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Biochemical Responses of Salt-Sensitive and Salt-Tolerant Tall Fescue

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ABSTRACT

The Turfgrass industry in saline soil is expanding, making it important to use salinity-tolerant turfgrasses. In this experiment, the effect of salinity stress on some biochemical content in salt-sensitive and salt-tolerant tall fescue was evaluated. The Sanandaj and Daran populations with commercial tall fescue (TF) were evaluated as salt-tolerant tall fescues and the Sanajan population was used as salt-sensitive TF. Five salinity levels of irrigation water (0, 45, 90, 135, and 180 mM NaCl) were applied to turfgrasses to identify the tolerance mechanisms in tolerant tall fescue under salinity stress. Results showed that salinity affected all turfgrasses in proline, chlorophyll, 1-1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, as well as sodium and potassium in their shoots. Sanajan population in 90, 135, and 180 mM salinity had the lowest chlorophyll content among all turfgrasses. Salt stress leads to an increase in the activity of proline compared to the control at the first stage (for evaluating osmotic stress) of measurement. In the second stage (to evaluate ionic stress), at concentrations of 135 and 180 mM NaCl, maximum proline was recorded in Daran and Sanandaj populations, respectively. The interaction effect of salinity and TF was significant for DPPH activity. The Na^+/K^+ ratio in the Sanajan population was the highest at all salinity levels. In conclusion, the growth and antioxidant capacity of *Festuca arundinaceae* populations differ in their response to NaCl treatments. In salt-tolerant TF, proline and antioxidant activity increased with increasing NaCl. These may be a mechanism to protect tolerant TF in salt stress, leading to lower accumulated Na+ in tolerant TF, high K+ uptake, and high chlorophyll content. Based on these results, proline content, DPPH radical scavenging activity, chlorophyll contents, and potassium content could use to distinguish tolerant TF from sensitive TF.

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1. Introduction

Quality and quantity of water in water shortage areas are legitimate concerns worldwide (Lee *et al.*, 2005). The higher amount of NaCl causes osmotic and ionic stresses in plants leading to photosynthetic damage (Rezazad Bari *et al.*, 2022), so in order to develop turfgrass industry in arid and semiarid regions, there is a need to use salinity tolerant turfgrasses (Mousavi Bazaz *et al.*, 2015). Salinity causes much damage to plants, such as growth inhibition, necrosis, impaired metabolism, loss of production and quality, (Miladinova *et al.*, 2013) and decreasing uptake of other nutrients, especially K^+ as an essential and necessary element (Mousavi Bazaz *et al.*, 2015). Salinity stress interferes with ion homeostasis by increasing Na^+ and decreasing K^+ (Munns and Tester, 2008). The excessive accumulation of Na^+ in plants under salinity stress causes leaf damage (Rezaei *et al.*, 2017). Salinity stress decreased K^+ and increased Na^+ in tall fescue (Pan *et al.*, 2021). In the presence of salinity, in many plant species, photosynthetic ability is reduced, which is related to stomatal closure, structural damages and destructive effects of excessive energy on photosynthetic systems (Lee *et al.*, 2004). One of the main reasons for the impact of salinity on plants is the reduction of photosynthesis activity which is due to chlorophyll reduction and the reduction of Co_2 absorption and photosynthesis capacity (Namrudi *et al.*, 2014).

In some plants, there are different bio-physical and biochemical strategies to cope with salinity stress on plant growth, such as the production of proline which can act as a protective agent for cellular organelles and enzymes as well as being a compatible solute. Moreover, proline can be a membrane stabilizer and scavenger for free radicals (Shakeri and Emam, 2018). The production of reactive oxygen species (ROS) is another consequence of salt stress which often causes oxidative damage (Parvaiz and Satyawati, 2008). The antioxidant system can protect against the toxic effects of ROS (Queirós *et al.*, 2011). Antioxidant activity could be measured by scavenged DPPH radical. DPPH can be expressed as its magnitude of antioxidation ability (Sharma and Ramawat, 2014). The Rate and the peak value of DPPH disappearance can defines as the ability of radical scavenger (Deng *et al.*, 2011). For turfgrass managers and breeders in saline sites, understanding the salinity tolerance mechanisms of the most tolerant ecotypes as well as their maximum salinity tolerance range is important (Lee *et al.*, 2005). 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 (Mousavi Bazaz *et al.*, 2015). This study aimed to evaluate the responses and mechanisms of salt-tolerant and sensitive tall fescue under different salinity levels.

