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Screening of Eleven *Festuca arundinacea* Native Populations for NaCl Tolerance in Order to Use in Green Space

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Abstract

In the turfgrass industry, the need for salinity tolerant turfgrasses is increasing because of the increased use of saline and non-potable water. Greenhouse container experiments were conducted to determine the relative salinity tolerance and growth responses of eleven native populations of tall fescue (*Festuca arundinacea* Schrub) (TF), including: Semirom, Mashhad, Sanandaj, Yasuj, Yazd Abad, Daran, Kamyaran, Gandoman, Borujen, Nasir Abad and Alborz to 8 weeks of salinity stress. Also, commercial TF was used as control. Four salinity levels of irrigation water (0, 45, 90, 135 mM NaCl) were applied to turfgrasses. Results showed shoot and root dry weight, total leaf area, leaf length, leaf width and leaf firing percentage was significantly affected by salinity for all turfgrasses. The lowest leaf firing percentage at 90 and 135 mM, was related to Sanandaj population and commercial TF, and the highest leaf firing percentage was related to Alborz at 45 mM and Gandoman population at 90 and 135 mM. Shoot dry weight, total leaf area and leaf length of Sanandaj population was less affected by salinity compared to other populations. Based on data on growth parameters, the salinity tolerance ranking of selected populations was: Sanandaj >Daran >Yasuj >Kamyaran >Nasir Abad >Semirom >Mashhad >Alborz >Yazd Abad >Borujen >Gandoman . These results showed the potential and competetive role of TF native populations compared to commercial TF.

Keywords: Growth Parameter, Native Population, Salt tolerance, Tall Fescue.

INTRODUCTION

One of the most important environmental factors limiting plant growth is salinity (Bayat *et al.*, 2013). Worldwide, more than one-third of irrigated land is salinized (Diedhiou *et al.*, 2009; Chen *et al.,* 2009). On the other hand, rapidly expanding population growth is occurring in many arid regions, where soil and water salinity are problems and there are increased demands on limited fresh water resources (Marcum, 2006).

The United Nations predicts that 2.7 billion people will suffer severe water deficiency by 2025 if consumption continues at current rates (Montaigne, 2002). In the turfgrass industry, the increased use of saline and non-potable water, the development of turfgrass landscapes in arid and seashore regions where saline soil is common, and the use of salt for deicing roadways, the need for salinity tolerant turfgrasses is so important. (Chen *et al*., 2009). The major compound contributing salinity in soils is sodium chloride (NaCl), and salt-tolerant turfgrasses are required to cope this problem (Uddin *et al.*, 2012). Under soil salinity, high concentration of Na+ competes with the uptake of other nutrients, especially K+ as a necessary element (Sharbatkhari *et al*., 2013). The earliest plant response of salt stress is a reduction in leaf surface expansion, followed by cessation of expansion as the stress intensifies (Wu *et al*., 2006). However, little studies have been reported regarding leaf growth and shoot density. Under salinity stress, shoot biomass is often reduced. As salinity stress level increases further, root growth is also decreased (Adavi *et al*., 2006; Alshammary *et al.*, 2004; Marcum and Murdoch, 1994; Marcum, 1999). Other detrimental effects of salinity on turfgrass growth include: osmotic stress, nutritional disturbances, and ion toxicity. Salt tolerant plants have the ability to decrease these detrimental effects by producing a series of anatomical, physiological, and morphological adaptations such as an extensive root system and salt secreting glands on the leaf surface (Alshammary *et al*., 2004).

Differences in salt tolerance between turfgrasses have been demonstrated in many studies (Horst and Taylor, 1983; Marcum and Murdoch, 1994; Qian *et al.*, 2001; Alshammary *et al.,* 2004; Chen *et al*., 2009; Uddin *et al*., 2012). Much research has been conducted on the physiological responses and growth to salinity stress in warm season turfgrasses (Adavi *et al*., 2006; Alshammary *et al.*, 2004; Marcum and Murdoch, 1994; Marcum, 1999; Chen *et al*., 2009; Uddin *et al.,* 2012). But there are some reports on responses cool season turfgrasses to salinity (Alshammary *et al*., 2004; Simkunas *et al.*, 2007; Dianati Tilaki *et al.*, 2010).

