



## Salinity affects leaf physiologic and biochemical traits of Vetiver grass (*Chrysopogon zizanioides* L.)

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Article Info	Abstract
<b>Article type:</b> Research Article	Salinity is the second most important abiotic stress in the world, especially in Iran. This research focused on <i>in vitro</i> physiological and biochemical responses (proline content, carbohydrates, total protein and peroxides enzymes) of <i>Chrysopogon zizanioides</i> to soil salinity. Prior to salinity application, the plant seedlings were placed in greenhouse for adaptation. To apply soil salinity stress on <i>Chrysopogon zizanioides</i> , sodium chloride solution was used in 6 different concentrations over a period of 4 days. All measurements, including leaf proline content, soluble and non-soluble sugar, protein content, and peroxidase enzyme, were conducted after 2 months of salinity treatment. The results indicated that salinity stress could have a significant increasing effects on proline content, soluble and non-soluble sugar of leaves ( $p \leq 0.05$ ). Leaf total protein content and peroxidase enzyme were also significantly affected by salinity stress, showing an increasing trend up to 32 ds/m followed by a decreasing trend at 44 ds/m. According to the physiologic and biochemical parameters, the results confirm the high tolerance of Vetiver grass against 32 ds/m salinity in short term.
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### Introduction

Historically, rangeland ecosystems have faced environmental stresses such as drought and salinity in arid and semi-arid regions (Mane et al., 2013) and adapted to thriving under such stresses. Salinity has negative impacts on plants' growth and production in natural ecosystems (Acir and Gunal, 2020). Rangelands response to soil salinity depends on both plant species and salts concentration (Dell'Aversana et al., 2021). Sodium concentration increases in soil solution and consequently decreases the

porosity and permeability, destroys the aggregates, scattering the clay particles; and finally leading to the crust and swelling in soil (Mane et al., 2013). In addition, soluble salt in soil solution could cause the osmotic stress and ion imbalance, constraining the plant growth and productivity (Munns, 2003; Dell'Aversana et al., 2021). Some plant species tolerate salt-affected soils via different mechanisms such as amino acids production like proline through osmotic adjustment during the salinity stress (Vendruscolo et al., 2007; Dell'Aversana et

al., 2021). Therefore, proline concentration could be a criterion for the plant adaptation to salinity and play a protective role (Kuznetsov et al., 1999; Ashraf et al., 2005).

The literature supports the salinity problem in dry regions such as Iran (Qadir et al., 2008; Habibi et al., 2020). Almost 12.5% of Iran consists of rangeland ecosystems seriously affected by salinity. Thus, reclamation and rehabilitation of saline rangelands are a management challenge in Iran. Successful rangeland reclamation also depends on choosing suitable plant species. For example, the halophyte plants with the high capability of salt tolerance ( $\sim 20 \text{ dS m}^{-1}$ ) are a good recommendation for rangeland reclamation but they are rare (Stuart et al., 2012). By the late 1980s, a permanent grass species named Vetiver grass (*Chrysopogon zizanioides*) was introduced for the reclamation and rehabilitation of saline soils by the World Bank (Truong, 2004; Moosikapala and Te-chato 2010; Chitra et al., 2014). Vetiver grass prefers the well-drained soils with rainfall and temperature ranges of 1000-2000 mm and 21 to 44.5 °C, respectively (Maffei, 2002). While vetiver grass is not nutritional forage (Ahmadi Beni et al., 2014), it recommended as a soil conservation plant tolerating varieties of climate and extreme soil conditions (Arun et al., 2014) such as drought, flood, and salinity (Truong, 2004; Moosikapala and Te-chato 2010) and highly Pb contaminated soils as suggested by Prasad et al. (2014). As the salinity tolerance is a complex response due to biotic and abiotic conditions, vetiver salinity resistance have been reported differently in studies suggesting mortality in the  $> 6 \text{ ds/m}$  (Edelstein et al., 2009),  $> 10 \text{ ds/m}$  (Deifel et al., 2006; Liu et al., 2016), and  $0.005 \text{ ds/m}$  (Mane et al. 2011), and no salinity tolerance (Klomjek and Nitorisavut, 2005). While a wild ecotype of Vetiver was found in China in the 1950s, the information about its adaptability to saline stress is scarce (Liu et al., 2016), and even in some areas, the plant may stimulate soil salinity traces (Ahmadi Beni et al., 2016).

