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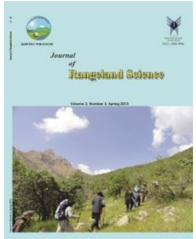

Full Length Article:

Effects of Salinity on Seedling Growth and Physiological Traits of Vetiver Grass (*Vetiveria zizanioides* Stapf)

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Abstract. Vetiver grass (*Vetiveria zizanioides*) is known to be survived under the diverse soil and water conditions. In order to study the effects of salinity stress on the seedling growth of the desired species, a completely randomized design was conducted with 3 replications. Seeds were sown in the salty-soil plots in the greenhouse. Salinity levels were specified as 4 control ones including 20, 30 and 40 dS/m. Results showed that the seedling growth and yield were progressively declined by the increase of NaCl concentration levels. The concentrations of both chlorophylls a and b were dramatically increased by increasing NaCl and the highest and lowest values have been obtained for the levels of 40 and 4 dS/m, respectively. There were no significant differences for the leaf area and water content with the salinity of 20 dS/m as compared to the control treatment. The concentrations of chlorophylls a and b were 0.73 and 0.41(mg/g FW) and 0.35 and 0.11 (mg/g FW) in the 40 and 4 dS/m salinity treatments, respectively. Leaf water content for 4 and 20 dS/m salinity levels has been calculated as 11.3 and 9.8 percent. But there was a significant reduction in the leaf water content with 30 and 40 dS/m salinity levels. Leaf water content with 30 and 40 dS/m salinity level was 6.3 and 5.2 %. Leaf area in 4, 20, 30 and 40 dS/m salinity levels has been computed as 172, 168.7, 81.7 and 65.2 cm^2, respectively. Compared to the control treatments, there was a significant reduction in leaf area with 30 and 40 dS/m salinity levels. Our results suggest that in an EC between 4 to 20 dS/m, *Vetiveria zizanioides* could grow.

Key words: Halophytes, Rangelands, Saline, Salinity stress

1. Introduction

Vetiver grass was first developed by the World Bank for the soil and water conservation in India in 1980s. In addition to the important application of it in the agricultural lands, scientific studies conducted on Vetiver grass have had much wider applications. This is due to its unique morphological, physiological and ecological characteristics that enable it to adapt with a wide range of climatic and soil conditions (Truong *et al*., 2002) including salt affected lands (Truong, 1994). This plant has been used for the soil rehabilitation in Golestan, Kermanshah, Fars, Tehran, Boosher, Systan and Baluchestan and Khozestan provinces (Fadaei and Aleebrahim, 2012). Vetiver grass hedges can be harvested without sacrificing the erosion control. This will provide the farmers with the extra income. This plant produces massive odorous root system which can be used for the extraction of essential oil which is of great economic importance (Islam *et al*., 2008).

Its roots go down up to 70cm while penetrating into the saline soil and reaching subsurface moisture which the other plants cannot reach and the dissolved salt content has been greatly reduced (Du and Truong, 2000). Vetiver grass has short rhizomes and massive finely structured root systems that grow very quickly. It has been reported that it may grow up to the depth of 1 m during the first year of growth (Truong *et al.*, 2002). Vetiver grass is well known as being a salt tolerant plant (Vimala and Kataria, 2005). Salinity reduces the ability of plants to take up water, and this causes the reductions in the growth rate along with a suite of metabolic changes similar to those caused by water stress. Hence, the ability of a plant to grow under these environmental stress conditions is a key factor to improve rangeland vegetation in the saline and arid rangelands (Sharp and Davies, 1979; Sharp and Davies, 1985; Netting, 2000; Bruce *et al*., 2002). So, salinity alters the plant growth rate and nutrient uptake (Sharp and Davies, 1979; Sharp and Davies, 1985; Netting, 2000; Bruce *et al*., 2002). The objectives of this study are to evaluate the salinity tolerance ability of *Vetiveria zizanioides*.