2. Material and Methods

The greenhouse experiments were conducted at the Faculty of Agriculture, Ferdowsi University of Mashhad. In this study, the seeds of three tolerant tall fescues, including Sanandaj population, Daran population, commercial TF (C.TF) and a salinity-sensitive population named Sanajan were used. Tolerant and sensitive TF's selected from earlier research by Mousavi et al. (2015). The seeds were planted in pots with a mixture of sand, humus and field-soil (in equal proportions). After germination, ten seedlings of TF were transplanted into plastic pots (20 cm diameter) for eight weeks with non-saline irrigation water. Pure sand used as the growing media and Hoagland solution (Hoagland and Arnon, 1950) used as nutrient. Grasses were clipped throughout the experiment to 5 cm. Irrigation waters of different salinities were prepared by addition of NaCl to the tap water. Saline waters of 45, 90, 135 and, 180 mM along with tap water as the control treatment were applied to TF. To avoid salinity shock, salinity levels were increased by 22.5 mM per day. Water was applied including 30% excess water as leaching requirement (400 ml). The irrigation water was applied on alternate-day basis for a period of 8 weeks. The treatments were set up by following a factorial experiment based on completely randomized design with four replicates per treatment.

Data on proline, chlorophyll, DPPH radical scavenging activity, sodium and potassium in shoot tissues were recorded after the salinity treatment.

2.1. Determination of Proline Content

Samples for proline analysis were taken 2 (for osmotic stress) and 50 (for ionic stress) days after the initiation of salt stress. Proline was measured as described by Bates et al. (1973).

2.2. Determination of Ion Content

To measure ion concentrations (Na+ and K+), leaves were dried in an oven at 70^{oc} and 100 mg of the dried plant materials was homogenized using a pestle. The powdered leaves were suspended in HNO3 for 24 hours for ion extraction and the mixture was incubated at 80^{oc} for 1 hour. It was then measured by flam-photometer (UK-Jenway,Masashi Miyama, 2010).

2.3. Determination of Chlorophyll Content

Samples were collected from the fully-developed leaves of each replication in varying concentrations of NaCl and control plants. One hundred mg of fresh material was extracted with 4 ml 80% acetone and centrifuged for 5 minutes in 3000 G. The pigment content was determined spectrophotometrically (Lightenthaler, 1987).

2.4. Determination of DPPH radical scavenging activity

The 1-1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the method described by Abe et al. (1998). In brief, one hundred mg of fresh material was homogenized in ethanol 90% and then incubated at 4^{o}c for 24 hours; insoluble materials were removed by centrifugation 3500 G for 5 minutes. Then, 20 ml of extracted material was mixed with 800 µl DPPH in ethanol (0.5 mM). The remaining DPPH was measured by absorbance at 517 nm. The radical scavenging activity was calculated in percentage (Abe *et al.*, 1998).

The experimental data was analyzed using the JMP 8 software. Treatment were compared by Fisher's protected LSD. Before analysis of variance, the assumption of normality, homogeneity of variance of treatments, etc., been checked.

3. Results

3.1. Leaf chlorophyll content

Interaction effect of salinity and population was not significant for chlorophyll-b content (Table 1). In all tall fescue chlorophyll content decreased as salinity increased. Sanajan population in salnity levels of 90, 135 and 180 mM had the lowest amount of chlorophyll-a content among all turfgrasses (Fig.1). In all tall fescues, the greatest content for chlorophyll-b was seen in commercial tall fescue (Fig.2). For salinity levels, no significant difference was observed at 135 and 180 mM salinity (Fig.3). Total chlorophyll content decreased under salt stress in all TF's. Interaction effect of salinity and all TF's was significant for total chlorophyll (Table 1). Sanajan population had least total chlorophyll content among all turfgrasses at 90, 135 and 180 mM salinity (Fig 4). Commercial TF had greatest total chlorophyll content at 45, 90, 135 and 0 mM salinity, but at 180 mM salinity, Daran population had highest total chlorophyll content among all TF's (Fig 4).