Since the information on salinity threshold is necessary to rehabilitate the salt-affected rangeland soils, local studies are necessary to evaluate the success of the local and regional reclamation projects. The main objective of the present study is to address the extreme *in vitro* salinity conditions effects on Vetiver grass (*Chrysopogon zizanioides* L.) in terms of physiological and biochemical traits of Vetiver leaves (total protein, peroxidase enzymes, proline content, soluble and non-soluble sugar).

### Materials and Methods

The experiment was conducted in the research greenhouse of Malayer University. A completely randomized block design with three replicates at 6 different levels of salinity treatments (T), EC of 0 (control, T0), 4 (T1), 8 (T2), 16 (T3), 32 (T4) and 44 (T5) ds/m (2.56, 5.12, 10.24, 20.48 and 28.16 g/L NaCl) (Kachout et al. 2009) was set up in 5L pots (a 1:1:3 ratio of soil, sand, manure) during 2013-2015. To simulate the salinity stress, different concentrations of sodium chloride (NaCl) solution were gradually added to the pots, starting with 250 cc solution every four days. Following the salinity treatment application, some plant physio-biochemical leaf parameters were measured. Leaf proline content was measured using the method presented by Bates et al. (1973). After preparing the samples and calibrating the spectrophotometer (JENUS UV-1200), the intensity of leaf proline variations was read at the 520 nm wavelength. Leaf soluble sugar, non-soluble sugar, and protein content were measured following the methods presented in Dubois et al., 1956, Nelson 1994, Bradford 1976, respectively. To measure the leaf antioxidant enzymes, the extraction buffer was first prepared and then the leaf extraction was used to measure the peroxidase enzyme as suggested in Abeles and Biles 2005.

The data were subjected to the analysis of variance (ANOVA) and multiple-range Duncan's means comparison to detect the significant effects ( $p \leq 0.05$ ). It should be noted that prior to analyses, normality and homogeneity of variances were investigated

using Shapiro-Wilks and Levene methods, respectively. All the statistical analyses were conducted using SAS v.9.1 (SAS Institute, 1990).

## Results and Discussion

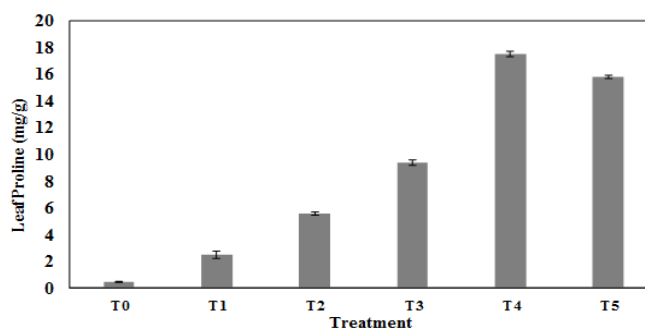
### Salinity Treatments Effects on Leaf

#### Proline Content

Generally, salinity levels affected the proline content of Vetiver's leaf (*Chrysopogon zizanioides* L.) ( $p \leq 0.05$ ) (Table 1). According to the results, minimum significant proline content (0.4 mg/g FW) was observed at control level (Table 1). Salinity led to the gradual increase of proline up to 32 ds/m content with the maximum proline content (17.5 mg/g FW) at 32 ds/m. The effect of salinity on proline content showed a linear increase up to 32 ds/m (Figure 1). Overall, the increase of salinity stress up to 44 ds/m resulted in 97.5% increase of proline concentration compared to 0 ds/m in control level (Figure 1).

The overproduction of proline content under salinity stress had earlier been suggested in Vetiver grass at the 300 mm

salinity level (Mane et al., 2011). Similar trends in different species have been recorded, e.g., salt-tolerant wheat (Kafi et al., 2003), *Brassica zarfam* L. (Farhoudi et al., 2015), *Tagetes minuta* (Moghaddam et al., 2020), and Barley Genotypes (Dell'Aversana et al., 2021). The increase of proline synthesis along with low dissimilation could be attributed to its aggregation when water potential is low. However, the impact of proline production on plant resistance to salinity stress is still controversial and it may act regardless of salinity intensity (Pellegrini et al., 2020). Despite the mentioned studies, the decreasing trend of leaf proline was reported in some other plant species; including, wheat, barley, and sorghum (Zaki and Radwan, 2011). Proline content may decompose right after confronting the stress in order to provide the required energy (ATP) for the plant resistance potential. The proline mitigating effect in salt stress is highlighted even by exogenous proline during plant growth (El Moukhtari et al., 2020).



