali, 1997). For determination of water uptake, seeds were removed from the Petri dishes at regular intervals, were quickly surface-dried with a paper tissue and weighed and then returned to the original conditions. Measurements for water uptake were taken at 3, 6, 12, 24, 36, 48 and 72 h (Alam et al., 2003). Water uptake percentage was calculated with the following formula:

$$\text{Water uptake (\%)} = W_2 - W_1 / W_1 \times 100$$

Where, W_1 = initial weight of seed and W_2 = weight of seed after absorbing water in a particular time.

Germination rates were determined on the 14th day. A seed was considered to have germinated when the emerging radicle elongated to 1 mm. Germination rate was calculated with the following formula:

$$\text{Germination rate (\%)} = (\text{Number of germinated seeds} / \text{Number of total seeds}) \times 100$$

Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980) as follows:

$$\text{Mean germination time (day)} = \sum(fx) / \sum f$$

Where, f is the number of newly germinated seeds on each day and x is the day of counting.

The number of root, root length, root fresh and dry weight, coleoptile length, leaf number, shoot length, shoot fresh and dry weight were measured on the 14th days after germination. Dry weights were determined after drying samples at 70°C for 48 h in an oven (Böhm, 1979). Data of fresh and dry weight of root and shoot were measured as sum of 15 plants. Then, data of fresh and dry weights were calculated as a plant weight.

The parameters given as percentage were subjected to arcsine transformation before statistical analysis. Water uptake was statistically analyzed according to randomized split-plot design and other characteristics were statistically analyzed according to randomized plot design. All data obtained from this experiment was statistically analyzed by using the MSTAT-C packet computer program. The statistical significance of differences among the mean values was determined by least significant difference (LSD) test at 5% probability (Duzgunes et al., 1987). The seeds did not germinate enough to measure the root and shoot growth in the 24 dS m^{-1} salinity treatment. So, 24 dS m^{-1} salt level was not calculated at statistical analysis for root and shoot growth.

RESULTS AND DISCUSSION

Results were presented and discussed under three titles as: germination tests, root growth, and shoot growth.

Germination tests

Water uptake of seeds

Statistical analysis showed that water uptake of perennial ryegrass seeds was significantly influenced ($P \leq 0.01$) by salt levels, time, and salt level x time interaction (Table 1).

Water uptake of seeds of perennial ryegrass was the highest at 4 dS m^{-1} salt concentration. The lowest water uptake was determined at 20 and 24 dS m^{-1} salt levels. According to the control, at 4 dS m^{-1} salt level, it was clear that water uptake was stimulated by a little increasing salinity. According to the control, seeds had a higher water uptake after 48 h at 4 dS m^{-1} salt level, then water uptake of seeds at 4 dS m^{-1} salt level was the same with the control application. Salt concentrations until 4 dS m^{-1}, prompted the germination of seed and accelerated the process of germination. On the other hand, at high level salt concentration, the osmotic pressure of the environment increased so water absorption of the seed decreased. We found that seeds of the perennial ryegrass carried on water uptake during the first 72 h. Therefore, approximately half of the absorbed water was uptaken in the first 3 h by seed. Water uptake ratio decreased in subsequent hours. It was obvious that water uptake of seed was further limited in high salinity levels according to the control after the first 24 h. Taiz and Zeiger (2002) reported that high concentration of NaCl increases its osmotic pressure. In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the germination. Kaydan and Yagmur (2008) reported that high NaCl concentrations caused lower water uptake by seed and so germination decreased. The result of the research is similar with the results of our research. In the same way, Munns (2002) reported that salinity reduces the ability of plants to absorb water.

Germination rate

Differences among NaCl treatment were statistically

Table 1. Water uptake (%) of seed on the germination of perennial ryegrass in different salinity levels.