Tall fescue (*Festuca arundinacea* Schrub.) is an important perennial cool-season grass in temperate regions and it is widely used for both forage and turf purposes (Zhao *et al*., 2007; Wu *et al.*, 2006; Alshammary *et al*., 2004). It has a high degree of self in compatibility, which makes conventional breeding quite difficult (Barnes, 1990), including breeding for salt tolerance (Zhao *et al*., 2007). On the other hand, tall fescue native populations are reach sources of variation including salt tolerance, and there is no study about evaluation of salinity on tall fescue native populations in Iran. The major objective of this study was to determine the relative salt tolerance and growth response of native populations of tall fescue to salinity in sand culture system.

MATERIALS AND METHODS

In this experiment seeds of some native populations of tall fescue (TF) (*F. arundinacea* Schreb) including: Semirom, Mashhad, Sanandaj, Sanajan, Yasuj, Yazd Abad, Daran, Kamyaran, Gandoman, Borujen, Nasir Abad, Alborz and commercial TF (C. TF) seeds were used. Seeds were germinated on pots filled with a mix of 1/3 soils: 1/3sand: 1/3 humus (v/v/v). After germination, ten seedling of TF were transplanted into plastic pots (20 cm diameter) in a greenhouse at Ferdowsi University of Mashhad and grown for 8 weeks with non-saline irrigation water. Pure sand was used as the growing media and Hoagland solution was used as nutrient. Grasses were clipped throughout the experiment to 5 cm.

Irrigation waters of different salinities were prepared by the addition of NaCl to tap water. Saline waters of 45, 90, and 135 mM along with tap water as the control treatment were applied to TF. To avoid salinity shock, salinity levels were gradually increased by daily increments of 22.5 mM. The amount of water applied including 30% excess water as leaching requirement (400 ml). The irrigation waters were applied on every other day basis for a period of 8 weeks.

The experimental treatments were set up by following a factorial experiment based on completely randomized design and each treatment had three replications. During the salinity treatment period, data were collected on leaf firing (in three stage: after 30, 45 days and at the end of salinity stress), shoot (green leaves) and root dry weight, leaf length, leaf width and total leaf area. Leaf firing was measured as the total percentage of chlorotic leaf area in plants at first and second stage, and percentage of chlorotic leaf dry weight in third stage of measuring. At the end of the experiment, shoots and roots were harvested separately. Both shoots and roots were washed with deionized water, and dried at 70 ◦C for 48 h to determine shoot and root dry weight. Leaf width was measured by calipers. Also, leaf area was measured by area measurement Delta-T device LTD. For relative water content (RWC) excised leaves from each pot (0.2 g) were measured for fresh weight, and leaf samples were rehydrated in a water-filled petri dish for 4 h at room temperature. Turgor weight was measured by allowing full rehydration, removing all water from leaf surface, and weighted. Leaf dry weights were recorded after oven drying for one week at 60 ◦C. The leaf relative water content was determined using the following formula (Whetherly, 1950): RWC = (Fresh weight − Dry weight) / (Fully turgid weight − Dry weight) × 100. The experimental data were analyzed using JMP 8 software. Treatment means were separated by Fisher's protected LSD.