**Figure 1.** The impact of salinity treatments on leaf proline contents of *Chrysopogon zizanioides* L.

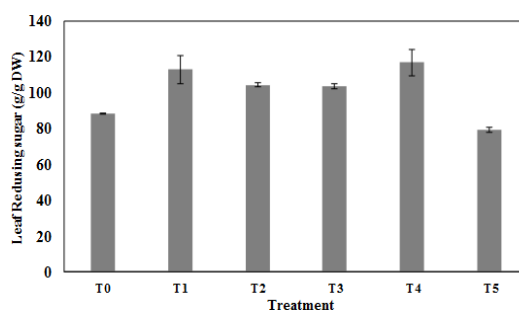
### Salinity Treatments Effects on Leaf Soluble/Non-Soluble Sugar Content

Different salinity levels affected the soluble sugar content of Vetiver's leaf (*Chrysopogon zizanioides* L.) ( $p \leq 0.05$ ) (Table 1). Compared to the control level, the salinity stress caused an increasing trend on the soluble sugar content up to 32 ds/m (T4) (Figure 2) with no significant changes at 44 ds/m (T5), showing the minimum value of 79.3 g/g DW (Table 1). The maximum soluble sugar with the value of 117 g/g DW was observed at T4 (32 ds/m).

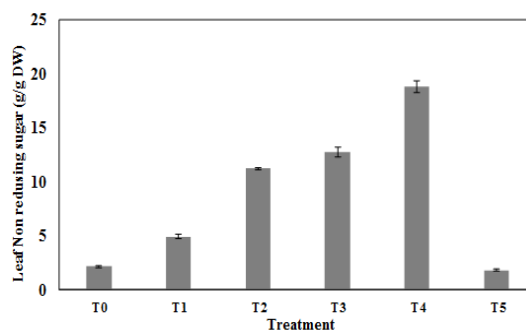
The results showed a significant difference between the control level and all the salinity levels except for salinity at 44 ds/m (Table 1). Another metabolic response to the salinity stress is more likely to be the change of leaf carbohydrates (Moghaddam et al., 2020). A sharp increase in the soluble sugar concentration confirmed in different plant species such as salt-tolerant cultivars of wheat (Kafi et al., 2003), seedless varieties of *Vitis vinifera* (Karimi and Yusef-Zadeh, 2013), and medicinal plant *Tagetes minuta* (Moghaddam et al., 2020)

under salinity. Sugar is one of the responsible parameters in osmotic adjustment and oxidative stress protection in the cytosol (Dell'Aversana et al., 2021). Among different organic compounds, sugar constitutes over 50% of the total osmotic potential (Kumar et al., 2007). A rise in soluble sugar could be a response to the changes of leaf relative water content (RWC) and leaf water potential. Therefore, higher sucrose concentration and soluble sugar levels play important role in plant resistance and adaptation due to the greater leaf water content (Dubey and Rani, 1999). Sugar constitutes the substrates of necessary energy and structural material for efficient defensive responses in plants under stress (Kafi et al., 2003; Morkunas and Ratajczak, 2014; Formela-Luboińska et al., 2020). However, lack of negative fluctuation in Vetiver's soluble sugar may be a result of appropriate photosynthetic conditions in the plant (Sabbagh et al., 2014). In fact, the decreasing trend at the 44 ds/m salinity level was observed (Figure 2); though the fluctuation was not significant. Significant changes may be observed in higher salinity stress or longer-term study

in Vetiver grass. The reduction of leaf soluble sugar concentration might occur because of harsh environmental conditions and low effective enzyme activity in photosynthesis (Sabbagh et al., 2014). However, the non-soluble sugar content showed an overall increasing trend in this study. This phenomenon could be explained through different mechanisms such as increasing leaf carbohydrates to decrease the osmotic potential and ions existence, like potassium ion, to regulate the photosynthetic activity and the ratio of soluble to non-soluble sugar (Vidal et al., 1990). A reduction in non-soluble sugar at 44 ds/m in this study could be a result of overproducing the soluble sugar at this salinity stress level (Figure 3). In fact, non-soluble sugar decomposition and conversion to the soluble sugar to maintain the osmotic potential and reduce dehydration risk during salinity stress is an important strategy in halophytic plants (Kumar et al. 2007; Sabbagh et al., 2014). The two-way reaction of the complex carbohydrates; polysaccharides and oligosaccharides also help to adjust the osmotic potential (Dubey and Rani, 1999).