Salt level (dS m^{-1})	Time (h)							Mean
	3	6	12	24	36	48	72	
0	31.23^{y}	40.27^{wx}	47.45^{r-v}	55.96^{k-p}	61.40^{g-k}	66.2^{b-g}	78.26^{a}	54.40^{b}
2	31.79^{y}	42.45^{vw}	49.76^{p-t}	57.36^{i-n}	61.14^{g-l}	65.27^{b-g}	69.92^{bc}	53.96^{b}
4	34.94^{xy}	46.69 $^{s-v}$	55.09^{l-q}	61.92^{f-k}	67.94^{b-f}	71.44^{b}	79.08^{a}	59.59^{a}
8	33.91^{y}	43.11^{uvw}	49.06^{q-u}	56.36^{j-n}	58.62^{h-n}	62.42^{e-j}	67.96^{b-f}	53.06^{b}
12	33.09^{y}	43.11^{uvw}	52.98^{n-r}	57.50^{i-n}	61.94^{f-k}	64.72^{c-h}	68.93^{bcd}	54.61^{b}
16	31.20^{y}	41.52^{vw}	50.04^{o-s}	56.10^{k-o}	60.53^{g-m}	63.03^{d-i}	68.45^{b-e}	52.98^{b}
20	30.39^{y}	41.45^{vw}	43.66^{t-w}	53.06^{n-r}	54.72^{m-q}	60.78^{g-m}	64.66^{c-h}	49.82^{c}
24	31.38^{y}	43.10^{uvw}	45.76^{s-w}	55.83^{k-p}	56.90^{i-n}	58.50^{h-n}	60.10^{g-m}	50.22^{c}
Mean	32.24^{g}	42.71^{f}	49.23^{e}	56.76^{d}	60.40^{c}	64.05^{b}	69.67^{a}	
LSD 5%	Salt level: 2.210		Time: 2.362		Salt level × time: 6.250			
CV (%)	Salt level: 51.0315		Time: 50.1120		Water uptake: 24.5721			

Means followed by the same letters are not significantly (P≤0.05) different.

Table 2. Germination rate and germination time of perennial ryegrass in different salinity levels.

Parameter	Salt level (dS m^{-1})								LSD 5%
	0	2	4	8	12	16	20	24	
Germination rate (%)	96.5^{a}	95.5^{a}	92.5^{a}	88.0^{ab}	78.0^{b}	67.0^{c}	53.0^{d}	20.0^{e}	10.795
Mean germination time (day)	6.21^{e}	6.14^{e}	6.56^{e}	7.68^{d}	8.82^{c}	10.79^{b}	10.61^{b}	11.77^{a}	0.890
CV (%)	Germination rate: 35.2839				Mean germination time: 25.8054				

Means followed by the same letters are not significantly (P≤0.05) different.

significant (P≤0.01) for germination rate (Table 2). Germination rate decreased greatly over 8 dS m^{-1} salinity levels. The highest germination rate was measured as 96.5, 95.5, 92.5, and 88.0% at 0, 2, 4, and 8 dS m^{-1} salt concentrations, respectively. The lowest germination rate was found as 20.0% at 24 dS m^{-1}. As a result, germination rate declined with increasing salinity. Germination rate decreases at high concentrations of NaCl (Taiz and Zeiger, 2002). Since the high salt level limited the water absorption, it affected the germination negatively. Our results are relevant to the findings of Dai et al. (2009), Akhtar and Hussain (2008) and Kaydan and Yagmur (2008) that worked on different plants. For example, Akthar and Hussain (2008) reported that germination of all three grasses (*Bothriochloa pertusa* (L.) A. Camus, *Dichanthium annulatum* Forssk, and *Panicum antidotale* Retz) significantly declined even at 5 dS m^{-1} level of treatment that further decreased at 10 and 15 dS m^{-1}.

Mean germination time

Mean germination time of perennial ryegrass was significantly influenced (P≤0.01) by salinity. Increasing salinity levels increased mean germination time of perennial ryegrass. 24 dS m^{-1} NaCl level had the longest mean germination time as 11.77 days. Whereas, seeds of perennial ryegrass germinated approximately in 6 days at 0, 2, and 4 dS m^{-1} NaCl levels. Germination time was 4 to 5 days longer at 16 dS m^{-1} and had higher NaCl concentrations than the control. It is obivious that germination time of perennial ryegrass elongates with increasing salinity stress. Seed of ryegrass germinated in a short time because of higher water uptake at the low salinity concentrations. The delay and prevention of water absorption also delayed the germination. Delayed germination causes both increased irrigation cost, and irregular and weak seedling growth in the establishment of perennial ryegrass. As similar with our results, some researchers claimed that as a result of increasing osmotic pressure, water uptake is delayed and, so germination time is elongated and germination rate is decreased (Quila, 1992; Gunjaca and Sarcevic, 2000; Almansouri et al., 2001; Taiz and Zeiger, 2002; Carpıcı et al., 2009).

Root growth

Root morphology is not only a very important factor for nutrient absorption by roots, but also it is very important

Table 3. Root number, root length, root fresh weight, and root dry weight of perennial ryegrass in different salinity levels.