RESULTS

Leaf firing percentage

Interaction of salinity and population had a significant effect on leaf firing. Leaf firing percentage for all populations increased as salinity increased. After 30 days (Fig. 1. A) of salinity treatment, reaching ~ 25% for Nasir Abad population, 30% for commercial TF, ~ 30% for Kamyaran, Yasuj, Semirom and Sanadej population and ~ 35% for remaining population at 45 mM. Gandoman population had greatest leaf firing percentage between all turfgrasses at 90 and 135 mM. Among all of populations, Sanandaj population had lowest leaf firing at 90 and 135 mM, respectively. Results showed after 45 days use of NaCl, at 45 mM, commercial TF and Sanadaj population, at 90 mM, Sanadaj population and at 135 mM, Daran population were least affected by salinity (Fig. 1. B). On the other hand Alborz population, Alborz and Borujen populations, and Borujen and Gandoman populations were most affected at 45, 90 and 135 mM, respectively. At the end of the study at 45 mM, Alborz, at 90 mM, Gandoman population and at 135 mM, Gandoman population had the highest leaf firing percentage (Table 1). Also, the lowest leaf firing percentage was related to Sanandaj and commercial TF at 90 and 135 mM, and there were no significant difference between them (Table 1).

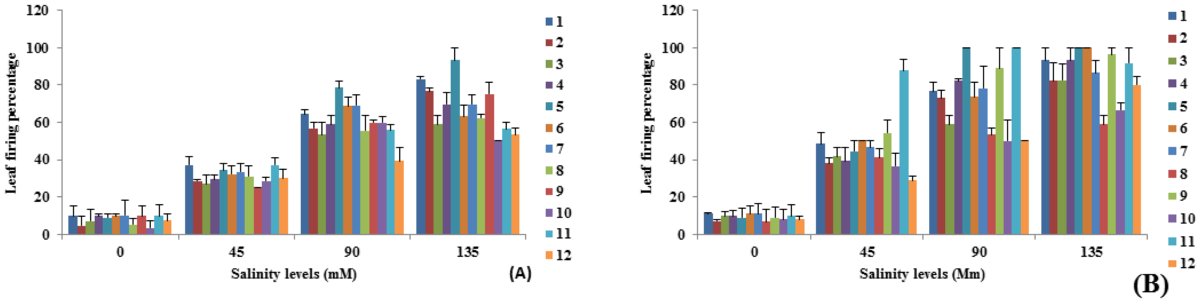

Fig. 1. Leaf firing percentage of *Festuca arundinacea* populations after 30 (A) and 45 (B) days salinity stress (1. Yazd Abad, 2. Kamyaran, 3. Yasuj, 4. Semirom, 5. Gandoman, 6. Borujen, 7. Mashhad, 8. Daran, 9. Nasir Abad, 10. Sanandaj, 11. Alborz TF native populations, and 12. Commercial TF). Vertical bars indicating standard error.

Table 1. Leaf firing percentage of *Festuca arundinacea* populations at the end of salinity stress.

TF populations	**Salinity levels (mM)**			
	0	**45**	**90**	**135**
Yazd Abad	13 .0 ^{no}	62.4 ^{gh}	88.5 $^{a-e}$	95.6 ^{abc}
Kamyaran	9.1 ^{o}	66.3 ^{gh}	88.7 $^{a-e}$	86.8 ^{cde}
Yasuj	13.0 ^{no}	54.0 ^{hij}	81.0 ^{ef}	84.0 ^{cde}
Semirom	5.9 ^{o}	43.1 ^{jk}	80.1 ^{ef}	89.9 $^{a-e}$
Gandoman	12.1 ^{no}	85.1 ^{cde}	99.7 ^{a}	100 ^{a}
Borujen	8.7 ^{o}	69.5 ^{fg}	88.2 $^{a-e}$	99.2 ^{ab}
Mashhad	11.1 ^{no}	41.6 ^{kl}	83.1 ^{de}	89.2 $^{a-e}$
Daran	2.6 ^{o}	37.8 ^{kl}	58.1 ^{gh}	69.7 ^{fg}
Nasir Abad	7.2 ^{o}	65.5 ^{gh}	94.9 $^{a-d}$	87.1 $^{b-e}$
Sanandaj	4.2 ^{o}	37.5 ^{kl}	44.5 ^{ijk}	61.8 ^{gh}
Alborz	11.1 ^{no}	86.1 ^{cde}	94.9 $^{a-d}$	93.6 $^{a-d}$
C. TF	8.2 ^{o}	22.0 ^{mn}	37.9 ^{kl}	58.7 ^{hi}

The data with the same letter are not significantly different at P<1%.

