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Original Research

Impact of Salinity on Germination and Seedling Growth of Four Cool-Season Turfgrass Species and Cultivars

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Abstract

Salinity is the main environmental factor accountable for decreasing crop productivity and turf quality worldwide. This study was carried out to determine and compare the salinity (sodium chloride, NaCl) tolerance in newly developed eight cultivars of four cool-season turf grass species (perennial ryegrass, slender creeping red fescue, tall fescue and Kentucky bluegrass) at germination and seedling stage. All experiments were arranged as randomized plots design with three factors (species, cultivar and salinity level) and four replications. The obtained results showed that the seed germination of perennial ryegrass, slender creeping red fescue, tall fescue and Kentucky bluegrass cultivars were highly affected by the highest level of salinity (200 mM NaCl). Statistically significant were observed differences among NaCl levels for root fresh mass, shoot fresh and dry mass, water retention capacity, relative water content, tolerance index and K^+/Na^+ ratios of cultivars. Based on their tolerance index (TI), five cultivars, 'Ringles' perennial ryegrass, 'Abercharm' slender creeping red fescue, 'Prafin' Kentucky bluegrass, 'Fesnova' and 'Golden Gate' tall fescue demonstrated good salt tolerance. The tolerance indexes of these cultivars were supported by the K^+/Na^+ ratios. The cultivars 'Ringles', 'Abercharm', 'Prafin', 'Fesnova' and 'Golden Gate' exhibited potential salt tolerance and could compete with other cool-season turfgrass varieties regarding productivity under salt stress.

Keywords: germination, seedling growth, tolerance index, turfgrass, salinity

Introduction

Salinity is one of the abiotic stresses and it is a big problem of both irrigated and non-irrigated turfgrass areas in several parts of the world, with a predominance in arid and semi-arid regions [1]. Especially, being near the coast should never restrict to grow turf of the high quality. Even in locations that are distant from the sea, salinity can be a problem caused by rising levels of salt in groundwater and an increasing reliance on recycled water for irrigation. Salinity also affects the verges of winter-salted roads and it inhibits the growth of turfgrass species [2].

Most cool-season turfgrass species do not establish or survive long in highly saline areas, allowing salt-tolerant weeds to invade. Salinity limits plant growth and productivity through the toxic effects of sodium (Na^+)

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and chloride (Cl^-) ions, which leads to ionic imbalances, osmotic and oxidative stress [3]. Salinity tolerance is a complex trait, governed by several physiological and biochemical parameters and these parameters greatly influence the normal growth and development of plants [4]. Salinity makes it difficult for the roots to take up water due to the decreased osmotic potential [5]. Plants may have difficulty withdrawing water from what appear to be moist soils. A build-up of salt in the leaves, especially old leaves, can lead to necrosis. The overall effect on the plant depends on the rate of new growth compared with the rate of leaf necrosis. High Na^+ and Cl^- concentrations in the soil can affect the uptake of other nutrients, e.g., nitrogen, phosphorus, potassium and, magnesium, which are essential for plant growth [6]. Besides, salinity accelerates the production of active oxygen radicles, such as H_2O_2 (hydrogen peroxide), $•O_2^-$ (superoxide), $^1O_2$(singlet oxygen), and $•OH^-$(hydroxyl radicle), which can damage or even kill plants [7].

Cool-season turfgrass species and other species vary widely in their tolerance to salts in soil and irrigation water [5, 8]. In most plants, salt tolerance is lowest at germination and during early seedling establishment [9]. Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface. These salts interfere with seed germination and crop establishment. Lower levels of salinity delayed germination whereas higher levels in addition, reduced the final percentage of seed germination [10]. Salt tolerance levels can also change with soil and environmental conditions. Minimizing the salt stress on plants, particularly during early development, promotes successful establishment and growth [11]. Salt tolerance of any individual species is demonstrated as the ability to maintain an optimal physiological and biochemical equilibrium under NaCl treatment [3]. In addition to salinity tolerance, tolerance to heat and drought stress is required, because much of the salinized area around the world is found in hot and dry regions [12].