**Figure 2.** The impact of various salinity levels on leaf soluble sugar amount in *Chrysopogon zizanioides* L.



**Figure 3.** The impact of various salinity levels on leaf soluble non- sugar amount of *Chrysopogon zizanioides* L.

### Salinity Treatments Effects on Leaf Total Protein

Total protein content of leaf is considered as one of the physiological parameters in terms of plant salinity resistance. The results of mean comparison showed a significant effect of salinity levels on leaf protein content only at 8, 16, and 44 ds/m in comparison to control level (Table 1). However, no clear trend responses were observed along different salinity stresses (Figure 4, Table 1). The minimum significant amount was measured at 44 ds/m (T5) around 74 mg/g FW. In general, the salinity levels of T2 and T5 presented the increasing and decreasing trends by 16% and 60% comparing to control, respectively (Table 1).

Total leaf protein content is another important trait to understand plant behaviour under salinity stress as suggested by previous studies (Ashraf and Haris 2004) also confirmed by the results of this study, specifically at 32 ds/m salinity. Protein synthesis may be motivated in response to the salinity stress (Ashraf and

Haris, 2004). There are several salt responsive proteins in different plant species which could be classified into two groups: salt stress proteins and general stress associated protein (Hurkman et al., 1989; Mansour, 2000). Protein accumulation under salinity stress provides an evidence of nitrogen storage utilizing post-stress conditions and adjusts osmotic pressure (Singh et al., 1987; Ashraf and Haris, 2004). Therefore, salt responsive proteins may be increased in Vetiver under salt stress (Pareek et al., 1997). Contrary to our results, salinity could cause a decreasing trend in total leaf protein (Dasgupta et al., 2010; Sohrabi et al., 2011). It seems that a higher leaf protein concentration helps the Vetiver grass to maintain life cycle and resist the harsh abiotic conditions in salinity. Considering all the above results in this research, Vetiver grass showed the ability to adapt to salinity levels at least up to 32 ds/m because of a significant reduction at 44 ds/m (Figure 4).

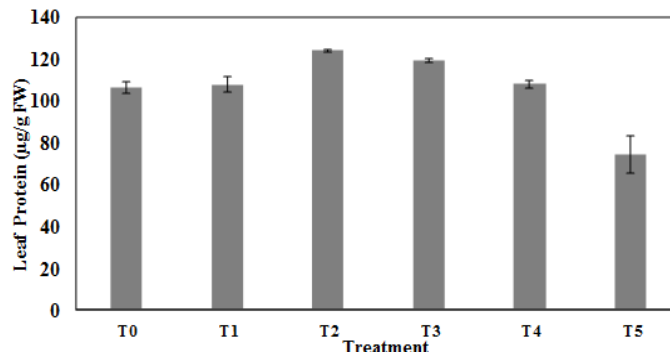


Figure 4. The impact of various salinity levels on total leaf protein amount in *Chrysopogon zizanioides* L.

### Salinity Treatments Effects on Leaf Peroxidase (PRX) Enzymes

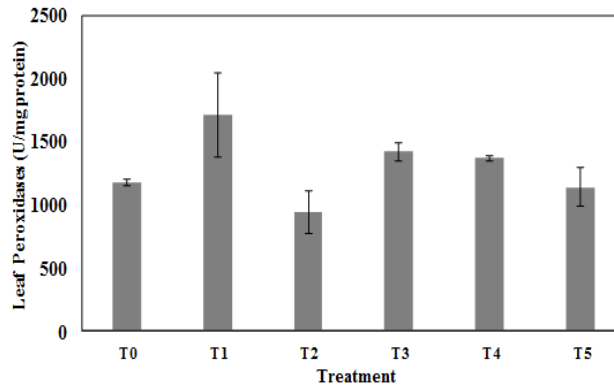
As it is shown in Table 6, the salinity level showed no significant influence on peroxidase enzymes in Vetiver's leaf ( $p \leq 0.05$ ). The results demonstrated no significant variations between average peroxidase enzymes activity of leaf under the salinity treatments in comparison with control (Table 1). This finding might be due to the sampling time or quick reaction of the enzyme against the stress. However, the

decreasing effect of salinity on the enzyme activity can be observed at salinity levels of 8 and 44 ds/m rather than 4 ds/m (Figure 5).