Table 1. Main and interaction effects on different variables by salinity and population in Turfgrasses

Variable	Salinity	Population	Salinity × Species
Chlorophyll-a	82.56**	12.14**	2.07*
Chlorophyll-b	31.56**	6.15**	0.60 ns
Total chlorophyll	146.61**	23.16**	2.15*
Proline (Stage 1)	665.53**	285.62**	198.60**
Proline (Stage 2)	297.73**	310.35**	60.05*
DPPH activity	2443.13**	3529.77**	1211.88**
Potassium	38.47**	17.42**	4.08**
Sodium	68.32**	3.00 ns	1.91 ns
Na+/ K+ ratio	5.65**	2.35**	0.56**

ns, * and ** show non-significant at 5% and 1% probability levels, respectively.

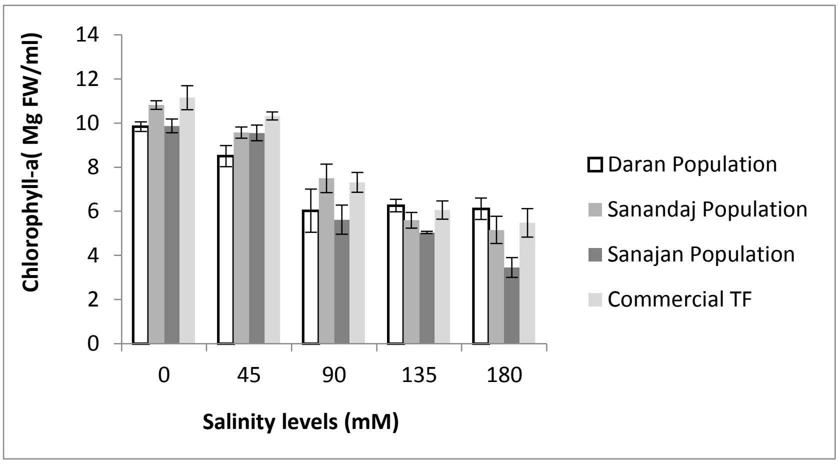

Fig. 1. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of chlorophyll-a content. (Vertical bars including standard error)

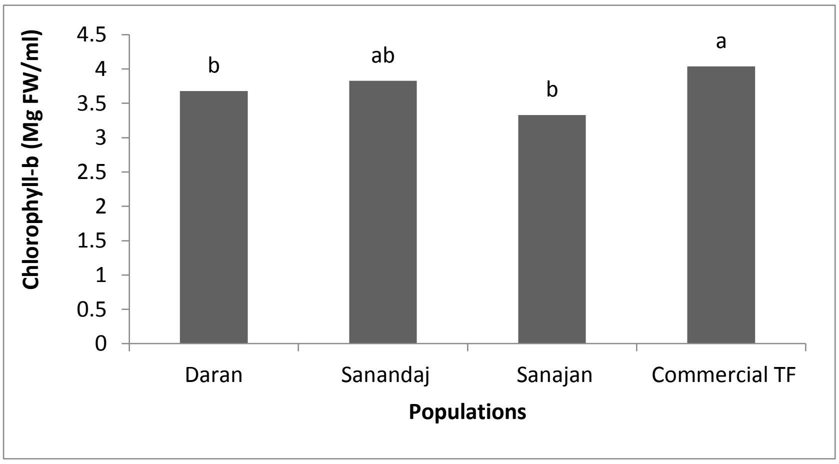

Fig. 2. Comparison of the mean main effect of tall fescue populations for the trait of chlorophyll-b content. (The data with the same letter are not significantly different at P<1%)

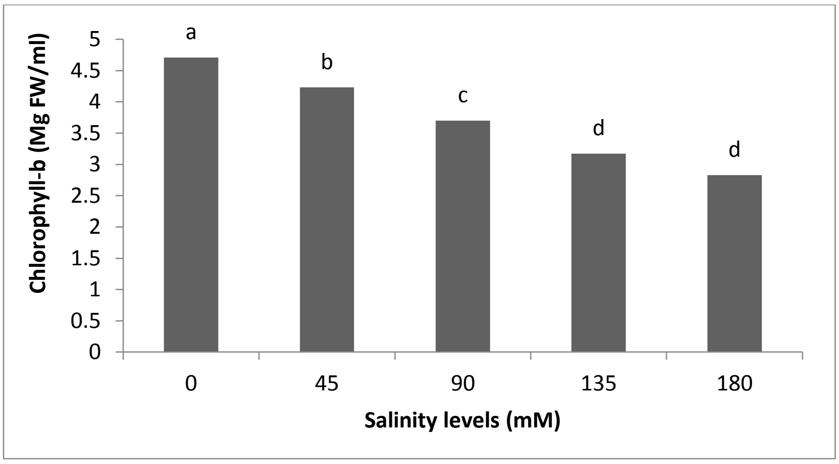

Fig. 3. Comparison of the mean main effect of salinity levels for the trait of chlorophyll-b content. (The data with the same letter are not significantly different at P<1%)