Nevertheless, the new cultivars of cool-season turfgrass species are constantly being developed by breeding companies in Europe, USA and etc. [13]. These cultivars tolerance to abiotic and biotic stresses at different climates must be known for turf management. Besides, the relative salt tolerance among most of the widely used new developed these cultivars has not been adequately studied and it is important to determine cultivars which can have a chance in salt-affected lands [14]. Because of this reason use of salt-tolerant cultivars offers a useful approach for increasing turfgrass performance. The first step in the program of identification and screening for salinity tolerance in turfgrass species and cultivars are a conduct a germination examination [15]. Literature suggests that in the seedling stage varieties of perennial ryegrass (*Lolium perenne* L.), slender creeping red fescue (*Festuca rubra* L. ssp. *trichophylla* Gaud.), tall fescue (*F. arundinacea* Schreb.) and Kentucky bluegrass (*Poa pratensis* L.) show different tolerance of salinity stress. Because of that, screening and breeding cool-season turfgrass varieties that are salt tolerant during both seed germination and vegetative growth becomes important [16]. Besides, the need for salinity tolerant turfgrasses is increasing because of the increased use of effluent or other low-quality waters for turfgrass irrigation. This study, therefore, was carried out to determine and compare salinity (sodium chloride, NaCl) tolerance in newly developed cultivars of four cool-season turf grass species (perennial ryegrass, slender creeping red fescue, tall fescue and Kentucky bluegrass) at germination and seedling stage.

Materials and Methods

Materials

Germination experiment was carried out in 2017 at the Department of Field Crops, Faculty of Agriculture, University of Tekirdag Namik Kemal in Turkey. Greenhouse experiments were carried out in 2017-2018 at same department. Salt tolerance was screened between two cultivars of four cool-season turfgrass species: (I) Perennial ryegrass: cv. 'Ringles' and cv. 'Sun', (II) Slender creeping red fescue: cv. 'Abercharm' and 'Spartan 2', (III) Tall fescue: cv. 'Fesnova' and cv. 'Golden Gate', (IV) Kentucky bluegrass: cv. 'Prafin' and cv. 'Heatmaster'. The certified seed of these cultivars belongs to Semillas Fitó S.A.U in Barcelona (Spain). Before experiments, viability of the seeds was tested by tetrazolium (2, 3, 5-triphenyltetrazolium chloride) test (TTC test) [17]. All experiments were arranged as randomized plots design with three factors (species, cultivar and salinity level) and four replications.

Methods

Laboratory Experiment

For surface sterilization, seeds were soaked in 2% NaClO (sodium hypochlorite) for 15 minute and rinsed with sterile water four times and then quickly dried with a sterile paper tissue. Subsequently, four replicates of twenty-five viable seeds for each cultivar of four species were placed on one layer of sterile 'germ test' paper (neutral pH) in closed sterile petri dishes (diameter of 15 cm) and moistured with 10 ml of deionized water at five concentrations of NaCl (Merck 106404 for analysis EMSURE® ACS, ISO, Reag. Ph Eur), namely 0 (control), 50, 100, 150 and 200 mM [5, 18]. Petri dishes were sealed with parafilm to prevent evaporation. According to the results of preliminary studies and standard seed laboratory protocols for perennial ryegrass, slender creeping red fescue, tall fescue and Kentucky bluegrass seed germination tests

[17, 19, 20] were conducted for forty-five days when no further germination was observed [21]. Tests were then incubated at 20±2.0ºC under 1.63Wm^{-2} of fluorescent lighting at a 12h light/12h dark diurnal cycle. Seeds were considered to have germinated when their radicles have emerged, after which the number of seeds germinated was recorded. During testing, contamination was not found. In salinity stress conditions, because of slow germination of seeds, germination tests were conducted over a longer period (forty-five days) than foreseen in the rules of [17, 19, 20]. Final germination percentage is described by FGP (%) = $100\frac{\Sigma n}{45}$ [22].

