

MORPHOLOGICAL ADAPTATIONS OF SOME GRASSES FROM THE SALT RANGE, PAKISTAN

MANSOOR HAMEED*, NARGIS NAZ, MUHAMMAD SAJID AQEEL AHMAD, ISLAM-UD-DIN¹ AND ATIF RIAZ²

Department of Botany, University of Agriculture, Faisalabad

¹*Department of Mathematics and Statistics, University of Agriculture, Faisalabad*

²*Institute of Horticultural Sciences, University of Agriculture, Faisalabad.*

Abstract

Three potential salt tolerant forage grasses (*Cynodon dactylon*, *Imperata cylindrica* and *Sporobolus arabicus*) were collected from the salt affected habitats in the Salt Range, Pakistan. Ecotypes of all the three grasses were also collected from normal non-saline habitats within the Faisalabad region. Mechanism of adaptation to saline environments is very specific, which is not only for grass species but also for ecotypes. Ecotypes of all three grasses from Faisalabad showed stunted growth under salt stress, perhaps to save energy for normal developmental and other metabolic processes. Salt tolerant ecotypes from the Salt Range had better growth and survival under saline conditions and had less reduction in shoot growth at the highest salt level as compared to those collected from non-saline habitats. Leaf area of tolerant genotypes was less affected by salinity than salt sensitive genotypes. Increased root growth as observed in *C. dactylon* and *S. arabicus*, can be attributed to better salt tolerance in the populations from the Salt Range. The ecotypes of all three grasses, *C. dactylon*, *I. cylindrica* and *S. arabicus* from the Salt Range performed better under high salinities than their counterparts from the Faisalabad region. On the basis of various morphological characteristics and growth attributes, the ecotype of *S. arabicus* from the Salt Range has been categorized the most tolerant among all the grasses and ecotypes. It was followed by the ecotypes of *C. dactylon* and *I. cylindrica* from the Salt Range.

Introduction

The Salt Range is facing multiple stresses simultaneously, especially drought and salinity (Hameed and Ashraf, 2008). It is the oldest series referred to as Cambrian (Yeats *et al.*, 1984; McKerrow *et al.*, 1992), in which salt layers normally lied at the lowest levels. Severe tilting of salt layers during the post-Pleistocene deformations resulted in exposure of these salt layers near the surface at many places. As a result soil lying between the Salt Range and River Jhelum is heavily salt infested due to runoff water during rainy season. Mainly sodium chloride (about 90%) is responsible for the increased salinity, the rest are the chlorides, carbonates and bicarbonates of sodium, potassium, magnesium and calcium (Qadir *et al.*, 2005).

Uchhali complex (Khabeki, Uchhali and Jahlar lakes) is of international importance because these wetlands are the wintering sites of rare or vulnerable waterfowl species, especially the white-headed duck (Nawazish *et al.*, 2006). Uchhali Lake is the largest of them, where the water is brackish to hyper-saline (Afzal *et al.*, 1998).

Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of anatomical, morphological, and physiological adaptations, such as

* Corresponding Author: hameedmansoor@hotmail.com; Hameedmansoor@yahoo.com

an extensive root system and salt secreting glands on the leaf surface (Marcum & Murdoch, 1990; Marcum *et al.*, 1998).

Naturally adapted salt tolerant plants provide an excellent material for investigating the adaptation mechanisms they use to encounter high concentrations of salt (Ashraf, 2003), which can be used as a model for the study of adaptive mechanism of stress tolerance. The present studies were focused to examine morphological adaptations of the three potential grass species to high saline conditions.

Cynodon dactylon, *Sporobolus arabicus*, *Imperata cylindrica*, *Aeluropus lagopoides* and *Cenchrus ciliaris* dominated the saline or saline arid habitats in the Salt Range. These grasses are expected to be well adapted against very high salinity. Thus, three grasses, *Cynodon dactylon*, *Imperata cylindrica* and *Sporobolus arabicus*, were used in the present studies to investigate the adaptability of these potential salt tolerant grasses from the Salt Range, Pakistan against salt stress.

