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Studying the Effects of Salinity Stress on the Growth of Various Halophytic Plant Species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*)

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Abstract: Determining plant resistance to salinity is very important for selecting the favorable plant species for saline rangelands. The objective of this study was to compare the salinity tolerance of three rangeland species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*). The experiment was conducted in a greenhouse, with a factorial arrangement in a completely randomized design using 5 replications. Salinity levels of 4 (as control), 20, 30 and 40 dS/m were applied. The results showed that increasing salinity level caused reduction in shoot height, shoot dry weight, root length, root weight and survival scores in all studied plant species. The maximum length and weight of the roots and the maximum height and weight of the shoot and the survival scores were found in the lowest salinity level (4 dS/m) treatment. However, the minimum length and weight of the roots and the minimum height and weight of the shoots and the survival scores were observed in the highest salinity level (40 dS/m) treatment. Among the studied species, *Agropyron elongatum* had the maximum and *Kochia prostrata had* the minimum salt tolerance.

Key words: Shoot height · Shoot dry weight · Root length · Root weight · Survival scores

INTRODUCTION

Soils with electrical conductivity (EC) more than 2 dS/m are considered saline [1]. The plants that can be naturally established in saline soils called halophytes [2]. Salinity stress causes reduced in nutrient uptake by roots and eventually plant death [3]. Thus, soil salinity can reduce rangeland production potential [4]. Salt-affected lands that are covered with halophytic plant species and provide forage for livestock grazing are called saline rangelands [6]. Extreme utilization of the saline rangelands causes more destruction to these areas [5]. Therefore, saline rangeland management and species selection is needed for providing forage for the livestock [5, 7]. Vegetation management is the most important factor in saline lands management [5]. A significant portion of Iran's land is considered saline rangeland [8]. A vast part of the Golestan Province rangeland is affected by salinity [9]. Adaptation to the high salinity condition has been seen only in a few native plant species in Incheh Boroun rangeland and the rangeland vegetation of this area is very poor [9]. Thus, the forage production of plant species in Incheh Boroun is not sufficient for the livestock needs [9]. Identifications and introductions of the halophytic plant species that can be adapted to the salinity conditions of this region are essential for increasing vegetation production in this area.

Therefore, according to salinity and grazing resistance, drought tolerance and palatability of the species, three halophyte plant species (*Agropyron elongatum, Kochia prostrata and Puccinellia distans*) have been selected in this study for the Incheh Boroun rangeland improvement. Thus, the effects of salinity on the growth of the above three species were performed with the following 2 hypotheses.

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- There is no significant difference among the growth of the studied species in the same salinity level.
- There is no significant difference among the growth of the studied species in different salinity levels.

There are several studies found on the effects of salinity stress on the growth of halophytic plant species [12,16-18].

Salinity tolerance of three rangeland grasses in the greenhouse condition was investigated [13]. Their results showed that the dry weights of the roots and the shoots in *Agropyron elongatom* were more than that of *Agropyron desertorum* and *Hordeum fragilis.* Adaptation (establishment and survival) of some rangeland plants of Chapar Ghoymeh saline rangeland was investigated [14]. The results of this research [14] showed that *Atriplex canescens, Artemisia sieberi* and *Agropyron elongatum*had better establishments and higher survival scores than other studied plant species.

Resistant to salinity, shoot height and root length of *Agropyron desertorum* was more than that of *Agropyron elongatom* [15]. The results of the studies [18, 19] showed that *Agropyron elongatom* had high resistance to soil salinity. Soil salinity caused reduced in shoot height, shoot dry weight, shoot fresh weight, root dry weight and root fresh weight at various stages of phenology in *Agropyron elongatom* [20]. The effect of soil salinity on *Puccinellia distans* was studied [11]. The results of this research [11] showed a significant reduction in shoot dry weight and shoot fresh weight. The adverse effect of salinity on shoots was reported more than that on the roots in *Puccinellia distans* [21].

The objectives of this study were to compare and evaluate the growth responses of 3 halophytic plant species (*Agropyron elongatum, Kochia prostrata and Puccinellia distans*) under salt stress conditions.

MATERIALS AND METHODS

The seeds of *Agropyron elongatum* and *Puccinellia distans* were taken from around Aqala City. *Kochia prostrata* seeds were prepared from Isfahan Agriculture and Natural Resources Research Center.