Greenhouse Experiments

In each year (2017 and 2018), eight seeds of each cultivar were initially planted in each plastic pot (each replication consisted of twenty pots, 18 cm high x 16 cm in diameter) filled with an 1800 g heat-sterilized (95°C, 72 h) soil consisting of 60% sand, 25% silt, and 15% clay, and after the emergence of all the seedlings, four seedlings were allowed to growth in each pot [8]. To prevent resistance in the pots, the mortar soil was placed into polyethylene bags [23]. Pots and bags were sterilized with 2% NaClO for 15 minute and rinsed with sterile water four times. The base nutrient solution was applied in 100 ml to the all pots. The base nutrient solution consisted of quarter-strength Hoagland's solution, except for calcium and magnesium, which were at full strength. This concentration was according to the protocol for forage production under salt stress [24]. All fertilizers used were analytical reagent grade and their concentrations in the solution were as: 0.25 mM potassium dihydrogen phosphate (KH_2PO_4), 1.25 mM potassium nitrate (KNO_3), 5.0 mM calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), 2.0 mM magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 11.3 µM boric acid (H_3BO_3), 2.3 µM manganese (II) chloride ($MnCl_2 \cdot 4H_2O$), 0.2 µM zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 0.08 µM copper sulfate ($CuSO_4 \cdot 5H_2O$), 0.004 µM ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) and 30.0 µM ferric-EDTA (Fe-EDTA). Concentrations of NaCl (0, 50, 100, 150 and 200 mM) were applied in 100 ml of water after emergence of each cultivar, and all pots were irrigated to field capacity. Salinity level was measured twice a week throughout the experimental duration with EC meter and maintained with the addition of proper quantity of NaCl solution. Control pots were irrigated with distilled water. After the targeted salinity levels were achieved, the irrigation water depending on field capacity was applied for a period of seven weeks (March 07 to April 26 at each year) at greenhouse condition [25]. Fifty days after emergence, seedlings were taken by gentile washing the soil, and each seedling were separated into roots and shoots [16]. Then, the root and shoot fresh mass (RFM and SFM, mg per plant) were determined. Shoot samples were oven-dried at 65ºC for 48 h, to determine dry mass (SDM, mg per plant). The water retention capability of the shoot was calculated using the following formula: Retention capability (RCS) of shoot (mg) = (SFM-SDM)/SDM [26]. Relative water content (RWC) as percentage of SFM was calculated the following formula: RWC (%) = SFM-SDM/SFM x 100 [10]. Furthermore, a tolerance index (TI) was calculated for each cultivar of species; i.e., shoot dry mass (SDM) was calculated by adopting the following formula [27]: TI = SDM in salinity stress/SDM in control x 100.

Chemical Analyses

The oven-dried samples were ground to small (≤1 mm) pieces and 15 mg of this was transferred to a digestion flask (25 ml) containing an acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$), in the ratio 3:1 (v/v). The flask was heated gently over a sand bath. The cooled digest was then diluted by adding distilled water and the volume was made up as required. The potassium (K^+) and Na^+ concentrations were determined using a flame photometer (Shanghaiprecision and scientific instrument Co., Ltd., 6400 A type) following the methods described by [10], then K^+/Na^+ ratios of shoots were calculated. All samples were analyzed in triplicate.

Data Analyses

Data were analyzed using analysis of variance (ANOVA) (TARIST statistical computer package). There were no significant differences at $P>0.05$ in the year and interactions when comparing the year of greenhouse study or salinity levels. The percentages were subjected to arcsine transformation. Same program was used for the comparison test (Fisher's Least Significant Difference, LSD) of the means from the two years and Standard Deviation (SD) was calculated.