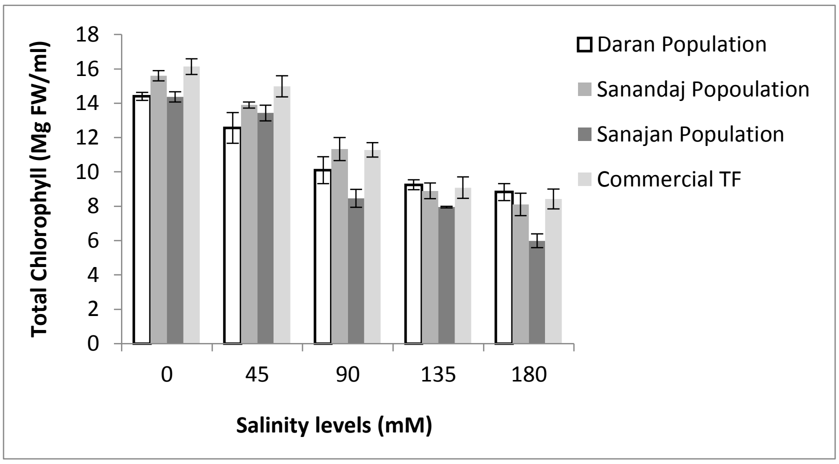

Fig. 4. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of total chlorophyll content. (Vertical bars including standard error).

3.2. Proline Content

Interaction effect of salinity and all TF's was significant for proline content after 2 (first stage) and 50 days (second stage) of salinity treatment (Table 1). Salt stress resulted in an increase in the activity of proline especially at 180 mM NaCl compared to the control at the first stage of measurement (Fig. 5). Maximum proline content was seen in Sanandaj population at 45 and 90 mM salinity. In addition, at 135 and 180 mM salinity, maximum proline content was recorded in commercial tall fescue and Daran population, respectively (Fig. 5).

At the second stage of measurement, salinity increased proline content in all tolerant TFs', but in sanajan population (sensitive TF) there were noticeable decrease in proline content at 135 and 180 mM salinity compared to 45 and 90 mM salinity (Fig. 6). At the second stage of measurement, there was a significant difference between tolerant TF and sensitive TF (sanajan population) at 135 and 180 mM salinity (Fig. 6). Maximum proline content was evidenced in commercial tall fescue at 45 and 90 mM salinity. Moreover, at 135 and 180 mM salinity, maximum proline content was recorded in Daran population and Sanandaj population, respectively (Fig. 6).

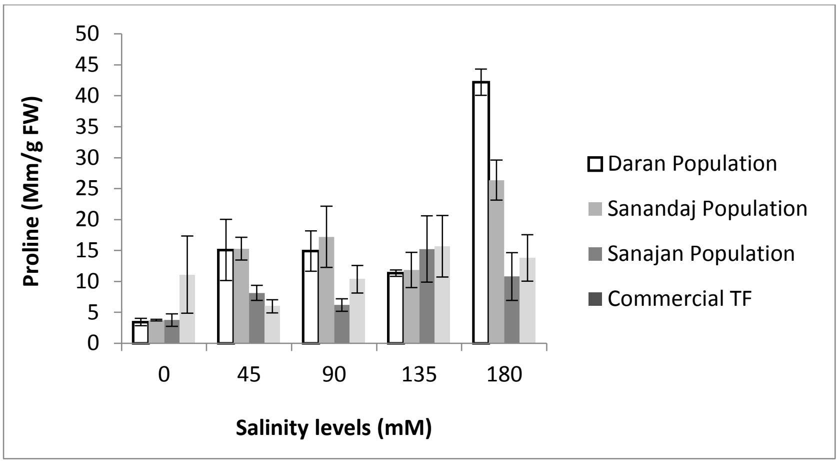

Fig. 5. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of proline content after 2 days of salinity treatment.
(Vertical bars including standard error)