Shoot (green leaf) dry weight

Interaction effect of salinity and population was significant on shoot dry weight. Shoot dry weights of turfgrass populations decreased as the level of salinity increased (Table 2). Commercial TF and Sanandaj population were less affected compared to other turfgrasses, and at 45 mM there were 30% and 37% decrease, respectively in shoot dry weight compared to control treatment. At 90 mM NaCl, decrease in shoot dry weight reached 63% for commercial TF and 65% for Sanadaj population. Also, at 135 mM NaCl, decrease in shoot dry weight reached 73% for commercial TF and 84% for Sanadaj population. Among native populations, after Sanadaj population, Daran population had greatest shoot dry weight at different salinity levels compared to control. As it was shown in Table 2, Gandoman population had lowest shoot dry weight among all TF populations at all different salinity levels.

Root dry weight

As salinity increased from control to 135 mM, root mass decreased (Table 2). At 45 mM and 90 mM salinity levels, Sanadaj population produced much higher root than other populations. At 45 mM salinity level (Table 2), decrease in root mass reached to 51% and 42% for commercial

Table 2. Shoot and root dry weight of *Festuca arundinacea* populations after salinity stress.

Treatments	**Salinity levels (mM)**							
	Shoot dry weight (g/pot)				**Root dry weight (g/pot)**			
	0	**45**	**90**	**135**	**0**	**45**	**90**	**135**
Yazd Abad	5.89 ^{fgh}	2.13 ^{lm}	0.31 $^{q-u}$	0.10 ^{tu}	3.5 ^{ef}	1.3 ^{lmn}	0.69 ^{tuv}	0.76 $^{s-v}$
Kamyaran	6.18 ^{efg}	2.15 ^{lm}	0.27 $^{q-u}$	0.23 $^{r-u}$	2.9 ^{hi}	0.97 $^{l-p}$	0.45 ^{uv}	0.58 ^{tuv}
Yasuj	6.59 ^{de}	2.32 ^{kl}	0.75 ^{pqr}	0.20 ^{stu}	3.4 ^{efg}	1.3 ^{lm}	1.2 $^{l-o}$	0.71 ^{tuv}
Semirom	6.42 ^{ef}	2.73 ^{jk}	0.67 $^{p-s}$	0.16 ^{stu}	3.2 ^{fgh}	0.97 $^{l-p}$	0.83 ^{pq}	0.75 $^{s-v}$
Gandoman	5.71 ^{gh}	0.58 $^{p-t}$	0.01^{u}	0.01 ^{u}	4.2 ^{bc}	1.2 $^{l-o}$	0.83 ^{pq}	0.80 $^{r-u}$
Borujen	6.37 ^{ef}	1.36 ^{no}	0.36 $^{q-u}$	0.02 ^{u}	3.7 ^{de}	1.7 ^{k}	0.72 ^{tuv}	0.69 ^{tuv}
Mashhad	7.85 ^{c}	2.71 ^{jk}	0.80 ^{pq}	0.12 ^{tu}	2.9 ^{hi}	0.95 $^{m-p}$	1.2 $^{l-p}$	0.45 ^{uv}
Daran	7.10 ^{d}	3.30 ^{i}	1.67 ^{mn}	0.68 $^{p-s}$	3.0 ^{ghi}	1.3 ^{lmn}	0.67 ^{tuv}	0.40 ^{v}
Nasir Abad	6.37 ^{ef}	1.40 ^{no}	0.20 ^{stu}	0.28 $^{q-u}$	4.0 ^{cd}	1.3 $^{l lmn}$	0.82 ^{qr}	0.90 ^{nop}
Sanandaj	8.62 ^{b}	5.41 ^{h}	2.98 ^{ij}	1.36 ^{no}	4.2 ^{bc}	2.0 ^{j}	1.9 ^{k}	0.80 $^{r-u}$
Alborz	9.04 ^{ab}	0.91 ^{op}	0.29 $^{q-u}$	0.36 $^{q-u}$	4.5 ^{ab}	1.1 $^{l-o}$	$^{1.2}$l-o	0.67 ^{tuv}
C. TF	9.32 ^{a}	6.51 ^{e}	3.41 ^{i}	2.46 ^{jkl}	4.8 ^{a}	2.9 ^{i}	2.3 ^{j}	0.85 ^{op}

The data with the same letter are not significantly different at P<1%.