Results and Discussion

Effect of Salinity on Seed Germination

The obtained results showed that the seed germination of perennial ryegrass, slender creeping red fescue, tall fescue and Kentucky bluegrass cultivars were highly affected by highest level of salinity (200 mM NaCl) ($P<0.01$, Table 1). FGP of both species 'perennial ryegrass' (75.2%) and 'Kentucky bluegrass' (75.4%) significantly decreased in same salinity level. However, NaCl treatment resulted in decreasing the FGP in the perennial ryegrass and Kentucky bluegrass cultivars as compared to slender creeping red fescue and tall fescue cultivars. The lowest FGP was determined from perennial ryegrass cultivar 'Sun' with 200 mM NaCl level. Among tested eight cultivars of four turfgrass

Table 1. FGP (%) of *L. perenne*, *F. arundinacea*, *F. rubra* L. ssp. *trichophylla* and *P. pratensis* cultivars under different salinity levels.

Salinity level mM NaCl	*Lolium perenne*		*Festuca arundinacea*		*Festuca rubra* L. ssp. *trichophylla*		*Poa pratensis*		*Lolium perenne*	*Festuca arundinacea*	*Festuca rubra* ssp. *trichophylla*	*Poa pratensis*
	'Ringles'	'Sun'	'Fesnova'	'Golden Gate'	'Abercharm'	'Spartan 2'	'Prafin'	'Heatmaster'				
0	99.7a	99.5a	99.6a	99.7a	98.8a	99.0a	99.3a	99.0a	99.6A	99.7A	98.9AB	99.2A
50	99.5a	96.4b	98.0a	98.3a	98.6a	98.5a	95.4b	93.4c	98.0AB	98.2AB	98.6AB	94.4BC
100	89.3c	88.1c	95.0b	96.2ab	90.6b	91.0b	90.7d	89.0de	88.7D	95.6B	90.8CD	89.9D
150	85.0d	79.7f	92.3c	91.4c	88.7c	88.5c	85.0f	82.4g	82.4EF	91.9C	88.6DE	83.7E
200	82.3e	68.0g	88.0d	89.4d	83.0d	84.1d	78.5h	72.2i	75.2F	88.7DE	83.6E	75.4F

Mean values within cultivars of the same species followed by the same lowercase letter are not significantly ($P \leq 0.01$) different; mean values within species followed by the same uppercase letter are not significantly ($P \leq 0.01$) different.

species 'Golden Gate' (89.4%) and 'Fesnova' (88.0%) showed higher FGP in this salinity concentration. Genetic variability within a species offers a valuable tool for studying mechanism of salt tolerance. One of these mechanisms depends on the capacity for osmatic adjustment that allows growth to continue under saline conditions. With increasing salinity there was a decrease in germination of seeds in the cultivars. Inhibition of germination due to salinity has been reported earlier [5, 6]. It is suggested that decrease in seed germination under saline stress is attributed to decrease water uptake followed by limited hydrolysis of nutrient reserves from storage tissue as well as due to impaired translocation of nutrient reserves from storage tissue to developing embryo axis [10]. When salt-stressed, turfgrasses can suffer from indirect water stress, causing a block in seed germination. An increasing NaCl concentration is likely caused by a decrease in the water potential gradient between seeds and their surrounding media. Seed germination is negatively affected by salinity stress, either osmotically through reduced water absorption or ionically through Na^+ and Cl^- accumulation, resulting in an imbalance in nutrient uptake and toxicity effects [18]. Sahiade and Boelt [28] emphasized that tall fescue is a cool-season turfgrass with a moderate to high salinity tolerance, and it possess a wide range of variation in salinity tolerance. Contrary to our results, Borawska-Jarmułowicz et al. [16] reported that the germination of perennial ryegrass to be relatively the most salt-tolerant than Kentucky bluegrass. Sheikh-Mohamadi et al. [22] stated that germination of seeds should occur uniformly for a successful establishment of turfgrass seedlings and high FGP is indicator that there is a high potential for successful establishment. Turk and Alagoz [9] reported increased salt concentrations caused decreased the germination rate of tall fescue. Present findings coincide with those earlier ones.