Soils with the Electrical Conductivity (EC) more than 2 dS/m are considered as saline ones (Alizadeh, 1999.) The plants that can be naturally established in the saline soils have been called halophytes (Tabaee Aghdaee, 1999). Salinity stress leads to the reduction of nutrient uptake by roots and eventually, results in the plant death (Jafari, 1994). Thus, soil salinity stress can reduce the rangeland production potential (Kafi and Mahdavi, 2002).

Salinity has considerable adverse impacts on the plant productivity (Lauchli and Epstein, 1990). It adversely affects the plant growth and development. An excess of soluble salts in the soil leads to the osmotic stress, specific ion toxicity and ionic imbalances (Munns, 2003) and the consequences of these can be mentioned as the plant death or loss of plant production (Rout and Shaw, 2001). Ashraf *et al*. (2004) have found that increasing the salt concentrations caused a significant reduction in the fresh and dry masses of plants. Vetiver grass provides the vegetation cover in the situations where the vegetation is unlikely to be established due to the extremes of salinity and aridity soils (Loch, 2006). The saline threshold of Vetiver grass is EC= 8 dS/m and soil EC values of 10 and 20 dS/m would reduce its yield by 10 and 50%, respectively (Truong *et al*., 2002). Vetiver grass can grow in the salinity ranged as 1-10 dS/m (Patcharee and MongKon, 2001; Cook, 1993; Chaweevan *et al*., 1996; Nanakorn *et al*., 1996). So, *Vetiver* grass establishment and growth were extremely poor under the extremely saline conditions (Truong *et al*., 2002).

2. Materials and Methods

The accessions of *Vetiveria zizanioides* were provided from the natural resource gene bank in Research Institute of Forests and Rangelands, Tehran, Iran. Seeds were sown in the greenhouse of Malayer university at 15-40°C for a photoperiod of 16 h. Three seeds were sown in the plots with the diameter and depth of 14 and 25 cm, respectively; each plot has contained 3.5 kg of soil. The soil characteristics included the type of entisol, the texture of sandy clay loam, sand computed as 54%, silt as 7%, clay as 39%, pH=9.63 and organic matter as 1%. Four salinity levels of 4 dS/m as the control treatment and 20, 30 and 40 dS/m were applied on the plots. Sodium chloride (NaCl) was used to make the salinity treatments. For salty soil preparation, 0.64 gr of NaCl per one kg of soil was added for the increased one grade salinity level (dS/m). Plots used in this study were 3.5 Kg so that the weight of salt needed at each salinity level was calculated (Kachout *et al.,* 2009). The salinity treatments were made with regard to this method.

Salinity levels were 4, 20, 30 and 40 dS/m (Kachout *et al*., 2009). Sodium chloride (NaCl) was used to prepare the salinity treatments. 0.64 g of NaCl per one kg of soil has increased one grade of salinity level (in term of dS/m). Plots used in this study were 3.5 Kg. So, 8.96, 44.8, 67.2 and 89.6 gr of NaCl have been added to each plot in 4, 20, 30 and 40 dS/m salinity treatments, respectively. The shoot and root system lengths (cm) and leaf area (cm^2) were measured after 3 months. Leaf area (cm^2) has been measured by the portable leaf area meter. Then, the plants were washed with the distilled water and separated into the shoots and roots. The shoot and root dry weight (dw) was determined after being dried in the oven at 60°C for 48 h. The effects of salinity have been also studied with respect to the physiological parameters as chlorophyll a and b using spectrophotometer (Metzner *et al*., 1965). In this method, after doing the acetone extraction of the pigments from fresh leaves, chlorophyll concentration was calculated. A leaf sample of 0.1 g was ground and extracted with 5 ml of 80% (v/v) acetone in a dark room. The slurry was filtered and absorbencies were determined at 645 and 663 nm (Metzner *et al*., 1965). Chlorophyll a and b and total chlorophyll have been measured by the use of below equations (Porra *et al*., 1989):

Chl a = 12.25 A 663.6 - 2.55 A 646.6

Chl b = 20.31 /1646.6 -4.91 A 663.6

Chl a + b = 17.76 A 646.6 + 7.34/1663.6

The leaf water content (TWC) was calculated through the following equation:

TWC % = (FW-DW)/FW (Jungai *et al*., 2011). The plots were arranged according to the completely randomized design using three replications. ANOVA was employed for the statistical analysis of data. Mean comparison was performed on the basis of Duncan ($P<0.05$) method.