While there was no clear trend in leaf peroxidase enzyme activity, a suppression was obvious by salinity levels higher than 4 ds/m. The reduction of enzymes' activity could be a reason of the production of active oxygen varieties in plant cells during stress. Unlike the above findings, some studies have emphasized the positive increasing effect of salinity on leaf

peroxidase enzyme (Gulen et al., 2006; Turhan et al., 2008; Al-Sammarraie et al., 2020). Generally, the increase of peroxidase enzyme activity in the plant under salinity stress resulted in the reduction of active

oxygen which is more likely to decrease the destruction of cell membranes and damages of plant and even, decomposes the hydrogen peroxide to oxygen and water while adjusting the negative effects.



**Figure 5.** The impact of various salinity levels on leaf peroxidase enzymes in *Chrysopogon zizanioides* L.

**Table 1.** Summary of Duncan's multiple ranges test on salinity effects on leaf physiologic and biochemical traits of Vetiver Grass (*Chrysopogon Zizanioides* L.).

Variants	Pr> F	Treatment					
		control	4ds/m	8ds/m	16ds/m	32ds/m	44ds/m
Shoot Length	0.80	25.5±3.2 <sup>a</sup>	26.5± 3.1 <sup>a</sup>	27.5± 3.1 <sup>a</sup>	28.7±3.2 <sup>a</sup>	25± 1.2 <sup>a</sup>	23.5±2.26 <sup>a</sup>
Shoot Weight	0.003	2.2± 0. 7 <sup>c</sup>	3.3±0.49 <sup>b</sup>	3.5±0. 6 <sup>b</sup>	3.7±0.4 <sup>ab</sup>	4/2±0.1 <sup>ab</sup>	4/7± 0.47 <sup>a</sup>
Proline Content	<0.0001	0.4±0.03 <sup>f</sup>	2.5±0.27 <sup>e</sup>	5.5±0.14 <sup>d</sup>	9.4±0.2 <sup>c</sup>	17/5±0.2 <sup>a</sup>	16±0.15 <sup>b</sup>
Soluble sugar	0.0005	88.3±0.3 <sup>b</sup>	113±7.8 <sup>a</sup>	104±1 <sup>a</sup>	103±1.2 <sup>a</sup>	117±7.5 <sup>a</sup>	79.3±1.3 <sup>b</sup>
Non-soluble sugar	<0.0001	3.2±0.1 <sup>e</sup>	4.9±0.2 <sup>d</sup>	11.2±0.1 <sup>c</sup>	12.7±0.4 <sup>b</sup>	18.6±0.6 <sup>a</sup>	1.8±0.1 <sup>e</sup>
Protein	<0.0001	106±2.9 <sup>b</sup>	108±3.7 <sup>b</sup>	123.8±0.6 <sup>a</sup>	119±1.05 <sup>a</sup>	108±1.65 <sup>b</sup>	74±8.8 <sup>c</sup>
Peroxidase	0.085	1182± <sup>ab</sup> 26	1711±33 <sup>a</sup>	943± 166 <sup>b</sup>	1421±70 <sup>ab</sup>	1372±21 <sup>ab</sup>	1142±156 <sup>b</sup>

## Conclusion

The overall results of this study showed a quick adaptation to salinity stress at 4 to 16 ds/m salinity levels in *Chrysopogon zizanioides* L. in greenhouse experiment. This adaptation was somehow evident up to 32 ds/m. Therefore, the induced concentration of 44 ds/m sodium chloride was higher than *Chrysopogon zizanioides* L. tolerance due to sharp significant reduction

in the physiochemical traits. As the salinity stress was imposed by NaCl solution in alkaline soils, the overall results may suggest *Chrysopogon zizanioides* L. is a suitable plant for rehabilitation of the degraded salt-affected soils in natural ecosystems. However, a long term study is highly recommended for further in situ investigation to generalize the results.

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