Effect of Salinity on Root and Shoot of Seedlings

Among four cool-season turfgrass species and their cultivars, differences were observed in the root fresh mass, shoot fresh and dry mass (Table 2). Increasing NaCl concentration resulted in a significant decrease in root fresh mass, shoot fresh and dry mass ($P<0.01$). It was noticed that salinity had more negative effects on root fresh mass, shoot fresh and dry mass of slender creeping red fescue, Kentucky bluegrass and perennial ryegrass plants than on tall fescue. Concentration of 200 mM NaCl resulted in significantly lightweight shoots (39.6 mg of shoot fresh mass per plant and 10.0 mg of shoot dry mass per plant) of slender creeping red fescue and root dry mass (14.6 mg per plant) of Kentucky bluegrass. At same salinity level, Kentucky bluegrass cultivar 'Heatmaster' had lower root fresh mass than among cultivars of other species while slender creeping red fescue cultivars 'Abercharm' and 'Spartan 2' had lower shoot fresh mass and shoot dry mass than other cultivars of species. Massahi et al. [29]

Table 2. Shoot (SFM) and root fresh (RFM) mass, shoot dry mass (SDM) of seedlings of *L. perenne*, *F. arundinacea*, *F. rubra* L. ssp. *trichophylla* and *P. pratensis* cultivars under salinity conditions (Means of two years).

Salinity level mM NaCl	*Lolium perenne*		*Festuca arundinacea*		*Festuca rubra L.* ssp. *trichophylla*		*Poa pratensis*		*Lolium perenne*	*Festuca arundinacea*	*Festuca rubra ssp. trichophylla*	*Poa pratensis*
	'Ringles'	'Sun'	'Fesnova'	'Golden Gate'	'Abercharm'	'Spartan 2'	'Prafin'	'Heatmaster'				
	SFM mg per plant											
0	109.2a	109.9a	127.9a	128.0a	60.7b	62.8a	77.4ab	78.9a	109.6C	128.0A	61.8M	78.2H
50	108.5b	107.3c	127.0b	127.8a	59.7bc	60.5b	75.1b	75.3b	107.9D	127.4B	60.1MN	75.2J
100	90.8d	89.4de	100.4cd	101.7c	50.4c	49.7cd	68.0c	67.5c	90.1F	101.1E	50.1O	68.8K
150	78.1e	75.5f	89.9de	91.1d	45.8d	45.4d	60.1d	58.2de	76.8I	90.5F	45.6Q	59.2N
200	65.1g	63.4h	80.4e	80.6e	39.7e	39.5e	52.7e	45.5f	64.3L	80.5G	39.6R	49.1OP
	RFM mg per plant											
0	52.1a	52.4a	65.7a	66.0a	33.7b	34.5a	41.7a	42.0a	52.3B	65.9A	34.1G	41.9E
50	41.2b	40.6b	65.5ab	65.6ab	30.4c	30.7c	39.8b	40.4ab	40.9EF	65.6A	30.6H	40.1F
100	20.3c	18.9c	50.9bc	51.3b	25.7d	25.0d	30.2c	29.7c	19.6JK	51.1C	25.4I	30.0H
150	16.5d	15.8de	43.5cd	43.7c	20.1f	20.0f	25.1d	24.8d	16.2K	43.6D	20.1J	25.0I
200	15.4e	14.7f	39.7d	40.0d	15.2e	16.3e	15.0e	14.2f	15.1M	39.9F	15.8L	14.6N
	SDM mg per plant											
0	21.0a	21.5a	25.6a	25.9a	14.5b	15.1a	19.8a	20.4a	21.3B	25.8A	14.8H	20.1D
50	20.7b	20.4b	25.4ab	25.5ab	14.0bc	14.2bc	17.5b	17.8b	20.6C	25.5A	14.1I	17.7E
100	17.3c	17.0c	20.5bc	21.0b	11.7c	11.5c	16.7c	16.6c	17.2F	20.8C	11.6K	16.7FG
150	14.5d	14.2d	17.1d	17.8c	10.8d	10.4de	13.4d	13.2d	14.4HI	17.5E	10.6LM	13.3J
200	13.3e	12.8f	15.2e	15.4e	9.9e	10.0e	11.3e	10.7f	13.1J	15.3G	10.0M	11.0L

Mean values within cultivars of the same species followed by the same lowercase letter are not significantly ($P \leq 0.01$) different; mean values within species followed by the same uppercase letter are not significantly ($P \leq 0.01$) different.