3. Results

3.1. Effects of salinity on leaf growth

Salt stress has significantly affected the leaf area of vetiver. The highest leaf area values have been achieved in the control treatment. Compared to the 4 dS/m treatment, leaf area was reduced by 28.32, 38.22 and 47.26 percent in the 20, 30, and 40 dS/m salt treatments, respectively. There was no significant effect on the leaf area in 20 dS/m salinity level as compared to that of control treatment. But there was a significant reduction in leaf area regarding 30 and 40 dS/m salinity levels.

3.2. Effects of salinity on dry weight and growth

Shoot height, shoot dry weight, root length and root weight were progressively reduced as the salinity concentrations increased. The relative percentages of shoot height, shoot dry weight, root length and root weight of the salinized plants in comparison with those of the control treatment were computed as (salinized plants/control plants) x100 illustrated in (Fig. 1).

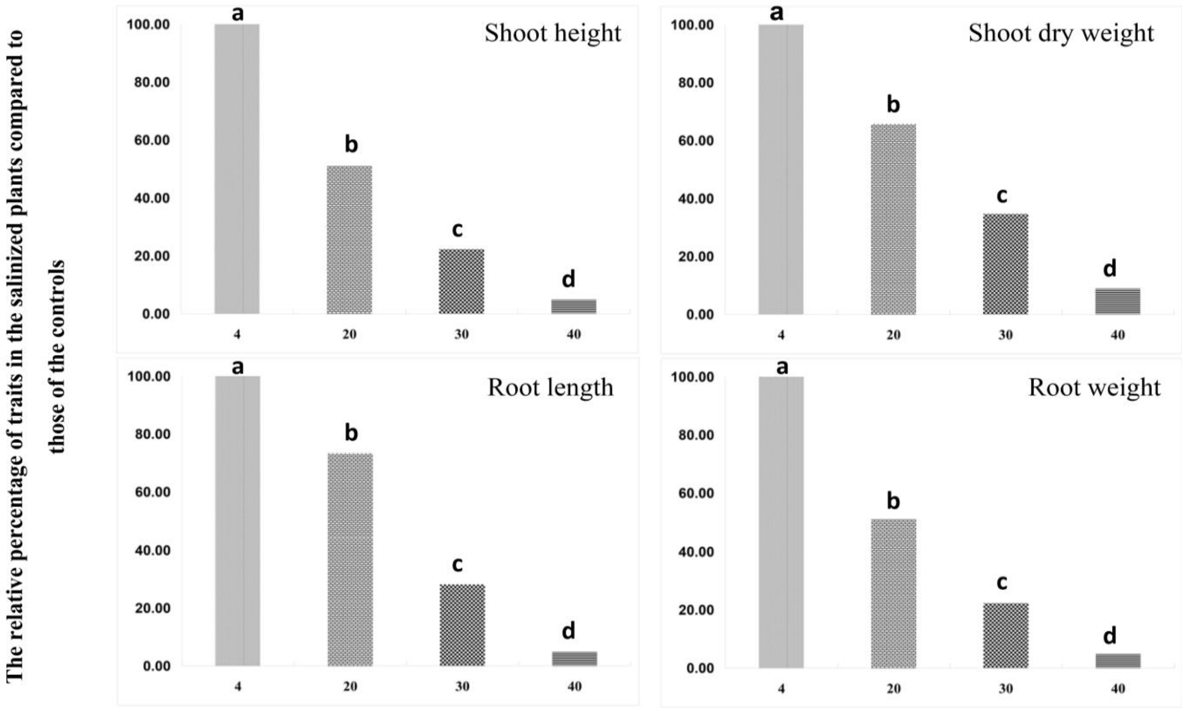

Fig. 1. Relative percentage of the A-shoot height, B-shoot dry weight, C-root length and D- root weight of salinized plants as compared to those of the control treatment.
Different letters represent a significant difference (P<0.05) between treatments.