Table 3. Leaf relative water content and leaf area of *festuca arundinacea* populations at the end of salinity stress.

TF populations	**Salinity levels (mM)**							
	Leaf relative water content				**Leaf area**			
	0	**45**	**90**	**135**	**0**	**45**	**90**	**135**
Yazd Abad	92 ^{ab}	82 $^{c-h}$	78 $^{f-j}$	67 ^{kl}	724 ^{fg}	161 $^{i-p}$	19 ^{opq}	3 ^{pq}
Kamyaran	94 ^{a}	87 $^{a-f}$	83 $^{b-h}$	81 $^{e-j}$	1254 ^{ab}	202 $^{i-l}$	50 $^{l-q}$	15 ^{opq}
Yasuj	93 ^{a}	83 $^{b-h}$	80 $^{e-j}$	72 ^{jk}	929 ^{de}	317 ^{hi}	89 $^{k-q}$	7 ^{pq}
Semirom	93 ^{a}	77 $^{g-j}$	58 ^{lm}	46 ^{n}	1004 ^{cd}	167 $^{i-o}$	27 $^{n-q}$	4 ^{pq}
Gandoman	89 $^{a-e}$	85 $^{a-g}$	48 ^{n}	51 ^{mn}	792 ^{ef}	37 $^{m-q}$	0.6 ^{q}	0.1 ^{q}
Borujen	92 ^{ab}	81 $^{e-j}$	42 ^{n}	44 ^{n}	977 ^{cd}	116 $^{j-q}$	19 ^{opq}	1 ^{q}
Mashhad	92 ^{ab}	80 $^{e-j}$	76 $^{h-k}$	75 $^{h-k}$	1027 ^{cd}	262 ^{ij}	24 $^{n-q}$	6 ^{pq}
Daran	91 ^{abc}	85 $^{a-g}$	81 $^{e-j}$	78 $^{f-j}$	1205 ^{ab}	441 ^{h}	104 $^{k-q}$	42 $^{m-q}$
Nasir Abad	92 ^{ab}	81 $^{d-i}$	63 ^{l}	44 ^{n}	1129 ^{bc}	117 $^{j-q}$	14 ^{opq}	14 ^{opq}
Sanandaj	93 ^{a}	88 $^{a-e}$	88 $^{a-e}$	86 $^{a-f}$	1289 ^{a}	629 ^{g}	187 ^{ijk}	122 $^{j-q}$
Alborz	93 ^{a}	82 $^{c-i}$	75 $^{h-k}$	73 ^{ijk}	1267 ^{ab}	47 $^{l-q}$	17 ^{opq}	21 $^{n-q}$
C. TF	90 $^{a-d}$	83 $^{b-h}$	83 $^{b-h}$	^{88} a-e	1218 ^{ab}	620 ^{g}	231 ^{ijk}	180 $^{i-n}$

The data with the same letter are not significantly different at P<1%.

TF and Sanadaj population, respectively compared to 0 mM salinity. Also, at 90 mM salinity, decrease in root mass reached to 53% and 56% for commercial TF and Sanadaj population, respectively. At 135 mM, the lowest decrease in root mass belonged to Semirom population and reached to 77%, and highest decrease for this factor belonged to Semirom, Kamyaran and Daran populations, at 45, 90 and 135, respectively (Table 2).

Leaf area

Interaction effect of salinity and population was significant on leaf area. Leaf area of turfgrass populations decreased as the level of salinity increased (Table 3), and was significantly decreased in all populations compared to control treatment (Table 3). Among native populations, in different salinity levels, the greatest leaf area was belonged to Sanandaj population. On the other hand, there were no significant difference between Sanandaj population with commercial TF in all levels of salinity. Also, Gandoman population was more affected by NaCl treatment, and decrease in leaf area reached 95%, 99%, and 100% at 45, 90, and 135 mM, respectively (Table 3).