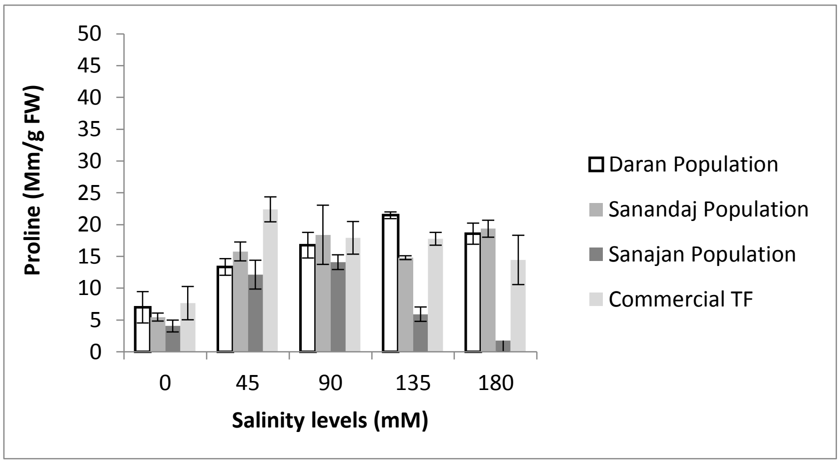

Fig. 6. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of proline content after 50 days of salinity treatment.
(Vertical bars including standard error)

3.3. DPPH radical scavenging activity

Interaction effect of salinity and all TF's was significant for DPPH activity (Table 1). Salt stress resulted in a noticeable increase in DPPH activity at 45, 90, 135 and 180 mM NaCl compared to the control for all tolerant TF's (Fig. 7). Maximum DPPH radical scavenging activity was evidenced in commercial tall fescue at 45 and 180 mM salinity and Sanandaj population at 90 and 135 mM salinity (Fig. 7). In Sanajan population (sensitive TF) there was noticeable decrease for this factor at 135 and 180 mM salinity compared to 45 and 90 mM salinity (Fig. 7).

3.4. Ion Content

Interaction effect of salinity and all TF's was significant for potassium content (Table 1). Potassium content decreased with increasing salinity (Fig. 8). The lowest potassium content was recorded at 45, 90, 135 and 180 mM salinity in Sanajan population (Fig. 8). Maximum

Potassium content was evidenced in commercial tall fescue at 90, 135 and 180 mM salinity and Daran population at 45 mM salinity, although at 135 and 180 mM salinity levels, there were no significant difference with following TF (Fig. 8).

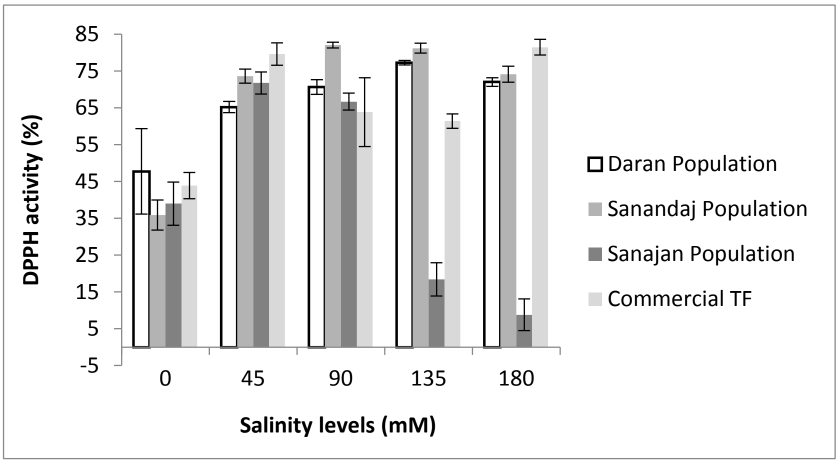

Fig. 7. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of DPPH radical scavenging activity. (Vertical bars including standard error).

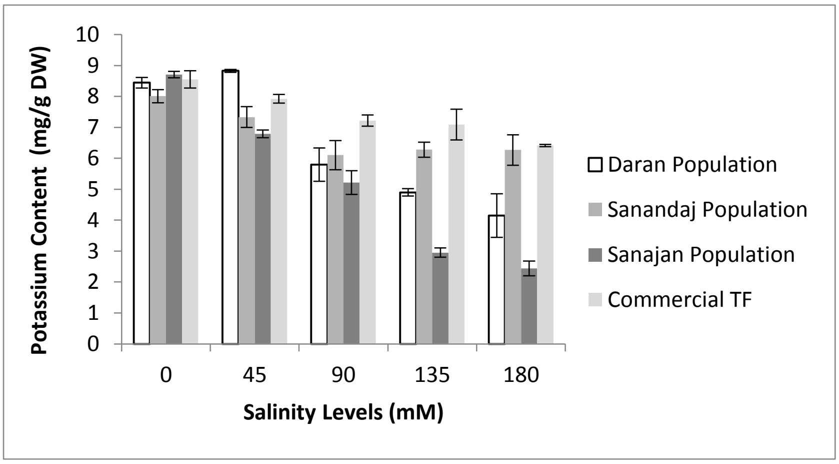

Fig. 8. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of potassium content. (Vertical bars including standard error)