Salt level (dS m^{-1})	Root number (number/plant)	Root length (cm)	Root fresh weight (mg/plant)	Root dry weight (mg/plant)
0	2.23	5.44^{a}	0.915^{ab}	0.118^{ab}
2	2.38	5.70^{a}	1.135^{a}	0.120^{a}
4	2.30	4.27^{b}	1.047^{ab}	0.118^{ab}
8	2.50	4.32^{b}	0.920^{ab}	0.110^{abc}
12	2.10	2.87^{c}	0.750^{b}	0.090^{bcd}
16	2.08	1.98^{cd}	0.683^{bc}	0.085^{cd}
20	1.83	1.43^{d}	0.338^{c}	0.068^{d}
24	-	-	-	-
LSD 5%	ns	0.969	0.384	0.029
CV (%)	17.6696	44.9666	41.2363	25.7356

Means followed by the same letters are not significantly (P≤0.05) different.

for water uptake by roots from saline soils (Schleiff, 2008). So, it will be usefull to examine root growth under different salinity stress conditions. Root number, root length, root fresh weight, and root dry weight of germinated perennial ryegrass at different salinity levels are given in Table 3. Germination and seedling growth was not enough to examine the root growth at 24 dS m^{-1} salt level.

Root number

The number of root was not significantly affected by salt stress (Table 3). Therefore, root number increased till 8 dS m^{-1} salinity level, then over 8 dS m^{-1} salt levels it decreased. As the osmotic pressure increases at the germination environment, water carry most of the water soluble nutrition (especially soluble carbohydrate) to the root in order to increase osmotic pressure of roots to get enough water (Balkan, 2011). This increases the root number. Similarly, in this study the salt concentration increased up to 8 dS m^{-1} and led to an increase in the number of root. On the other hand, at the higher salt concentration, since the plant growth was affected negatively, root number decreased. As similar with our results, Rubinigg et al. (2002) reported that relative growth rate of *Festuca rubra* was reduced by exposure to a sodium chloride concentration as 50 mol m^{-3}.

Root length

There were statistically significant differences in root length of perennial ryegrass for various NaCl levels (Table 3). We found that according to the control at 2 dS m^{-1}, root length was increased, and it decreased over 2 dS m^{-1} salt concentrations. Our results confirm the findings of Dai et al. (2009) for *Poa annua* and Alshammary et al. (2004) for *Puccinellia distans* with increased root length at lower salinity levels but decreased root length at higher levels. In our study, the longest root was measured at 0 and 2 dS m^{-1} salt concentrations, as 5.44 and 5.70 cm, respectively. Root length decreased as much as 1.43 cm at 20 dS m^{-1} NaCl concentration. According to the control application, while root length increased by 4.8% at 2 dS m^{-1} salt concentration (0 dS m^{-1}), it decreased by 21.5% at 4 dS m^{-1}. Root length also decreased by 47.2, 63.6 and 73.7% at 12, 16 and 20 dS m^{-1} salt concentrations, respectively. Pessarakli and Kopec (2009) also stated that root length of perennial ryegrass decreased linearly with increased salinity levels. It is seen that salinity stress affected root length more than root number of perennial ryegrass in this research. The number of the roots increased for water uptake under low level salt stress. However, the roots did not lenghten. On the other hand, at high salt concentrations, both the root number and root length were affected.

Root fresh weight

Root fresh weight of perennial ryegrass was affected (P≤0.01) by different salt levels (Table 3). Root fresh weight increased with increasing salinity at 2 dS m^{-1} and it started to decrease in higher salt levels over 2 dS m^{-1}. In 2, 4 and 8 dS m^{-1} NaCl levels, root fresh weight was higher than 0 dS m^{-1} application. The lowest root fresh weight was determined at 20 dS m^{-1}. The increase of root number till 8 ds m-1 level caused the increase of fresh root growth. At higher level of salt concentrations, since the the root number and root length decreased densely, the fresh root growth also decreased. The findings of our results confirm some researchers' reports which claimed that root weight increased at low NaCl salt levels for some plants (Kaya et al., 2005). Likewise, Alshammary et al. (2004) also reported that salt tolerant plants have the

Table 4. Leaf number, coleoptile length, shoot length, shoot fresh weight, and shoot dry weight of perennial ryegrass in different salinity levels.

Salt level (dS m^{-1})	Leaf number (number/plant)	Coleoptile length (cm)	Shoot length (cm)	Shoot fresh weight (mg/plant)	Shoot dry weight (mg/plant)
0	1.63^{a}	0.88^{a}	5.02^{a}	4.082^{bc}	0.400^{ab}
2	1.80^{a}	0.84^{a}	5.26^{a}	5.383^{a}	0.457^{a}
4	1.65^{a}	0.79^{a}	4.80^{a}	5.348^{a}	0.455^{a}
8	1.55^{ab}	0.80^{a}	5.04^{a}	4.915^{ab}	0.435^{a}
12	1.28^{bc}	0.77^{a}	3.62^{b}	3.700^{c}	0.363^{ab}
16	1.00^{c}	0.76^{a}	3.44^{bc}	3.083^{c}	0.308^{b}
20	1.00^{c}	0.59^{b}	2.75^{c}	1.467^{d}	0.193^{c}
24	-	-	-	-	-
LSD % 5	0.317	0.152	0.697	1.143	0.098
CV (%)	25.4367	16.3570	23.1320	37.4048	28.9215

Means followed by the same letters are not significantly (P≤0.05) different.

ability to minimize detrimental effects by producing a series of morphological and physiological adaptations, such as an extensive root system.