Leaf relative water content (RWC)

Relative water content (RWC) of all turfgrass populations was significantly influenced by salinity (Table 3). As salinity increased, RWC decreased. (Table 3). Relative water content significantly decreased at 90 mM salinity level, except for commercial TF, Sanandaj, Daran, Yasuj and Yazd Abad populations compared to 45 mM salinity level. At 135 mM salinity level, commercial TF, Sanandaj and Kamyaran populations had higher RWC compared to other populations (Table 3).

Leaf width

Interaction effect of salinity and population was not significant for leaf width. Leaf width of all turfgrass populations was significantly influenced by salinity. Among all populations, the greatest and lowest leaf width was related to Semirom and Gandoman populations, respectively (Fig. 2).

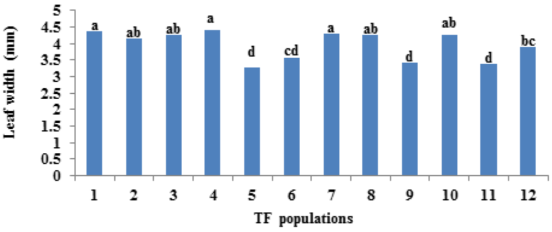

Fig. 2. Leaf width of *Festuca arundinacea* populations after 30 day salinity stress (1. Yazd abad, 2. Kamyaran, 3. Yasuj, 4. Semirom, 5. Gandoman, 6. Borujen, 7. Mashhad, 8. Daran, 9. Nasir abad, 10. Sanandaj, 11. Alborz TF native populations, and 12. Commercial TF). (The data with the same letter are not significantly different at P<1%).

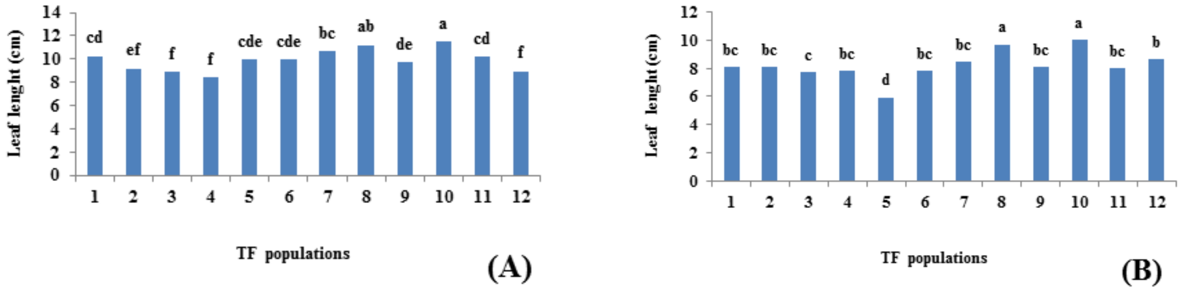

Fig. 3. Leaf length of *Festuca arundinacea* populations after 15 (A) and 45 (B) days salinity stress (1. Yazd abad, 2. Kamyaran, 3. Yasuj, 4. Semirom, 5. Gandoman, 6. Borujen, 7. Mashhad, 8. Daran, 9. Nasir abad, 10. Sanandaj, 11. Alborz TF native populations, and 12. Commercial TF). The data with the same letter are not significantly different at P<1%).

Leaf length

Interaction of salinity and population had no significant effect on leaf length. At first stage of measuring (after 15 day salinity stress) (Fig. 3. A), turfgrasses that had the greatest leaf length included: Sanandaj and Daran populations, whereas Semirom population had the lowest leaf lenght (Fig. 3. A). All turfgrasses had lower leaf lenght at stage 2 of measuring (after 45 day salinity stress) compared to stage 1 (Fig. 3. B). Gandoman population had the lowest leaf length, whereas turfgrasses that had the greatest leaf lenght included: Sanandaj and Daran populations (Fig. 3.B).