Materials and Methods

Three potential salt tolerant forage grasses (*Cynodon dactylon*, *Imperata cylindrica* and *Sporobolus arabicus*) were collected from the salt affected habitats in the Salt Range, Pakistan. *Cynodon dactylon* (L.) Pers. was collected from the heavily salt affected soils in the vicinity of natural salt lake, Uchhali Lake, in the Salt Range (coordinates 32°46'35.46"N, 72°10'23.98"E, pH 6.62, ECe 19.92 dS m⁻¹, Na⁺ 4034.86 mg kg⁻¹, Cl⁻ 2021.32 mg kg⁻¹). *Sporobolus arabicus* Boiss was collected from the valley near the foothills, which is greatly affected by the salt deposition as a result of dissolved salts from the exposed hills (coordinates 32°76'23.45"N, 72°62'27.58"E, pH 8.12, ECe 34.36 dS m⁻¹, Na⁺ 5314.62 mg kg⁻¹, Cl⁻ 2467.28 mg kg⁻¹). *Imperata cylindrica* (L.) Raeuschel was collected from the edges of agricultural fields, which is seasonally inundated and the plants face diluted less saline waters during the rainy season (coordinates 32°46'35.92"N, 72°10'23.41"E, pH 7.38, ECe 15.40 dS m⁻¹, Na⁺ 2904.02 mg kg⁻¹, Cl⁻ 1530.86 mg kg⁻¹). Populations of all three grasses, *C. dactylon* (coordinates 31°25'30.61"N, 73°03'53.84"E, pH 9.20, ECe 1.82 dS m⁻¹, Na⁺ 69.24 mg kg⁻¹, Cl⁻ 422.46 mg kg⁻¹), *S. arabicus* (coordinates 31°28'25.25"N, 73°12'42.87"E, pH 8.86, ECe 1.52 dS m⁻¹, Na⁺ 81.68 mg kg⁻¹, Cl⁻ 312.20 mg kg⁻¹) and *I. cylindrica* (coordinates 31°25'43.96"N, 73°04'17.40"E, pH 6.70, ECe 2.92 dS m⁻¹, Na⁺ 320.44 mg kg⁻¹, Cl⁻ 330.46 mg kg⁻¹) within the Faisalabad region from normal non-saline habitats were collected and considered as control.

Populations of all the three grass species were grown in normal non-saline soil for a period of six months. Nine-inches earthen pots filled with loam and sand in equal quantities were used for the plants. The plant were kept under full sunlight and irrigated daily till their establishment in Faisalabad environments. The ramets each with three tillers of uniform size from each plant were grown in half-strength Hoagland nutrient solution till their establishment in hydroponics. Containers of fibre-glass of 25 L capacity were used for hydroponics. Twenty liters of Hoagland's nutrient solution was filled in each container. The containers were aerated with air pumps about twelve hrs daily and placed under plastic covering to check rain affect. Five salinity levels were maintained after the establishment of grass species for eight weeks, viz., Control (no salinity treatment), 50, 100, 150 and 200 mM of NaCl salinity in solution culture. The experiment was planned in a Completely Randomized Design (CRD) with two factors (ecotypes and salinity levels) and twelve replicates. Plants were carefully uprooted from the

hydroponics after the completion of the project and washed with tap-water for morphological characteristics.

For dry weight, plants were oven-dried at 65°C until constant weight was achieved. Morphological characteristics recorded at the end of the experiment were plant height, number of leaves per plant, leaf area, root length, fresh weight of plant (shoot and root) and dry weight of plant (shoot and root).

Analysis of variance of the data from each attribute was computed using the MSTAT Computer Program (MSTAT Development Team, 1989). The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1997).

Results

All the differences among grass species and salt levels were highly significant, as were their interactions. However, ecotype differences were non-significant for root and shoot length. *Imperata cylindrica* from the Faisalabad region was unable to survive at the highest salt level, i.e., 200 mM NaCl treatment.