Table 3. Retention capability (RCS), relative water content (RWC) and tolerance index (TI) of shoots of seedlings in *L. perenne*, *F. arundinacea*, *F. rubra* L. ssp. *trichophylla* and *P. pratensis* cultivars under salinity conditions (Means of two years).

Salinity level mM NaCl	*Lolium perenne*		*Festuca arundinacea*		*Festuca rubra L.* ssp. *trichophylla*		*Poa pratensis*		*Lolium perenne*	*Festuca arundinacea*	*Festuca rubra ssp. trichophylla*	*Poa pratensis*
	'Ringles'	'Sun'	'Fesnova'	'Golden Gate'	'Abercharm'	'Spartan 2'	'Prafin'	'Heatmaster'				
	RCS (mg)											
0	4.4a	4.1b	4.0b	3.9b	3.2a	3.2a	2.9c	2.9c	4.3A	4.0C	3.2E	2.9G
50	4.2a	4.3a	4.0b	4.0b	3.3a	3.3a	3.3b	3.2b	4.3A	4.0C	3.3E	3.3E
100	4.2a	4.3a	3.9b	3.8b	3.3a	3.3a	3.1b	3.1b	4.3A	3.9C	3.3E	3.1F
150	4.4a	4.3a	4.3a	4.1a	3.2a	3.4a	3.5a	3.4a	4.4A	4.2B	3.3E	3.5D
200	3.9c	3.9c	4.3a	4.2a	3.0b	3.0b	3.7a	3.2b	3.9C	4.3A	3.0F	3.5D
	RWC (%)											
0	80.8b	80.4b	80.0c	79.8c	76.1b	76.0b	74.4f	74.1f	80.6B	79.9C	76.1H	74.3K
50	80.9a	81.0a	80.0c	80.0c	76.5b	76.5b	77.0c	76.4d	81.0A	80.0C	76.5G	76.7F
100	80.9a	81.0a	79.6c	79.4d	76.8a	76.9a	75.4e	75.4e	81.0A	79.5D	76.9F	75.4I
150	81.4a	81.2a	81.0a	80.5b	76.4b	77.1a	77.7b	77.3b	81.3A	80.8B	76.8F	77.5E
200	79.6c	79.8c	81.1a	80.9a	75.1c	74.7c	78.6a	76.5d	79.7C	81.0A	74.9J	77.6E
	TI (%)											
50	98.6a	94.9b	99.2a	98.5a	96.6a	94.0ab	88.4a	87.3a	96.8B	98.9A	95.3BC	87.9D
100	82.4c	79.1d	80.1b	81.1b	80.7b	76.2bc	84.3b	81.4bc	80.8F	80.6F	78.5G	82.9E
150	69.0e	66.0f	66.8c	68.7c	74.5c	68.9d	67.7c	64.7d	67.5I	67.8I	71.7H	66.2JK
200	63.3g	59.5h	59.4d	59.5d	68.3e	66.2e	57.1e	52.5f	61.4L	59.5L	67.3I	54.8M

Mean values within cultivars of the same species followed by the same lowercase letter are not significantly ($P \leq 0.01$) different; mean values within species followed by the same uppercase letter are not significantly ($P \leq 0.01$) different.