Root dry weight

Root dry weight of perennial ryegrass was significantly changed between different salinity levels (Table 3). The highest root dry weight was obtained from 2 dS m^{-1} salt levels as 0.120 mg/plant. The lowest root dry weight was determined in 20 dS m^{-1}. Root dry weight of perennial ryegrass did not change significantly on conditions where salinity levels increased up to 8 dS m^{-1}, but decreased significantly after 8 dS m^{-1} salinity levels. The data is in agreement with previous studies (Alshammary et al., 2004; Kaya et al., 2005). Dai et al. (2009) reported that root dry weight of perennial ryegrass declined with increasing salinity. In parallel with the increase of fresh root weight, root dry weight also increased in this study.

Shoot growth

Leaf number, coleoptiles length, shoot length, shoot fresh weight, and shoot dry weight of the germinated perennial ryegrass under different salinity stress conditions are given in Table 4. Germination and seedling growth was not enough to examine the shoot growth at 24 dS m^{-1}.

Leaf number

Leaf numbers of ryegrass were significantly affected (P≤0.01) by different salinity levels (Table 4). Leaf number significantly decreased with increasing salinity levels. The decrease in leaf number was more in over 8 dS m^{-1} salinity levels. Leaf number only increased according to the control at 2 dS m^{-1}. However, both applications (control and 2 dS m^{-1}) were statistically in the same significant groups.

Coleoptile length

In terms of coleoptile length of perennial ryegrass, differences among salt levels were significant (Table 4). Coleoptile length decreased when salinity stress increased. The longest coleoptile length was measured at the control treatment (0 dS m^{-1}) while the shortest coleoptile length was determined at 20 dS m^{-1} treatment.

Shoot length

Differences among the salinity levels were statistically significant for shoot length (Table 4). While shoot length did not significantly change from 0 to 8 dS m^{-1} salinity levels, it significantly decreased at the salinity levels over 8 dS m^{-1}. Shoot length at 2 dS m^{-1} salinity level was longer than for the control. The shortest shoot length was measured as 2.75 cm at 20 dS m^{-1}. Shoot growth of perennial ryegrass can be tolerated up to 8 dS m^{-1} salinity level. Pessarakli and Kopec (2009) found that shoot length decreased by the increasing salt concentrations.

Shoot fresh weight

As seen in Table 4, shoot fresh weight was significantly influenced (P≤0.01) by salinity levels. The highest shoot fresh weight was obtained from 2 and 4 dS m^{-1} salinity levels. The lowest shoot fresh weight was found at 20 dS m^{-1}. At 2, 4 and 8 dS m^{-1} salinity levels, shoot fresh weight was higher than at 0 dS m^{-1}. Since the root growth was stimulated at salt concentrations till 8 dS m^{-1}, plant growth

was affected positively up to this salt level. Thus, shoot fresh weight was higher than the control application at 2, 4, and 8 dS m^{-1} salt concentrations. However, shoot fresh weight significantly decreased in the salinity levels over 8 dS m^{-1}. This result was supported by the findings of some researches which showed that shoot weight increased in low NaCl levels for some plants (François 1994; Kaya et al., 2005).

Shoot dry weight

Salinity stress significantly (P≤0.01) affected shoot dry weight. Shoot dry weight significantly decreased at salt levels over 8 dS m^{-1}. The highest shoot dry weight was obtained from 2, 4, and 8 dS m^{-1} salinity levels which were statistically in the same significant group. The lowest shoot dry weight was determined at 20 dS m^{-1} salinity level. Our findings are also in agreement with the results of other researchers (Rogers, 2007; Mohammadi et al., 2008; Pessarakli and Kopec, 2009; Dai et al., 2009). Shoot fresh and dry weight increased at low NaCl salt levels; same as in the root growth. This increase was prominent especially on shoot fresh weight.

Conclusion

In this research, perennial ryegrass seeds uptook approximately half of the absorbed water in the first 3 h during the first 72 h of germination. Water uptake of the seed was limited in high salinity levels according to the control after the first 24 h. As the germination rate decreased with increasing salinity stress, germination time elongated. Root and shoot growth of perennial ryegrass suffered seriously in salinity levels over 8 dS m^{-1} during the first 14 days of seed germination. Shoot and root growth also increased in 2 dS m^{-1} as compared to 0 dS m^{-1} (control). As a result, it can be said that perennial ryegrass has tolerance up to 8 dS m^{-1} salinity level at the germination and early seedling growth stages.

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