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ECO-MORPHIC RESPONSE TO SALT STRESS IN TWO HALOPHYTIC GRASSES FROM THE CHOLISTAN DESERT, PAKISTAN

NARGIS NAZ, MANSOOR HAMEED* AND MUHAMMAD ASHRAF†

Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan
†Second affiliation: King Saud University, Riyadh, Saudi Arabia
**Corresponding author's E-mail: hameedmansoor@yahoo.com*

Abstract

A study was conducted to evaluate the ecotypic difference in salinity tolerance in two halophytic grass species *Aeluropus lagopoides* and *Sporobolus ioclados* from the Cholistan Desert, Pakistan in a hydroponic system. Three populations of each of the two grass species were collected from ecologically different habitats, i.e., lesser salt-affected Derawar Fort (DF), moderately salt-affected Bailahwala Dahar (BD), and highly salt-affected Ladam Sir (LS). Genetic variation for salinity tolerance was found in all populations of both *A. lagopoides* and *S. ioclados*, its magnitude corresponded to the selection pressure of the habitats. The lesser saline DF populations of both grass species were the least tolerant to salt stress in relation to most of the agro-morphological characteristic measured. The moderately saline BD populations were relatively less affected due to salt stress and 100 mM NaCl stimulated their growth measured in terms of plant height, root length and fresh and dry weights of shoots and root. The highly saline LS populations were the most tolerant among all populations of both grasses and optimal growth was recorded at 200 and 300 mM NaCl of the growth medium. Of the two grasses, *A. lagopoides* was superior to *S. ioclados* in salinity tolerance because its all three populations were relatively more tolerant as compared to those of the latter grass species.

Introduction

The importance of variation among ecotypes within a plant species has been documented since long (Bradshaw, 1984; Kuiper, 1990; Ashraf & Ahmad, 1995; Ashraf *et al.*, 2003; Hameed *et al.*, 2008; Naz *et al*., 2009a). Such variation is of vital important in providing suitable genetic stock capable of coping with a multitude of environmental conditions (Pezeshki & DeLaune, 1997). Generally, the genetic heterogeneity among populations of a particular species is associated with evolutionary processes like interactions among natural selection, selection pressure, genetic drift and recombination, and mutation (Ashraf, 2004; Fjellheim & Rognli, 2005). Selection pressure of variable habitats during an evolutionary history of a species can evolve into quite diverse populations, which are genetically distinct and adapted to such specialized habitats (Eleuterius, 1989).

Genetically based variation has been reported in many salt tolerant and halophytic species e.g., in *Imperata cylindrica* (Cheng & Chou, 1997; Hameed *et al*., 2009), *Cenchrus ciliaris* (Hameed *et al*., 2002), *Cynodon dactylon* (Hameed & Ashraf, 2008), *Aeluropus lagopoides* and *Ochthochloa compressa* (Naz *et al*., 2009b), *Dactylis spicata* (Brewer & Bertness, 1996), *Panicum antidotale* (Hameed & Ashraf, 2008), and *Spartina alterniflora* and *Spartina patens* (Seliskar, 1995).

Saline flats of the Cholistan desert support a number of halophytic or salt tolerant plant species, among including two considerably important grasses *Aeluropus lagopoides* and *Sporobolus ioclados*. However, these two grasses were selected for the present study. *Aeluropus* is a genus of 6 species and represented by 3 species in Pakistan, whereas *Sporobolus* includes 250 species and represented by 10 species in Pakistan (Cope, 1982; Chaudhry, 1989). *Aeluropus littoralis* can tolerate up to 1,100 mM NaCl and *A. lagopoides* 1,500 mM NaCl (Bodla *et al.*, 1995; Gulzar *et al.*, 2003a&b). Many species in genus *Sporobolus* are highly salt tolerant and halophytic (Ashour & Mekki, 2006). Examples are *S. virginicus* (Ashour *et al*., 1997; Bell & O'Leary, 2003), *S. airoides* (Marcum, 1999; Grieve *et al*., 2004), *S. ioclados* (Gulzar *et al*., 2005), *S. madraspatanus* (Joshi *et al*., 2005), *S. spicatus* (Ramadan, 2001), *S. pyramidatus* (Tóth *et al*., 1997), and *S. arabicus* (Khan & Ungar, 2001).