reported that lawn quality of tall fescue was unaffected by saline irrigation at an EC of 12.0 dS·m^{-1} for 210 days. Wheatgrass (*Agropyron cristatum* L.), perennial ryegrass, red fescue (*F. rubra* L.), and switchgrass (*Panicum virgatum* L.) were also classified 'moderately tolerant' together with tall fescue. Among the cool-season turfgrasses, the authors only rated alkali grass (*Puccinellia* spp.) as 'tolerant': able to grow at salt levels equivalent to >10 ds m^{-1}. Salt stress involves osmotic and ionic stresses, and the suppression of growth is directly contingent on the total soluble salt concentration and soil osmotic potential. The detrimental effect can be seen at the whole-plant level as plant death or decreased productivity [18]. According to Borawska-Jarmułowicz et al. [16] and Souri and Tohidloo [30], the fresh mass and dry mass of seedlings were significantly increased under salinity conditions. Our data showed that under this salinity level the root mass of cultivars of four species reduced. Other values were similar to those designated by researchers [1, 8, 10, 31, 32].

Effect of Salinity on RCS, RWC and TI of Shoot of Seedlings

Statistically significant (P<0.01) were observed differences among NaCl levels for RCS, RWC and TI of shoots of seedlings in perennial ryegrass, tall fescue, slender creeping red fescue and Kentucky bluegrass cultivars (Table 3). TI of the species and cultivars significantly decreased in salt stress. When compared four turfgrass species, the lowest TI was calculated in the Kentucky bluegrass (54.8 %) in 200 mM NaCl level, whereas the lowest RCS (2.9 mg) and RWC (74.3 %) were determined in the same species in control treatment. The tall fescue cultivars 'Golden Gate' and 'Fesnova' exhibited higher values than the other cultivars for RCS of shoot (4.2-4.3 mg) under 200 mM salinity level. However, the cultivars showed diversity in RWC under salt stress. The Kentucky bluegrass cultivars showing a low RWC for shoot in control treatment were cultivar 'Heatmaster' (74.1 %) and cultivar 'Prafin' (74.4 %). These results were distinct with Ates and Tekeli [10]. They reported that the RWC of shoot were not affected in salinity treatments whereas Negrão et al. [33] stated that the water content and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity. Hniličková et al. [34] reported that the osmotic potential decreased with increasing NaCl concentration, while RWC decreased did not take place until 200 mM NaCl. A greater decline in osmotic potential compared with the total water content led to turgor maintenance in plants under progressive or prolonged salinity stress [35]. Soni et al. [36] emphasized that the water content was decreased by salinity stress conditions, but Gebauer et al. [37] reported that the leaves of salt-treated plants accumulated more water than leaves of control plants. In the study carried out by Lin and Qian [23], they were determined that TI was higher in low

Table 4. Effect of salinity on K^+/Na^+ ratio in shoots of *L. perenne*, *F. arundinacea*, *F. rubra* L. ssp. *trichophylla* and *P. pratensis* cultivars (Means of two years).

Salinity level mM NaCl	*Lolium perenne*		*Festuca arundinacea*		*Festuca rubra L.* ssp. *trichophylla*		*Poa pratensis*		*Lolium perenne*	*Festuca arundinacea*	*Festuca rubra* ssp. *trichophylla*	*Poa pratensis*
	'Ringles'	'Sun'	'Fesnova'	'Golden Gate'	'Abercharm'	'Spartan 2'	'Prafin'	'Heatmaster'				
0	10.7±0.3	11.0±0.2	12.1±0.2	12.4±0.3	9.9±0.2	10.0±0.3	9.5±0.2	9.8±0.3	10.8±0.2	12.3±0.2	9.9±0.3	9.6±0.2
50	10.2±0.2	10.5±0.3	11.9±0.3	11.6±0.4	9.4±0.2	9.7±0.2	9.4±0.2	9.6±0.2	10.3±0.3	11.8±0.3	9.5±0.3	9.5±0.2
100	8.7±0.3	9.1±0.3	10.3±0.4	10.0±0.4	9.5±0.3	9.6±0.3	8.9±0.3	8.5±0.3	8.9±0.2	10.2±0.2	9.5±0.2	8.7±0.2
150	5.2±0.4	4.9±0.2	6.1±0.3	6.7±0.2	5.4±0.2	6.3±0.4	5.0±0.2	4.8±0.2	5.0±0.3	6.4±0.3	5.8±0.4	4.9±0.3
200	1.70±0.3	1.55±0.2	2.1±0.2	2.2±0.3	1.9±0.2	2.2±0.2	1.5±0.3	1.6±0.3	1.6±0.3	2.2±0.2	2.1±0.2	1.5±0.3