Plants were grown in Gorgan University of Agricultural Sciences and Natural Resources greenhouse at 15-40°C under a photoperiod of 16 h. *Agropyron elongatum, Kochia prostrata and Puccinellia distans* seeds were planted in pots of 14 cm diameter and 25 cm depth; each pot contained 3.5 kg soil that prepared from Incheh Boroun area. The soil characteristics were as follows:

- Entisol in type, sandy clay loam in texture, sand 53.7%; silt 7.14%; clay 39.16%; pH 9.63 and organic matter 0.87%.

For the seed germination test in the laboratory, 100 seeds were planted in 4 Petri dishes on the moistened germination papers. Then, the 4 Petri dishes were placed in a germinator under alternating 20-30 ºC temperature. An initial seed germination count was made after 4 days and a final count after 7 days [22]. Twenty [15] seeds of each species were planted in each pot and five replicates were used for each treatment [12]. Irrigation was applied every other day to achieve soil water field capacity level.

The average soil surface salinity of Incheh Boroun is 28 dS/m [9]. Therefore, salinity levels of 20, 30 and 40 dS/m as different levels of salinity treatments and salinity level of 4 dS/m as control were applied on the pots. To better simulation of natural conditions in the pots, soil salinity was increased with the addition of salts in the bottom of the drainage channels of Incheh Boroun.

The salts were dissolved in distilled water in the laboratory as the stock saline solutions, prepared in high concentration level. Then, the desired salinity levels were made in a separate container with trial and error method. Finally, for each level of salinity the solution volume was calculated. The salinity treatments at 4 levels [4 (control), 20, 30 and 40 dS/m] were made with this method [12].

The variables and the levels of the salinity treatments based on completely randomized design for the pot culture are shown in Table 1.

At the end of the phonology stage of the halophytic plant species, the average of shoot height in each pot was measured in cm. The pot soil was rinsed with distilled water, then, the plant roots and shoots were divided into two parts. The dry weight was measured after oven drying the plants at 60 °C for 48 hours. The dry weights of the roots and shoots were also determined in g, separately.

The survival scores were recorded weekly by scoring in the scales of 0 to 4 (0 score for the dried plant and 4 for very vigor plant) during the greenhouse experiment [13].

Shoot height (cm), shoot weight (g), root length (cm), root weight (g) and survival scores data were standardized to compare the effect of salinity on *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans.*

Table 1: Variables and the levels of treatments based on factorial arrangement in a completely randomized design

Variables	Variable Numbers	Variable Names
Species	3	*Agropyron elongatum* *Kochia prostrata* *Puccinellia distans*
Salinity (dS/m)	4	EC = 4 (Control) EC= 20 EC= 30 EC= 40
Replications	5	-----

Equation 1 was used to standardize the data of this research.

$$S_t = \frac{N}{N\max} \quad (1)$$

S_t: Standardized data of studied treatments
N: Data in each replicate
N max: Maximum data of all replicates [23].

After the data standardization, ANOVA was performed for statistical analysis of the data. Statistical significance was considered at $P < 0.05$.

RESULTS

Shoot height, shoot dry weight, root length and root weight at all stages of development were reduced progressively with increasing salinity concentrations. The relative percentage of the shoot height, shoot dry weight, root length and root weight of the salinized plants compared to those of the controls were computed as (salinized plants/control plants) x100 and illustrated in Figure 1.

The interactions of different salinity treatments and the species types on the shoot height, shoot dry weight, root length and root weight were significant ($p < 0.05$) (Table 2).

Table 2: Results of the analysis of variance (ANOVA) of the salinity, species and the interactions of the salinity with species on shoot height, shoot dry weight, root length and root weight

	Salinity, species and interactions of salinity with species on shoot height				Salinity, species and interactions of salinity with species on shoot weight		
Sources of Variations	P	F	MS	df	P	F	MS
Species	*<.0001	15.02	0.28	2	*<.0002	10.21	0.49
Salinity Level	*<.0001	14.36	1.86	3	*<.0001	16.33	0.79
Salinity and species interactions	ns 0.07	2.03	0.03	6	ns 0.69	0.65	0.03
	Salinity, species and, interactions of salinity with species on root length				Salinity, species and interactions of salinity with species on root weight		
Sources of Variations	P	F	MS	df	P	F	MS
Species	*<.0002	25.25	0.37	2	*<.0001	11.08	0.81
Salinity Level	*<.0001	11.67	1.7	3	*<.0001	15.27	1.27
Salinity and species interactions	ns 0.06	4.88	0.07	6	ns 0.376	1.17	0.07