Identification of natural populations tolerant to high salinities is extremely vital in developing strategies for re-vegetation and reclamation of salt-affected areas. Salt tolerant ecotypes can provide exceptional means for investigating the adaptative mechanisms to tolerate environmental stresses (Ashraf, 2003), and therefore, can be used as a model plants (Hameed *et al*., 2008; Pessarakli & Kopec, 2009). The present study was focused on genetic variations among the ecologically different populations and the morphological response to salt stress in two grasses (*A. lagopoides* and *S. ioclados*) to elevated levels of salinity in a hydroponic culture solution under the Faisalabad environmental conditions.

Materials and Methods

Ecotypic difference in salinity tolerance of three populations was investigated of each of each of two halophytic grass species *Aeluropus lagopoides* (L.) Trin. ex Thw., and *Sporobolus ioclados* (Nees ex. Trin.) Nees from the Cholistan Desert, Pakistan. The experiment was conducted hydroponically in half-strength Hoagland's nutrient culture solution in the Botanical Garden, University of Agriculture, Faisalabad. Three populations of each of the two grass species were collected in the Cholistan Desert from ecologically different habitats, i.e., lesser salt-affected Derawar Fort (DF; coordinates 71º 27' 32.73" E, 29º 24' 31.65" N; ECe 15.21 dS m^{-1}; Na^+ 3246.46 mg L^{-1}; Cl^- 1483.21 mg L^{-1}), moderately salt-affected Bailahwala Dahar (BD; coordinates 70º 93' 23.34" E, 29º 38' 18.27" N; ECe 26.7 dS m^{-1}; Na^+ 4382.45 mg L^{-1}; Cl^- 2446.62 mg L^{-1}), and highly salt-affected Ladam Sir (LS; coordinates 72º 64' 25.11" E, 30º 53' 26.42" N; ECe 49.28 dS m^{-1}; Na^+ 5149.33 mg L^{-1}; Cl^- 2647.76 mg L^{-1}). Electrical conductivity of saturated soil extract (ECe) of the rhizospheric soil of all the populations of these grasses was measured using a electrical conductivity meter (WTW series InoLab pH/Cond 720), soil Na^+ by a flame photometer (Model Sherwood 410, USA) and soil Cl^- by a chloride meter (Model 926, Sherwood Scientific Ltd., Cambridge).

Populations of both grasses from all three habitats were established in loamy-clay soil. Ramets were detached from each population and grown in half-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950). Air pumps were used for about 12 h daily for the aeration of the hydroponic system.

The experiment was conducted in a 3-factor completely randomized design with 3 populations (DF, BD, and LS), 2 grasses (*A. lagopoides* and *S. ioclados*), and 4 salinity levels (0, 100, 200 and 300 mM NaCl) with 10 replications. Agro-morphological characteristics studied during the experiment were shoot and root length, top internodal length, number of tillers and leaves per plant, and fresh and dry weights of plant.

Results

All populations of both *Aeluropus lagopoides* and *Sporobolus ioclados* showed differential response under increasing salt levels. Shoot length in population of *A. lagopoides* from the least saline Derawar Fort (DF) consistently decreased with increase in salt level of the medium (Fig. 1). In contrast, the populations from moderately saline Bailahwala Dahar (BD) and highly saline Ladam Sir (LS) showed a slight increase up to 200 mM NaCl of external medium and thereafter a slight decrease was observed at 300 mM NaCl. Shoot length in all populations of *S. ioclados* were only affected by the highest salt level (300 mM NaCl), however, the population from the highest saline LS showed a consistent increase in shoot length up to 200 mM NaCl.

A similar response of root length was recorded in all populations of *A. lagopoides*, which depicted a gradual decrease with increasing salt levels of the hydroponic culture solution. The highly saline LS population, however, was relatively less affected in terms of root length as compared to the other populations by increasing salt levels. The *S. ioclados* population from lesser saline DF showed a considerable decrease in root length, while those from moderately saline BD and highly saline LS showed increased root length under elevated salt levels.

Top internodal length showed quite a variable behaviour under salt stress (Fig. 1). It was promoted by moderate salt level (100 mM NaCl) in all three populations of *S. ioclados*. Top internodal length in *A. lagopoides,* nevertheless, in the DF population consistently decreased, in BD population it increased up to 200 mM salt level, and in LS population, it consistently increased with increase in salt levels.

Number of tillers per plant and number of leaves per plant showed almost a similar response to elevated salt levels. The DF population of both *A. lagopoides* and *S. ioclados* were adversely affected in relation to these parameters by increasing salt levels. In contrast, BD population showed stability in these parameters and LS populations showed an increase in number of tillers and leaves with increase in salt levels.