Salt stress significantly reduced shoot length in all the grass species examined in the present study. However, the effect was more pronounced in Faisalabad ecotypes of all three grass species (Table 1). The most adversely affected was the population of *Cynodon dactylon* from Faisalabad, where a decrease from 77.02 to 37.29 cm was recorded with increase in salt level. In *I. cylindrica* from Faisalabad shoot length was decreased from 28.00 to 17.02 cm at 150 mM of NaCl and it was not survived at 200 mM NaCl. Moderate levels (100 mM NaCl) of salt improved shoot lengths of the Salt Range ecotypes of all grass species but thereafter at the higher salt levels shoot length decreased gradually. The Salt Range ecotype of *C. dactylon* had the maximum shoot length at 100 mM NaCl (49.46 cm) and a further increase in salt level reduced its shoot length to 37.29 cm. The ecotype of *Sporobolus arabicus* from Salt Range showed the maximum shoot length at 100 mM NaCl, but shoot length decreased to 51.92 cm at the subsequent higher salt levels.

Sporobolus arabicus had longer root system than the other grasses and it showed a considerable increase in root length up to 150 mM NaCl (56.17 cm in Faisalabad and 59.08 cm in Salt Range) and afterwards a slight decrease at 200 mM NaCl was observed in both Faisalabad and Salt Range populations. Root length in the ecotype of *C. dactylon* from the Salt Range increased markedly (nearly 300%) with increase in salt level where the length was measured as 40.67 cm (Fig. 2a). Root length in the Faisalabad population increased up to 150 mM NaCl (37.26 cm) but it decreased abruptly (16.83 cm) at higher salt levels. *I. cylindrica* from Faisalabad was the worst affected population in which root length was incessantly decreased with increase in salt level. Root length in the ecotype from the Salt Range, however, was slightly affected by the salt stress.

A progressive increase in the number of leaves per plant was recorded in *C. dactylon* from the Salt Range, ranging from 111.83 at 0 mM NaCl to 203.33 at 200 mM NaCl. *I. cylindrica* from Faisalabad was affected maximally where well over 50% decrease in number of leaves was recorded at 150 mM NaCl. *S. arabicus* showed enhanced number of leaves up to 150 mM NaCl (44.73 in population from Faisalabad and 58.97 in that from the Salt Range). Number of leaves in the ecotype of *C. dactylon* from Faisalabad

Table 1: Morphological characteristics of some grasses from the Salt Range, Pakistan