* significant at 0.05 MS: Mean of Squares F: Fisher Statistics
ns: not significant at 0.05 df: degree of freedome P: significant Level

Table 3: Means comparison of different levels of salinity treatments on shoot height, shoot dry weight, root length and root weight

Salinity Level	Salinity effect on root weight ---------- Mean ----------	Salinity effect on root length ---------- Mean ----------	Salinity effect on shoot weight ---------- Mean ----------	Salinity effect on shoot height ---------- Mean ----------
4	0.70 a	0.52 a	0.59 a	0.86 a
20	0.45 b	0.36 b	0.37 b	0.48 b
30	0.16 c	0.14 c	0.19 c	0.19 c
40	0.06 c	0.07 c	0.06 c	0.07 d

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level.

Table 4: Means comparisons of the species types effects on shoot height, shoot dry weight, root length and root weight

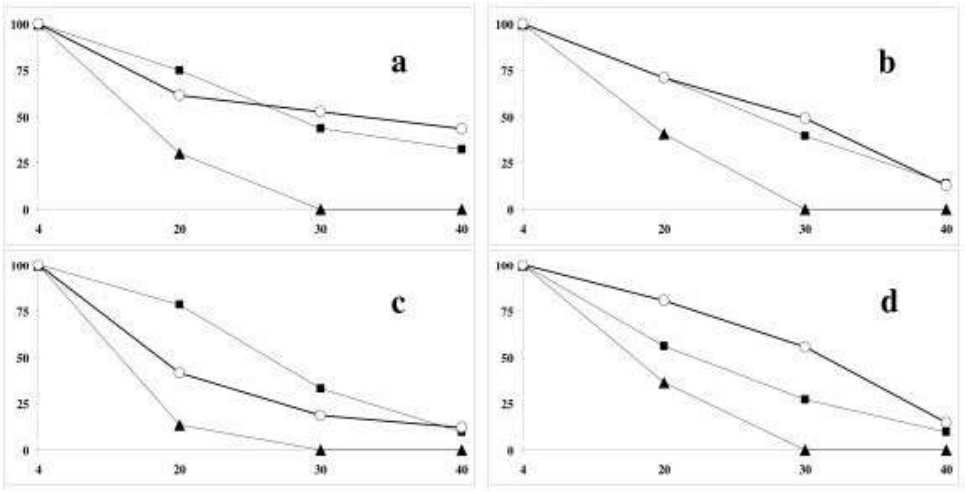
Species type	Species type effect on shoot height ---------- Mean ----------	Species type effect on shoot weight ---------- Mean ----------	Species type effect on root height ---------- Mean ----------	Species type effect on root weight ---------- Mean ----------
Agropyron elongatum	0.49 a	0.45 a	0.32 a	0.54 a
Puccinellia distans	0.45 a	0.33 a	0.41 a	0.37 a
Kochia prostrata	0.26 b	0.13 b	0.13 b	0.12 b

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level.

Table 5: Effects of salinity, species and the interactions of species with salinity on survival scores in plants phonology stages

Salinity and species interaction effects on survival scores				
Sources of Variations	df	MS	F	P
Salinity Level	3	1.96	120.30	*<.0001
Species	2	1.06	76.07	*<.0001
Salinity and species interactions	6	0.56	6.74	ns 0.063
Salinity effect on survival scores				
Salinity Level				Mean
4				0.83 a
20				0.53 b
30				0.21 c
40				0.08 c
Species type on survival scores				
Species				Mean
Agropyron elongatum				0.56 a
Puccinellia distans				0.53 a
Kochia prostrata				0.15 b
F: Fisher Statistics				MS: Mean of Squares
df: degree of freedome				P: significant Level
* significant at 0.05				ns: not significant at 0.05

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level.



a: shoot height, b: shoot weight, c: root length and d: root weight.
■ *Agropyron elongatum,* ▲ *Kochia prostrata and* ○: *Puccinellia distans*

Fig. 1: Relative percentage of the shoot height, shoot dry weight, root length and root weight of salinized plants compared to those of the controls.