Interaction effect of salinity and all TF's was not significant for sodium content. Also, among different TF's, there was no significant difference, but there was significant difference between salinity levels for this factor (Table 1). Sodium content increased with increasing salinity (Fig. 9). Maximum sodium content was recorded at 180 mM salinity, but there was no significant difference between 135 and 180 mM salinity (Fig. 9).

Consequently, the Na^+/ K^+ ratio in Sanajan population was the highest at all salinity levels. At 45 mM salinity, there was no significant difference among all TF's. At 45 mM salinity, the lowest Na^+/ K^+ ratio was evidenced in Daran population, and it was recorded in commercial tall fescue at 90 and 135 mM salinity (Fig. 10). Sanandaj population had lowest Na^+/ K^+ ratio at 180 mM salinity (Fig. 10).

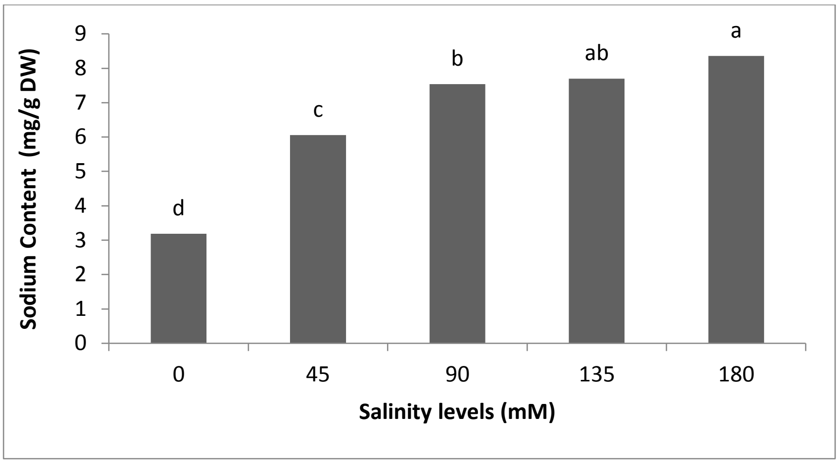

Fig. 9. Comparison of the mean main effect of salinity levels for the trait of sodium content. (The data with the same letter are not significantly different at P<1%.)

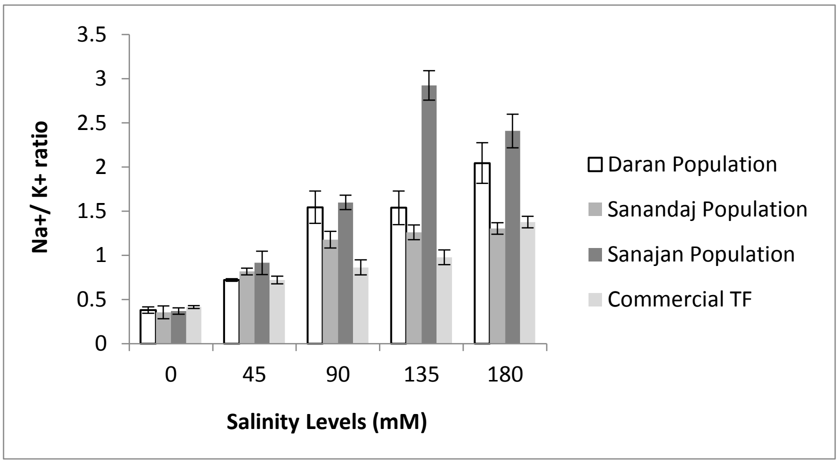

Fig. 10. Comparison of the mean interaction effect of tall fescue populations and different salinity levels for the trait of Na^+/ K^+ ratio. (Vertical bars including standard error)