Characteristics	Grasses	Faisalabad						Salt Range			
		0 mM	50 mM	100 mM	150 mM	200 mM	0 mM	50 mM	100 mM	150 mM	200 mM
Shoot length (cm)	<i>Cd</i>	77.02a	47.04a	44.08cd	41.58bc	37.29b	31.48a	45.67cd	49.46d	41.92b	37.92b
	<i>Ic</i>	28.00c	29.83c	21.33b	17.02b	0.00a	31.50c	37.67d	45.75e	42.46de	27.08c
	<i>Sa</i>	67.63d	73.58e	75.39a	51.00b	41.91a	44.58ab	50.43cd	63.78b	61.58cd	51.92cb
Root length (cm)	<i>Cd</i>	14.29b	26.84a	30.11e	37.26b	16.83b	10.75a	16.00b	21.17c	36.50b	40.67b
	<i>Ic</i>	14.83bc	10.13b	8.17b	7.83b	0.00a	11.58b	18.50c	16.58c	10.10b	9.25b
	<i>Sa</i>	20.92a	27.58b	43.99d	56.17e	45.67d	20.09a	32.25c	33.75c	53.08e	47.08d
Number of leaves (per plant)	<i>Cd</i>	85.40bc	85.40bc	162.67g	103.70c	57.95a	111.83d	123.02e	152.50i	152.50i	203.33h
	<i>Ic</i>	28.47d	22.37c	18.30c	11.18b	0.00a	14.23bc	21.35c	16.27bc	8.13b	10.17b
	<i>Sa</i>	21.35a	31.52b	33.55bc	44.73d	42.70d	26.43ab	33.55bc	39.65c	58.97e	40.67cd
Leaf area (cm ²)	<i>Cd</i>	117.34c	135.08d	193.70g	108.84b	59.38a	199.75g	233.81e	192.07g	157.15e	170.73f
	<i>Ic</i>	271.61h	163.90g	119.78f	46.10c	0.00a	156.45g	101.85e	64.57d	24.66b	30.11b
	<i>Sa</i>	174.37a	288.68c	421.98h	476.09i	404.16g	216.90b	307.31d	365.60d	832.68f	533.91e
Fresh weight of shoot (g plant ⁻¹)	<i>Cd</i>	8.75d	6.30bc	4.24ab	4.14a	2.44a	12.20e	14.23f	7.58cd	7.52cd	4.78ab
	<i>Ic</i>	2.95b	1.63ab	1.45ab	0.97a	0.00a	4.27c	4.47c	3.15bc	2.34b	1.63ab
	<i>Sa</i>	12.45b	10.57ab	6.41ab	5.70a	2.98a	10.19c	13.49c	9.32bc	7.09b	6.50ab
Fresh weight of root (g plant ⁻¹)	<i>Cd</i>	2.54ab	2.60ab	2.18ba	1.88a	1.18a	2.44ab	2.82ab	3.56b	4.94c	4.11c
	<i>Ic</i>	0.85a	1.36a	1.06a	0.85a	0.00a	0.78a	0.67a	0.82a	0.99a	1.16a
	<i>Sa</i>	3.38ab	4.42bc	5.55cd	3.68ab	3.20ab	2.82a	3.76ab	4.72bc	6.30d	5.05cd
Dry weight of shoot (g plant ⁻¹)	<i>Cd</i>	3.02c	2.42bc	1.80ab	1.25a	0.86a	2.76bc	2.84cb	2.66bc	2.47bc	2.27b
	<i>Ic</i>	0.79a	0.76a	0.40a	0.39a	0.00a	0.89a	0.70a	0.68a	0.46a	0.25a
	<i>Sa</i>	3.64c	3.24c	2.71b	2.51b	1.08a	2.66bc	3.40c	3.06c	2.93bc	2.76bc
Dry weight of root (g plant ⁻¹)	<i>Cd</i>	0.64ab	0.57a	0.57a	0.40a	0.39a	0.71ab	0.81ab	0.97ab	1.21c	1.08b
	<i>Ic</i>	0.25a	0.24a	0.27a	0.28a	0.00a	0.18a	0.20a	0.25a	0.19a	0.14a
	<i>Sa</i>	0.89ab	0.97ab	1.31b	0.78a	0.59a	0.56a	0.75a	0.92ab	0.98ab	0.80ab

Means showing similar letters are statistically non-significant, *Cd*=*Cynodon dactylon*, *Ic*=*Imperata cylindrical*, *Sa*=*Sporobolus arabicus*

increased up to 100 mM NaCl (increased from 85.40 to 162.67) and above that the number got an unexpected decrease (103.70 at 150 mM and 57.95 at 200 mM NaCl).

Total leaf area per plant was adversely affected by the increasing salt level in both ecotypes of *I. cylindrica*. The ecotype from the Faisalabad region was more severely affected (Table 1), in which a decrease from 271.61 cm² at 0 mM NaCl to 47.10 cm² at 150 mM NaCl was observed. Leaf area in *C. dactylon* from Faisalabad was enhanced up to 100 mM NaCl (193.70 cm²), but higher salt levels resulted in a sharp decline (108.84 cm² at 150 mM and 59.38 cm² at 200 mM NaCl). However, leaf area in the Salt Range ecotype remained more or less stable by the increasing salt levels. Salt level up to 150 mM promoted leaf area in both ecotypes of *S. arabicus*. In particular, the ecotype from the Salt Range showed an increase over 250% in leaf area as compared to that at control.

Fresh weight of shoot was one of the most adversely affected characters by the increasing salt level (Table 1). Ecotypes of all three grasses from the Faisalabad region showed a substantial reduction in shoot fresh weight with increase in salt level, all showing about 75% decrease at the highest NaCl level (200 mM) as compared to control. Shoot fresh weight in the ecotypes from the Salt Range was promoted at 50 mM NaCl but thereafter there was a gradual decrease in shoot fresh weight with increase in salt level. Overall, the ecotypes of all three grasses from the Salt Range were less affected by the increasing level of salt. The least affected was the population of *S. arabicus* from the Salt Range in which shoot fresh weight was decreased from 10.19 (at 0 mM NaCl) to 6.50 g (at 200 mM NaCl).