Increased salt concentration in the soil significantly decreased ($p < 0.05$) dry weight of the shoot and the shoot height (Tables 3 and 4).

Dry weight significantly decreased ($p < 0.05$) for root and the root length in response to increasing salt concentrations (Tables 3 and 4).

Effects of salinity, species and the interactions of species with salinity on *Agropyron elongatum, Kochia prostrata and Puccinellia distans* survival scores in plants phonology stages are shown in Table 5.

DISCUSSION

The results showed that *Agropyron elongatum* and *Puccinellia distans* could survive in 40 dS/m salinity level, but *Kochia prostrata* could survive only at 30 dS/m salinity level (Figure 1). The average soil surface salinity of Incheh Boroun is 28 dS/m [9]. Therefore, *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* can survive in a saline soil similar to that of the area in Incheh Boroun saline rangeland. However, for introducing

any plant species to this area and its adaptation in this region, the ecological, edaphical and climatical conditions of Incheh Boroun must be taken into consideration.

Among the studied species *Agropyron elongatum* exhibited the maximum salt tolerance and *Kochia prostrata* showed the minimum salt tolerance. Salt tolerance of *Puccinellia distans* was higher than that of *Kochia prostrata,* but lower than that of *Agropyron elongatum. Kochia prostrata* could not survive at 40 dS/m salinity level (Figure 1). Although *Kochia prostrata* is a halophytic plant species [21], high salinity levels of the study induced deleterious effects on the growth of this species. There are a few studies found on the tolerance of rangeland plants to salinity [16, 17 and 24]. Rangeland halophytic plant species can tolerate different degrees of salinity stresses. Therefore, the rangeland halophytic plant species must be classified into various salinity tolerance levels.

An important finding was that *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* could survive at 30 dS/m and *Agropyron elongatum* and *Puccinellia distans* could survive at 40 dS/m. Other researchers have not observed these plants growing at these ranges of salinity [11, 13-21].

In the present study, soil salinity increased with the addition of salts at the bottom of the drainage channels of Incheh Boroun that was probably due to the better simulation of the natural conditions. Other researchers have not used this method and only have studied the effects of mineral salts on plant growth [11-21].

In our study, *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* exhibited different degrees of salt t olerance. *Puccinellia distans* had the least decrease in root weight (80, 61 and 14% at 20, 30 and 40 dS/m, respectively, compared to the controlled plants). Other researchers have not observed these plants growing at these ranges of salinity stresses [11, 21].

The maximum Length and weight of the roots and the shoots were seen in the lowest salinity level (4 dS/m). However, the minimum Length and weight of the roots and the shoots were seen in the highest salinity level (40 dS/m) (Tables 3 and 4). In other words, under salinity stress conditions, nutrient and water absorption by roots and shoot growth are reduced [25]. Thus, there are significant differences between the growth of different species in the same salinity level. Therefore, the first hypothesis of this study is not accepted.

The highest survival scores [4, 10] have been seen in *Agropyron elongatum*. However, the lowest survival scores (1 and 0) have been seen in *Kochia prostrata* (Table 5). This is in agreement with the results of several other investigators [11-21].

The survival scores of *Puccinellia distans* were higher than that of *Kochia prostrata* and lower than that of *Agropyron elongatum* (Table 5). This can be due to the ecological nature of *Puccinellia distans*. *Puccinellia distans* is a hydrohalophytic plant species [26]. However, the results of the present study and those of the above investigators are in contrast to the results reported [11, 20].

The 28 dS/m average soil salinity of the Incheh Boroun rangeland compared with the salinity threshold of 2 dS/m reported by [1] indicates that this salinity threshold is not applicable to this area. This threshold salinity level can be used only to determine soil salinity in agricultural lands. Therefore, it is necessary to establish a new local soil salinity classification and threshold on the saline rangelands.

The results of our study showed that the growth of *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* have been curtailed in this saline soil rangeland. Thus, these species are Facultative halophytic plant species [12].

CONCLUSIONS

Overall, considering the results of this study, it could be concluded that among the studied plant species, *Agropyron elongatum* exhibited the maximum salt tolerance and *Kochia prostrata* showed the minimum salt tolerance. Salt tolerance of *Puccinellia distans* was between that of the above two species, higher than that of *Kochia prostrata* and lower than that of *Agropyron elongatum*.

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