Total leaf area was negatively affected in the populations of both grasses from lesser saline LS at higher salt levels. The leaf area of LS population of *A lagopoides* was consistently increased with increase in salt levels, while that of the LS population of *S. ioclados* was slightly affected at the highest 300 mM NaCl level (Fig. 1).

A similar response in terms of root fresh and dry weights was recorded in all populations of both grass species (Fig. 2). *Aeluropus lagopoides* from DF showed the maximum of these parameters at 100 mM NaCl, whereas that from BD showed the maximum value at 200 mM and that from LS at 300 mM NaCl. Fresh and dry weights of DF and BD populations of *S. ioclados*, on the other hand, were slightly increased at 100 mM NaCl, and these parameters decreased gradually with further increase in the salt levels. The LS population, however, was adversely affected only at 300 mM NaCl in relation to these parameters.

A significant decrease was recorded in shoot fresh and dry weight in the DF population of both *A. lagopoides* and *S. ioclados* by increasing salt levels. These parameters were adversely affected at 300 mM NaCl in the BD populations of both grasses, whereas, in the LS population, these parameters were consistently increased with increase in salt levels (Fig. 2).

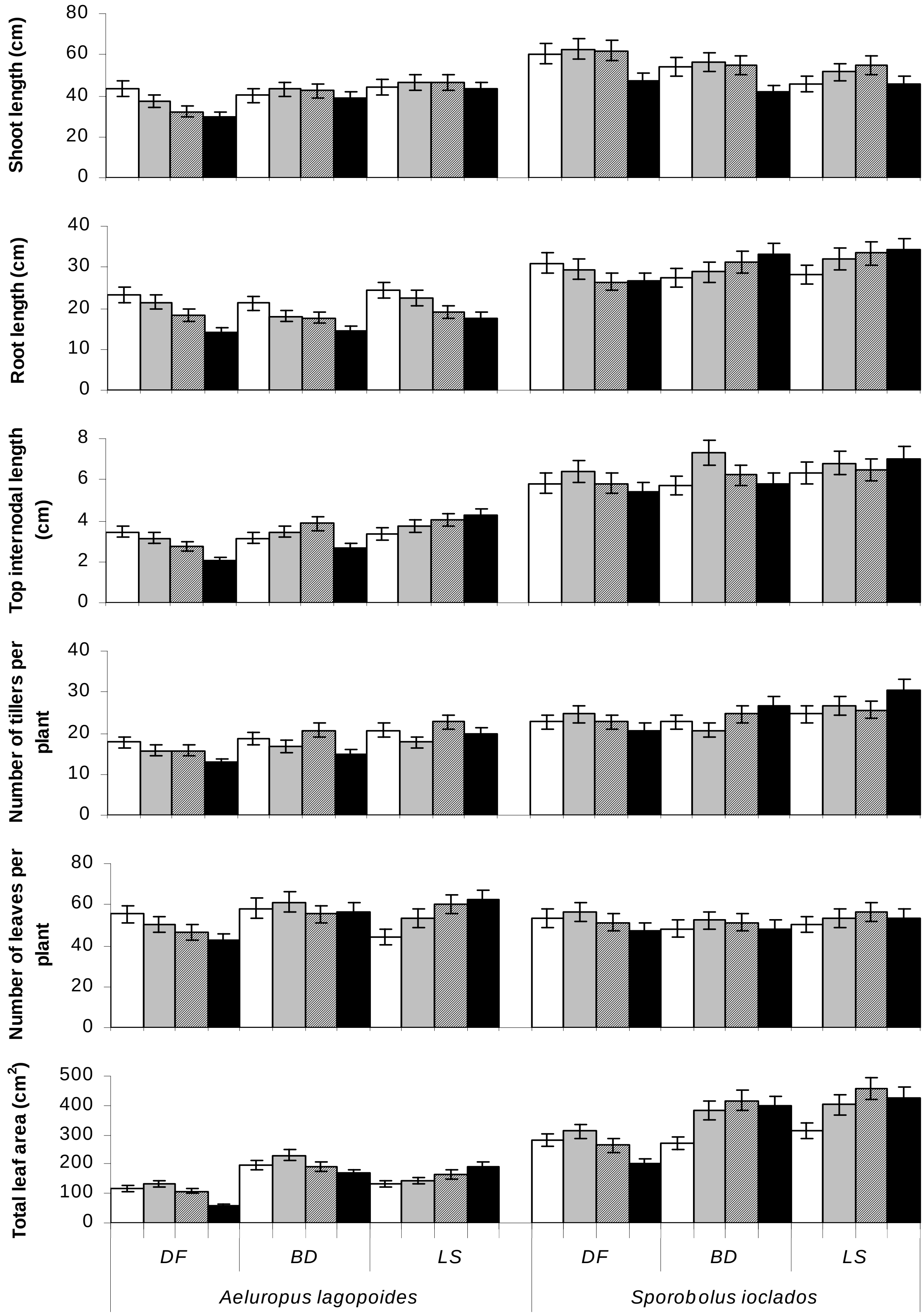

Fig. 1. Some agro-morphological characteristics recorded in ecologically different populations of two grass species under salt stress (n = 10, means ± SE, DF = Derawar Fort, BD = Bailahwala Dahar, LS = Ladam Sir).

0, 100, 200 and 300 mM NaCl.

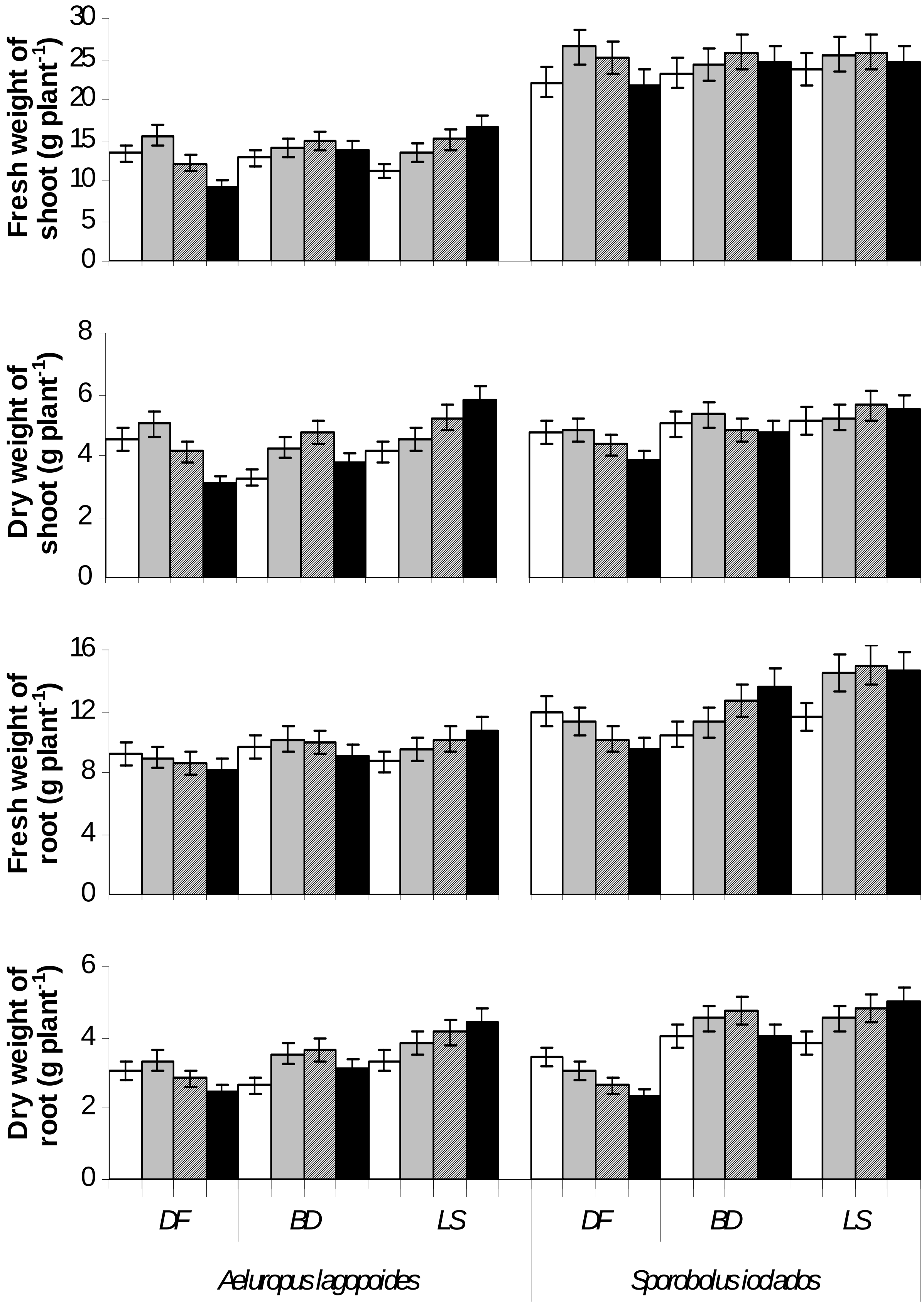

Fig. 2. Fresh and dry weights of roots and shoots recorded in ecologically different populations of two grass species under salt stress (*n* = 10, means ± SE, DF = Derawar Fort, BD = Bailahwala Dahar, LS = Ladam Sir).
0, 100, 200 and 300 mM NaCl.