Table 1. Effects of salinity on root and shoot dry weights and growth of *Vetiveria zizanioides*

Salt Treatments	Shoot Length	Shoot Dry Weight	Root Length	Root Weight	Leaf Area	Chlorophyll a	Chlorophyll b	Relative Water Content
Control 4 dS/m	78.5 a	180.8 a	93.7 a	56.2 a	172 a	0.35 c	0.11 c	11.3 a
20 dS/m	39.7 b	118.3 b	68.1 b	35.3 b	168.7 a	0.48 b	0.16 c	10.8 a
30 dS/m	17.3 c	62.4 c	26.1 c	9.1 c	81.7 b	0.62 a	0.23 b	6.3 b
40 dS/m	3.7 d	16.3 d	4.4 d	1.5 d	65.2 c	0.73 a	0.41 a	5.2 c

Columns with the same letter have no differences based on P<0.05 Duncan method

3.3. Effects of salinity on chlorophyll concentrations

Changes in leaf chlorophyll concentrations under different salinity stresses are shown in (Fig. 2). The concentrations of chlorophylls a and b have been calculated as the average values of 0.73 and 0.41(mg/g FW) in the 40 dS/m salinity treatment and 0.35 and 0.11 (mg/g FW) in 4 dS/m salinity treatment, respectively.

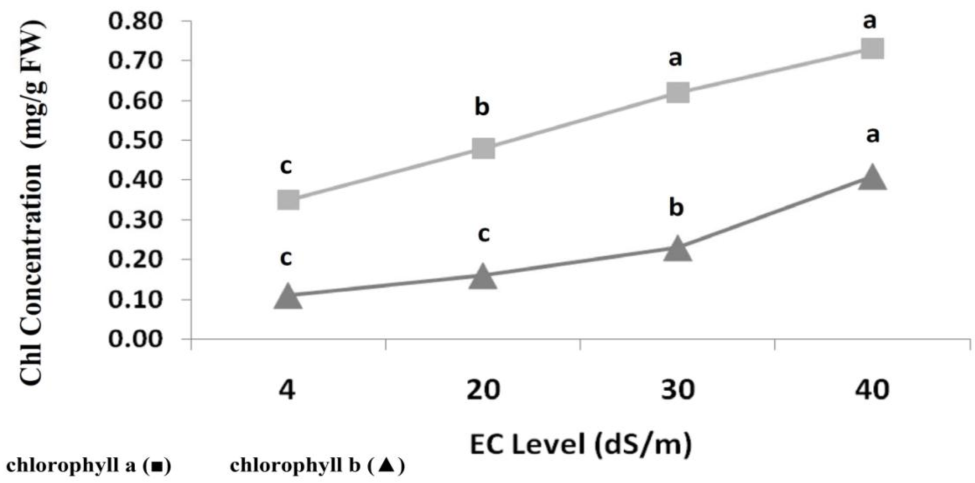

Fig. 2. Effects of various salinity levels on the concentrations of a and b.
Different letters represent a significant difference based on P<0.05 Duncan method.

3.4. Effects of salinity on water content

Leaf area and leaf water content with 4 and 20 dS/m salinity levels were 11.3 and 9.8 percent. There was no significant effect on leaf area and leaf water content with 20 dS/m salinity level as compared to that of control treatment (4 dS/m). But there was a significant reduction in leaf area and leaf water content with 30 and 40 dS/m salinity levels. Leaf area and leaf water content with 30 and 40 dS/m salinity levels were specified as 6.3 and 5.2 percent.