DISCUSSION

Growth parameters have been reported to be excellent criteria to determine salinity tolerance among turfgrasses (Alshammary *et al.,* 2004). Based on data on growth parameters (shoot and root mass, leaf firing, leaf width and length and leaf area) the salinity tolerance ranking of selected grasses was: Commercial tall fescue >Sanandaj >Daran >Yasuj >Kamyaran >Nasir Abad >Semirom >Mashhad >Alborz >Yazd Abad >Borujen >Gandoman TF native populations.

Our results show that salinity declined root and shoot mass of turfgrasses. Alshammary *et al.* (2004) reported that yield production of turfgrasses was reduced significantly when the salinity level increased from 4 to 12 dS m^{-1}. Dean et al. (1996) found that turf quality and shoot growth of turfgrasses were reduced as the salinity level increased from 1.1 to 6 dS m^{-1}. Exposure of plant roots to salinity altered the shape, dimensions and volume of cortical and epidermal cells and thus limit water uptake (Alshammary *et al.*, 2004). Plant biomass production depends on the presence of carbon products through photosynthesis, and salinity stress can reduced photosynthetic capacity of many plant species, which is associated with stomatal closure, increased mesophyll resistance for CO_2 diffusion, reduced efficiency of Rubisco for carbon fixation, damage to photosynthetic systems by excessive energy, and structural disorganization (Lee *et al*., 2004). In addition to reduced photosynthesis, the negative effect of salinity on plant growth is attributed to the decreased osmotic potential of the growing medium, specific nutrient ion deficiency, and ion toxicity (Luo *et al.,* 2005).

Leaves are the main organs of plant photosynthesis, and keeping leaf area stable under salinity stress can reduce the effect on photosynthesis (Chen *et al*., 2009). In this experiment, commercial TF, Sanandaj and Daran TF populations, maintained the higher shoot biomass among 12 turfgrasses at high salinity levels, Sanandaj and Daran populations were also the grasses that showed the greater quantity in leaf length and leaf area, but all of them decreased in leaf length and leaf area compared to control. The leaves of some glycophyte, such as *Sorghum bicolor* (L.), *Zea mays* L., *Triticum aestivum* L., and four turfgrasses (*Zoysia matrella* L., *Zoysia japonica* Steud., *Cynodon dactylon* L., and *Paspalum vaginatum* Sw.) often become short in length, narrow in width, light in weight, and reduced in number per shoot under salinity stress (Hu and Schmidhalter, 2007; Neves-Piestun and Bernstein, 2005; Chen *et al*., 2009).These studies suggest that salinity modified growth through its effects on the leaf expansion. Indeed, several authors have reported the strong implication of leaf expansion in glycophyte as well as in halophyte response to salinity (Tarchoune *et al.*, 2010). Salt-induced growth reduction might be related to salt osmotic effects, which affect expansion and cell turgor. Differently, it has reported that salinity affected initiation of new leaves without reducing cell expansion (Ben Amor *et al*., 2005). In this experiment, result showed over time leaf firing was increased. It may be explained with the fact that at first salinity affects growth with a short-term effect, including osmotic effects and, second long-term effects, including excessive salt uptake, which caused ionic stress, take part afterwards (Munns, 2002). High sodium levels disturb potassium (K+) nutrition and when accumulated in cytoplasm it inhibits many enzymes, and such secondary stresses as oxidative stress linked to the production of toxic reactive oxygen intermediates (Iqbal *et al.*, 2006). This leads to leaf firing which follows a reduction in the available photosynthetic area necessary to maintain growth. (Tarchoune *et al.*, 2010).

CONCLUSION

In conclusion, according to salinity problem and importance of tall fescue in the world, it is necessary to find salinity tolerance grasses in this species. Based on the present results, it is concluded that commercial tall fescue, Sanandaj and Daran tall fescue populations can be used under saline condition. These results showed the importance and competitive role of TF native populations compared to commercial seeds. In addition to detailed field experiments are needed to confirm this conclusion.

ACKNOWLEDGMENTS

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