Each value represents mean of three replicates. Values were rounded up to nearest whole figure and SD determined.

salt concentrations, as salt level increased TI decreased significantly, which is similar to the present findings.

Effect of Salinity on K^+/Na^+ Ratios in Shoot Dry Mass

The K^+/Na^+ ratios in shoots dry mass were also significant different ($P<0.01$, Table 4). The K^+/Na^+ ratios were decreased with the increase of NaCl concentrations. The 200 mM NaCl treatment had the lowest K^+/Na^+ ratio of 1.5 (ranging from 1.5 to 2.2 for all cultivars) in shoot dry mass of 'Prafin' cultivar of Kentucky bluegrass. The possible mechanisms of salt tolerance in plants include the following: (a) the uptake of no or little salt into the plant, (b) tissue tolerance, (c) the accumulation of salt in vacuoles without any physiological interference, (d) ion discrimination (e.g., K^+, sodium (Na^+), chloride (Cl^-), and sulfate ($SO4^-$)) during root uptake and translocation in plant shoots, and (v) the use of different biochemical processes, such as enzyme, hormone, or antioxidant production. One or more of these mechanisms may be responsible for the observed variations in the salt tolerance of plants with different genotypes and from different species. The K^+/Na^+ ratio has been established as a criterion by scientists to determine if a plant has salt tolerance. Thus, cultivars that maintain higher K^+/Na^+ ratios are considered salt tolerant [38]. Evelin et al. [39] reported that regulating K^+ uptake and preventing Na^+ entry and the efflux of Na^+ from cells are common strategies used by plants to maintain desirable K^+/Na^+ ratios in the cytosol. Rodrigues [40] demonstrated that the maximum ratios of photosynthesis and plant growth occurred when the leaf K^+/Na^+ ratios were between 1.0 and 2.0, which indicated that this parameter could be a good indicator in leaves for favorable K^+ homeostasis under high salinity conditions. The tall fescue cultivars and slender creeping red fescue cultivar 'Spartan 2' had K^+/Na^+ ratios greater than 2.0. Besides, our results were distinct with Gebauer et al. [36]. They reported that the K^+/Na^+ ratio increased as the salinity of the irrigation water increased for tall fescue cultivar 'Fawn' and perennial ryegrass cultivar 'Quartet'.

Conclusions

Salinity is one of the most important factors affecting turf of the high quality. The results from the different NaCl concentrations applied in four cool-season turfgrass species and cultivars can be summarized as follow: (a) Eight introduced turfgrass cultivars showed different adaptations for coping with different salinity levels. (b) Based on their tolerance index (TI), five cultivars, 'Ringles' perennial ryegrass, 'Abercharm' slender creeping red fescue, 'Prafin' Kentucky bluegrass, 'Fesnova' and 'Golden Gate' tall fescue demonstrated good salt tolerance. (c) In addition, maximum ratios of photosynthesis and plant growth occurred when the K^+/Na^+ ratios were between 1.0 and 2.0. The tolerance indexes of the these cultivars were supported by the K^+/Na^+ ratios. (d) The cultivars 'Ringles', 'Abercharm', 'Prafin', 'Fesnova' and 'Golden Gate' exhibited potential salt tolerance and could compete with other cool-season turfgrass varieties regarding productivity under salt stress.

Conflict of Interest

The authors declare no conflict of interest.

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