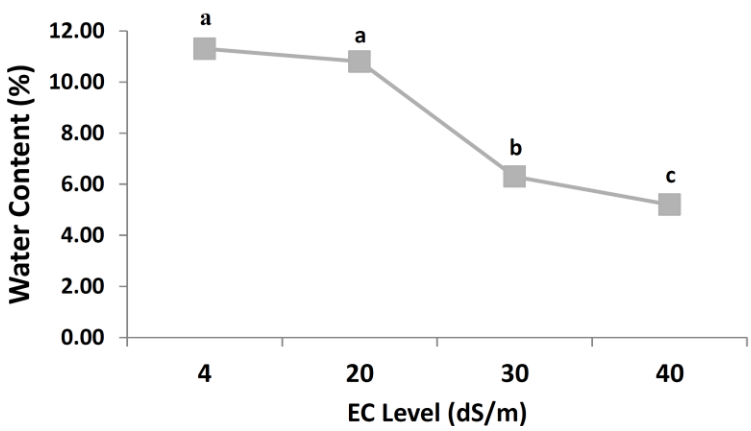

Fig. 3. Effects of salinity on total leaf area in vetiver.
Different letters represent a significant difference (P<0.05) between treatments.

4. Discussion

There are few studies found on the salinity tolerance of rangeland plants (Akhzari *et al*., 2012; Sepehry *et al*., 2012; Kafi and Mahdavi, 2002; Masoudi *et al*., 1997; Ashraf *et al*., 1986). 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.

A primary response in the salt stressed plants is a decrease in plant water potential resulting in the decreased water use efficiency and leading to the overall toxic damages and yield reduction (Chaum and Kirdmanee, 2009). Salt stress affects the water status (Amirjani, 2010). Water relationships and the ability to adjust osmotically are important determinants of the growth response (Munns, 2003). Salinity stress caused the decreased leaf water potential and a reduction in the relative leaf water content which has resulted in loss of turgor which in turn causes the stomatal closure, limits the dioxide carbon assimilation and reduces photosynthetic rate. The decrease in total glycineb etaine in high levels of NaCl may be related to the reduced tissue water content (Khan *et al*., 2000). This research results showed that vetiver is tolerant to high soil salinity. These results are consistent with the results of previous studies done by Loch (2006), Vimala and Kataria (2005), Patcharee and MongKon (2001) Chaweevan *et al*. (1996) Nanakorn *et al*. (1996) and Cook (1993). This is probably due to high root system widespread of this plant species. This result is in agreement with the results presented by Truong *et al*. (2002) and Du and Truong (2000). These results indicate that vetiver grass can be compared favorably with some of the most salt tolerant crop and pasture species grown in the world (Truong *et al*., 2000).

In our study, vetiver has exhibited different degrees of salt tolerance. Other researchers have not observed these plants growing in these ranges of salinity stresses (Loch, 2006; Vimala and Kataria, 2005; Truong *et al*., 2002; Patcharee and MongKon, 2001; Du and Truong, 2000; Chaweevan *et al*., 1996; Nanakorn *et al*., 1996; Cook 1993). These studies were done in Thailand, India and Europe. Soil salinity severity in some parts of Iran is very higher than these regions. Investigations in the field of natural resources have been done due to natural conditions in different countries. So, there is no need to test vetiver resistance to high salinity levels in Thailand, India and Europe.

The maximum length and weight of the roots and shoots were seen in the lowest salinity level (4 dS/m). However, the minimum length and weight of the roots and shoots were observed in the highest salinity level (40 dS/m). In other words, under salinity stress conditions, nutrient and water absorption by the roots and shoot growth are more likely to be reduced (Ashraf *et al*., 2004; Munns, 2003; Rout and Shaw, 2001; Lauchli and Epstein, 1990).