Although fresh weight of root was enhanced with increase in salt level up to 150 mM NaCl in populations of *S. arabicus* (2.82 to 6.30 g) and *C. dactylon* (2.44 to 4.94 g), both from Salt Range, it was slightly reduced at the highest salt level (200 mM). *Imperata cylindrica* from the Salt Range showed a progressive increase in root fresh weight with increasing salt level (range 0.67 to 1.16 g). Fresh weights of the ecotypes of all three grasses from the Faisalabad region responded positively only at moderate salinities, but significantly decreased at higher levels (150 and 200 mM NaCl). The worst affected was *C. dactylon* from Faisalabad in which the fresh weight decreased about 60% at the highest salt level.

The ecotypes of all three grasses from Faisalabad region responded to salt stress with respect to shoot dry weight (Table 1) as they did for fresh weights. Shoot dry weights in the populations from the Salt Range of both *C. dactylon* (range 2.27 g at 200 mM NaCl to 2.84 g at 50 mM NaCl) and *S. arabicus* (range 2.66 g at 0 mM NaCl to 3.40 mM at 50 mM NaCl) were slightly promoted by 50 mM NaCl and thereafter slightly reduced by the increasing salt levels. *Imperata cylindrica* was the most adversely affected grass by salt imposition, in which a sizeable reduction in shoot dry weight was recorded (from 0.79 to 0.39 g in the Faisalabad population and from 0.89 to 0.25 g in the Salt Range population).

The pattern of reduction in root dry weights of all the grasses was very much similar to that recorded for root fresh weight (Table 1). The ecotype of *C. dactylon* from Faisalabad was the most affected due to increase in external salt levels, in which a consistent decrease in root fresh weight was observed with increase in salt level (range 0.64 to 0.39 g). Root dry weight was gradually increased in *C. dactylon* and *S. arabicus* populations from the Salt Range up to 150 mM NaCl, and it was slightly reduced at the highest level. Root dry weights in the populations of *I. cylindrica*, both from the Faisalabad region and the Salt Range were least affected by the increasing salt level, although it had significantly lower values of root dry weight than the other two grasses.

Discussion

Salt Range in Pakistan originating in the Lower Cambrian has a very unique geological history (Butler *et al.*, 1987; Burbank & Beck, 1989). Salt layers were normally in the deeper levels, but the post-Pleistocene deformations have so tilted the layers that the salt layers have come near the surface and at many places these salt layers are exposed. Soil lying between the Salt Range and River Jhelum is heavily salt affected. Water from brine springs deposits salts along its route. The run-off water during the rainy season is the other source of salt deposition that is dissolved from the exposed salt rocks (Qadir *et al.*, 2005). Uchhali complex, including Khabeki, Uchhali and Jahlar lakes, is unique, because it comprises three internationally important wetlands in the Salt Range. These three lakes are hyper-eutrophic and their chemistry is controlled mainly by an evaporation-crystallization process and dissolved salts supplied by underlying sedimentary submerged springs. Uchhali Lake is hyper-saline, while Khabeki and Jahlar lakes are brackish (Ahmad *et al.*, 2002). Plants inhabiting there should have evolved very specific morpho-anatomical and physiological adaptations against several environmental stresses, mainly salinity and drought, in view of the long span of time they have been growing there. Such adaptations have been evaluated by several researchers in different grass populations from coastal areas, estuaries, salt marshes and dry-land salinities, e.g. in *Sporobolus virginicus* (Naidoo & Mundree, 1993), salt grass, Bermuda grass and seashore paspalum (Pasternak *et al.*, 1993), *Leptochloa fusca*, *Spartina patens* and *Sporobolus virginicus* (Ashour *et al.*, 1997), *Cenchrus pennisetiformis*, *Leptochloa fusca*, *Panicum turgidum*, *Pennisetum divisum* and *Puccinellia distans* (Ashraf & Yasmin, 1997), *Aeluropus lagopoides* (Gulzar *et al.*, 2003), *Urochondra setulosa* (Gulzar *et al.*, 2003a), *Sporobolus virginicus* (Bell and O'Leary, 2003), and Kentucky bluegrass, tall fescue, alkaligrass, and saltgrass (Alshammary *et al.*, 2004). The Salt Range has never been explored to assess the extent of adaptation in different grass species inhabiting there. Due to its long geological history and highly salt affected soils the grasses inhabiting there must have developed specific salt tolerance traits. However, the appraisal of extent of adaptation of the ecotypes to soil salinity was based on a number of morpho-anatomical and physiological attributes.