Discussion

Cholistan desert of Pakistan is a unique biome subjected to an interplay of several environmental stresses including drought, salinity, temperature and nutrient stresses (Arshad *et al*., 2008). Environmental heterogeneity and long evolutionary history of this desert is enough to impose significant genetic variations within the native species, making them perfectly adaptative to the harsh environmental conditions of the desert (Weber, 2009). Inter-dunal saline patches in the Cholistan desert have a significant amount of variation in soil physico-chemical characteristics (Naz *et al*., 2010), and this results in differential response of different ecotypes of the inhabiting species in diverse habitats. Such genetic variability within individual plant species has been reported by several authors in response to environmental variability (Toth *et al*., 1997; Ramadan, 2001; Bell & O'Leary, 2003; Hameed *et al*., 2009).

Genetic variation was found in all populations of both *A. lagopoides* and *S. ioclados*, which was the basis of their differential response to high salt stress (Naz *et al.*, 2009b; Hameed *et al*., 2009). The lesser saline DF populations of both grass species were the least tolerant to salt stress in relation to most of the agro-morphological characteristics. All agro-morphological characteristics of both grasses showed a significant decrease due to increasing salinity. Such decrease in less tolerant ecotypes due to salt stress has been reported by several authors (Ashraf & Ahmad, 1995; Ashraf *et al*., 2006; Hameed & Ashraf, 2008).

The moderately saline BD populations were relatively less affected by increasing salinity. However, moderate salt level (100 mM NaCl) resulted in increased growth in terms of plant height, root length and fresh and dry weights of shoots and root. There are only few reports on salt-induced growth stimulation in monocots, especially in grasses (Munns & Tester, 2008; Flowers & Colmer, 2008). However, optimal growth of salt tolerant or halophytic grasses has been reported by several authors at moderate salinities such as in *Puccinellia peisonis* (Stelzer & Laüchli, 1977), *Leymus arenarius* (Greipsson & Davy, 1996), and *Imperata cylindrica* (Hameed *et al*., 2009).

The highly saline LS populations were the most tolerant among all populations of both grasses with respect to agro-morphological characteristics measured in the present study. Optimal growth of the LS population of both grasses was recorded at high salinity levels, mainly at 200 mM NaCl of the growth medium. In particular, the LS population of *A. lagopoides* showed the maximum growth in terms of fresh and dry biomass at the highest salt level i.e., 300 mM NaCl. Similar results have been reported by Marcum & Murdoch (1994) in *Stenotaphrum secundatum* and Naz *et al*., (2009b) in *Ochthochloa compressa* at high salinities.

Ecological factors strongly affect the genetic variation within populations of specific species (Kolliker *et al*., 1998; Hsao & Lee, 1999), which in turn, is responsible for the development of an ecotype adapted to such specific environments (Gunter *et al*., 1996). From the present study it was evident that the variation in both grasses due to habitat differences is genetically based and this may be the basic requirement for the improvement of salinity tolerance in other plant species through conventional breeding and advanced molecular/genetic engineering techniques (Al-Khatib *et al*., 1993; Munns & Tester, 2008). Varying degree of salinity tolerance in a variety of species due to ecotypic differences has been reported by several researchers e.g., in *Lolium multiflorum* (Hannaway *et al.*, 1999), *Leymus arenarius* (Greipsson & Davy, 1996), *Cymbopogon* *jawarancusa* (Arshad *et al*., 1995), *Sporobolus ioclados* (Arshad *et al*., 1999) and *Cynodon dactylon* (Hameed & Ashraf, 2008).

Overall all populations (ecotypes) of both *A. lagopoides* and *S. ioclados* from highly saline LS were more tolerant as compared to those from moderately saline BD and lesser saline DF, as they showed optimal growth at moderate to high salinities in terms of various growth parameters studied.

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