The concentrations of chlorophylls a and b were the highest and lowest ones in the 40 dS/m and 4 dS/m salinity levels, respectively. So, salinity stress causes the increase in both chlorophylls a and b concentrations. These results are consistent with the previous studies of Jungai *et al*. (2011). Plant exposed to saline environment generally has the leaf area reduced. So, salinity concentration caused the considerable reduction of leaf area. This result is in agreement with the results reported by Beinsan *et al*. (2009) and Alamgir and Ali (2006). In the present study, salinity has reduced the leaf area and chlorophyll content as the salinity level has been increased with a significant effect. Salt stress reduced the leaf growth rate by shortening the length of the leaf elongating zone and decreasing the growth intensity in its central and distal portions (Bernstein and Lauchli, 1993). Leaf growth inhibition by the salinity must be expected to occur via an effect on this region (Lazof and Bernstein, 1998). NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase (Rao and Rao, 1981) may induce the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey, 1995).

5. Conclusion

Our results suggest that in an EC between 4 to 20 dS/m, *Vetiveria zizanioides* could grow.

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اثر شوری بر رشد نهال و صفات فیزیولوژیک و تیور گراس (***Vetiveria zizanioides*** **Stapf**)

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چکیده

Vetiveria zizanioides گونه‌ای است که در شرایط مختلف آب و خاک توان رشد دارد. به منظور مطالعه اثر تنش شوری بر رشد این گونه گیاهی طرح آزمایشی کاملا تصادفی در ۳ تکرار طراحی شد. بذور در گلخانه و در گلدان کشت شدند. سطوح شوری ۴ (شاهد)، ۲۰، ۳۰ و ۴۰ دسی زیمنس بر متر بودند. نتایج نشان داد که با افزایش شوری رشد نهال و تولید کاهش می‌یابد. با افزایش شوری غلظت کلرفیل‌های a و b بیشتر شد. به طوری که بیشترین غلظت کلرفیل‌های a و b در سطح شوری ۴۰ و کمترین مقدار آن در سطح شوری ۴ دسی‌زیمنس بر متر مشاهده شد. نتایج نشان داد که در سطح و شوری ۲۰ دسی‌زیمنس بر متر در مقایسه با شاهد تفاوت معنی‌داری وجود ندارد. غلظت کلروفیل‌های a و b در سطح شوری ۴۰ دسی‌زیمنس بر متر به ترتیب ۰/۷۳ و ۰/۴۱ mg/g FW بود. همچنین غلظت کلروفیل‌های a و b در سطح شوری ۴ دسی‌زیمنس بر متر به ترتیب ۰/۳۵ و ۰/۱۱ بود. محتوای آب برگ در سطح شوری ۴ و ۲۰ دسی‌زیمنس بر متر به ترتیب ۱۱/۳ و ۹/۸ درصد بود. ولی در سطوح شوری ۳۰ و ۴۰ دسی‌زیمنس بر متر کاهش معنی‌داری در محتوای آب برگ مشاهده شد. مقادیر محتوای آب برگ در سطوح شوری ۳۰ و ۴۰ دسی‌زیمنس بر متر به ترتیب ۶/۳ و ۵/۲ درصد بود. سطح برگ در سطوح شوری ۴، ۲۰، ۳۰ و ۴۰ دسی زیمنس بر متر به ترتیب ۱۷۲، ۱۶۸/۷، ۸۱/۷ و ۶۵/۲ سانتی‌متر مربع بود. در مقایسه با سطح شوری شاهد، در سطوح شوری ۳۰ و ۴۰ دسی‌زیمنس بر متر کاهش معنی‌داری در سطح برگ رخ داد. نتایج مطالعه نشان داد که گونه ***Vetiveria zizanioides*** در دامنه شوری ۴ تا ۲۰ دسی زیمنس بر متر می‌تواند رشد کند.

کلمات کلیدی: هالوفیت‌ها، مراتع، تنش‌شوری