Mechanism of adaptation to saline environments is very specific, which is not only for grass species but also for ecotypes. Ecotypes of all three grasses from Faisalabad showed stunted growth, perhaps to save energy for normal developmental and other metabolic processes (Mladenova, 1990; Mansour & Salama, 1996).

Of the number of growth variables affected by salt stress, shoot growth is of prime importance in appraising the salt tolerance of plants. However, it may vary with species, cultivars, and even populations. Alshammary *et al.* (2004) reported 50% reduction in shoot growth in Kentucky bluegrass, tall fescue, alkaligrass, and saltgrass at 49, 100, 200, and 349 mM, respectively. In a coastal salt marsh grass *Urochondra setulosa* salinities above 600 mM NaCl resulted in stunted growth (Gulzar *et al.*, 2003a). Salinity levels that caused shoot growth reduction were much lower for salt sensitive Kentucky bluegrass cv. Kenblue than for salt tolerant cv. Limousine (Qian *et al.*, 2001). Similar findings have been recorded in the present studies where salt tolerant ecotypes from the Salt Range had better growth and survival under saline conditions and had less reduction in shoot growth at the highest salt level as compared to those collected from non-saline habitats.

Leaf area is also one of the important growth attributes contributing to overall plant growth under normal or saline conditions. However, reduction in leaf area is the principal strategy that makes it possible to attenuate the effects of the reduction in the availability of water under saline stress (Alem *et al.*, 2002). The leaf area of tolerant genotypes, however, is less affected by salinity, as was reported by Greipsson & Davy (1996) in the coastal population than in inland population of *Leymus arenarius*. These reports are in accordance with the present findings where leaf area in the populations from the Salt Range was less affected than those from Faisalabad.

Salt stress has considerable effect on root length (Ashraf *et al.*, 2002; Gulzar *et al.*, 2003a), but this may vary with plant species or genotype. Alshammary *et al.* (2004) reported 50% root growth reduction in Kentucky bluegrass, tall fescue, alkaligrass, and saltgrass at 79, 215, 304 and 408 mM NaCl, respectively. In general, genotypes with high root number and root length under salinity stress are ranked highest in salt tolerance (Jaradat *et al.*, 2004). Increased root growth as observed in *Cynodon dactylon* and *Sporobolus arabicus*, therefore, can safely be attributed to better salt tolerance in the populations from the Salt Range. Similar findings have earlier been reported by Marcum (1999) in some salt tolerant grasses and by Qian *et al.* (2001) in Kentucky bluegrass.

In general, the ecotypes of all three grasses, *Cynodon dactylon*, *Imperata cylindrica* and *Sporobolus arabicus* from the Salt Range performed better under high salinities than their counterparts from the Faisalabad region. On the basis of various morphological characteristics and growth attributes, the ecotype of *S. arabicus* from the Salt Range has been categorized the most tolerant among all the grasses and ecotypes. It was followed by the ecotypes of *C. dactylon* and *I. cylindrica* from the Salt Range.

In conclusion, each individual grass ecotype had evolved some characteristic morphological features to thrive well under saline environments. However, the superiority of the *S. arabicus* ecotype from the Salt Range to the other grasses from the same habitat could have been due to the high impact of natural selection on the genetic variability of this ecotype for fixing the character during the entire evolutionary process.

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