4. Discussion

The aim of this study was to evaluate the responses of salt-tolerant and sensitive tall fescue to salinity. Under salinity stress, chlorophyll levels decreased, which may be recognized as a basic symptom of oxidative stress resulting in the inhibition of chlorophyll synthesis and chlorophyll degradation (Uddin *et al.*, 2012). Chlorophyll degradation may be related either to Mg deficiency and/or chlorophyll oxidation in salinity stressed conditions (Uddin *et al.*, 2012). The chlorophyll-a content of all TF's decreased much more with increasing salinity. Also, chlorophyll-b and total chlorophyll content decreased with increasing salinity levels. These results are consistent with the earlier reports for plants such as spinach (*Spinacia oleraceae*) (Di Martino *et al.*, 2003), *Hibiscus tiliaceus* (Santiago et al. 2000), paulownia (Miladinova *et al.*, 2013) and Tuberose (Salehi and Bahadoran, 2015). Salt-sensitive TF had lower chlorophyll content than salt-tolerant TF. Similar observation was made by other research such as the effect of salinity in six turfgrass (Uddin *et al.*, 2012). The proline concentrations in salt-tolerant TF were higher than Salt-sensitive TF, especially 50 days after salinity treatment. It is consistent with other reports such as cucumber and bean (Naliwajski and Skłodowska, 2014), (Stoeva and Kaymakanova, 2008). Proline, increases faster than other amino acids in plants under stress (Bates *et al.*, 1973). In salt stress, one of the first modifications in plants is proline accumulation, and it is often considered to be involved in stress-tolerance mechanisms (Mousavi Bazaz *et al.*, 2015). It is assumed that in *F. arundinacea*, the proline concentration is a genetically-determined factor, because this hypothesis was confirmed in a series of cultivars of *F. arundinacea* examined by variation in proline accumulation (Abernethy and McManus, 1998). Proline is a signaling molecule which can activate some responses for adaptation process (Ashraf and Harris, 2004). Proline is considered to be involved in the protection of cellular structures, enzymes, and to act as a free radical scavenger (Aghaleh *et al.*, 2011). In this experiment, results showed that as NaCl increased, DPPH increased in salt tolerant TF. It is consistent with some reports (Shabala and Mackay, 2011); (Sharma and Ramawat, 2014); (Cheng *et al.*, 2018). Basically, under salinity stress, it was seen that the activity of antioxidant systems is noticeably higher than normal conditions (Sharma and Ramawat, 2014). However, in some plants, no significant changes, or sometimes decreasing in activity of some antioxidant enzymes have been observed (Shabala and Mackay, 2011), which is consistent with sensitive TF's in this experiment. The correlation between antioxidant capacity and salt tolerance was shown in a large group of plants such as *Beta maritime*, *Cassia angustifolia and Crithmum maritimum* (Alam *et al.*, 2015). Sharma and Ramawat (2013) suggested elevated antioxidant potentials in callus culture of *Salvadora persica* under salinity conditions (Sharma and Ramawat, 2013). Increasing antioxidant capacity can decrease reactive oxygen species, because salt stress may increase in secondary metabolites (Sharma and Ramawat, 2014). Our results revealed that with increasing NaCl potassium decreased, which is consistent with (Tarchoune *et al.*, 2010), (Salehi and Bahadoran, 2015) and (Iqbal *et al.*, 2006). In soils, the basic compound contributing salinity is sodium chloride, which salt-tolerant TF must endure. High concentration of Na+ competes with the uptake of other nutrients, especially K+ as a necessary element (Mousavi Bazaz *et al.*, 2015). High NaCl produces effects that negatively affect plant growth and development. Osmotic stress and ionic toxicity are the primary effects. High concentrations of Na+ and Cl- in cells disturb several biochemical and physiological processes and lead to Ionic toxicity (Salehi and Bahadoran, 2015).

5. Conclusion

In conclusion, the growth and antioxidant capacity of *Festuca arundinaceae* populations differ in their response to NaCl treatments. Sanajan population had the most sensitive TF. It was seen that chlorophyll content was lower in sensitive TF compared to tolerant TF. In salt-tolerant TF, proline content and DPPH radical scavenging activity increased with increasing NaCl. Based on these results proline content, DPPH radical scavenging activity, chlorophyll contents and potassium content could be used to distinguish tolerant TF from sensitive TF. These may be a mechanism to protect tolerant TF in salt stress, leading to lower accumulated Na^+ in tolerant TF, high K^+ uptake and high chlorophyll content.

Acknowledgments

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