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Salinity Stress and its impact on Morpho-Physiological Characteristics of *Aloe Vera*

Robabeh Asghari[1]* and Rahim Ahmadvand[2]

[1]Department of Plant Production, Imam Khomeini Higher Education Centre, Research, Education and Extension Organization (AREEO), Karaj- Iran
[2]Institute of Seed and Plant Improvement, Agricultural Research, Education and Extension Organization Agricultural Research (AREEO) Karaj- Iran

ABSTRACT

Aloe Vera is a valuable medicinal plant. Its leaf and gel in particular are widely used as skin care and in medical applications. Salinity however, is an abiotic stress, and can negatively affect the plant's morphological characteristics as well as quality and quantity of its phyto-chemical compounds of, including total phenol, total soluble sugars and its components, namely sucrose, glucose, and fructose. In order to investigate the impact of salinity stress on morphological and physiological traits of plant, different levels of NaCl, namely 0 (control), 50, 100, 150, 200 and 250 mM were applied in a complete randomised design with three replications under greenhouse conditions. The results indicated that salinity stress has significant negative effect on the plant's morphological traits, such as its weight, leaf length, leaf weight, gel weight, root length; and biochemical traits such as total phenol, total soluble sucrose, glucose and fructose. The results of this study indicate that salinity stress has significant negative effect on *Aloe Vera*'s morphological traits which results in yield loss. Moreover, biochemical traits such as photosynthetic and defences of plants are also affected. It is thus, clear that *Aloe Vera* is susceptible to salinity stress.

Keywords: Medicinal Plant, Morphological traits, Phenol compounds, Phyto-Chemical, Soluble sugars

INTRODUCTION

Aloe Vera L., a valuable medicinal plant, is a perennial liliaceous which grows in tropical and sub-tropical regions. It has thick lace-shaped green leaves with jagged edges and sharp points, joined at the stem in a whirled pattern. Although more than 250 species of *Aloe* genus have been

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E-mail addresses:
fariba2022@yahoo.com (Robabeh Asghari),
ahmadvand2000@yahoo.com (Rahim Ahmadvand)
* Corresponding author

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identified worldwide, only two species, *Aloe barebadensis* and *Aloe aborescens*, are commercially important. *Aloe Vera* contains different nutritional contents such as vitamins, minerals, enzymes, sugars, phenol compounds, lignin, saponine, sterol as well as amino acids. It is widely used in healthcare and cosmetic products. Carbohydrate contents, which account for about 25% of its dry weight, are known to improve the immunity response of the human body (Green, 1996; Kahlon, 1991; Sheets, 1991). In recent years, studies have been conducted to identify the characteristics of the colourless gel available inside the leaves as well as substance produced by the outer layer of the leaves (Liu et al., 2011; Ni et al., 2004). The plant gel, which contains glucomannan as a type of emulsion polysaccharide, is a common moisturising ingredient in cosmetics (Chen et al., 2012; Zapata et al., 2013).

There are some phenol compounds such as anthraquinone found in the juice of *Aleo Vera*. This substance is commonly used as a laxative and it is known to have a strong antibacterial as well as sedative quality (Thu et al., 2013). The gel contains 99% water and a pH value about 4.5, and it is a common over-the-counter medicine for skin diseases. The extracts can be used to relieve cancer pain, digestive disorders and even AIDS. Due to its huge utilisation in pharmaceutical, cosmetic and food industries, the demand for quality planting material of *A. Vera* is increasing. (Bedini et al., 2009; Botes et al., 2008; Eshun & He, 2004; Grace et al., 2008; Lad & Murthy, 2013; Rodríguez et al., 2010). The clinical trials of *Aloe Vera* have been conducted for skin conditions, management of burn and wound healing, constipation, tumours, and gastrointestinal disorders (Rajasekaran et al., 2006). Mass propagation of uniform and healthy plants through tissue culture is the only available technique for large scale production of clonal plants in a short time. Several attempts have been made over the last few decades to develop tissue culture systems of *Aloe* spp., but still efficient regeneration protocols are requisite for large scale production of true-to-type plants of this commercially important species (Amoo et al., 2012; Das et al., 2010; de Oliveira & Crocomo 2009; Gantait et al., 2011; Haque & Ghosh, 2013; Rathore et al., 2011; Singh et al., 2009).

Water deficiency, salinity and temperature differences can cause a lot of damage to plants. Although, this plant is tolerant to unfavourable conditions such as poor soil, the adverse effects of salinity and drought on this plant is considerable (Tubabicer et al., 2004).

Salt stress retards plant growth and yield, and has become a a serious problem in the world (Horvath et al., 2007; Kirdmanee, 2009; Moghbeli et al., 2012). This stress is one of the most important abiotic stresses in arid and semiarid regions (Olfati et al., 2012; Sahu et al., 2011; Talebi et al., 2015; Zhang et al., 2010; Zheng et al., 2004). Better understanding of the mechanisms that enable plants to adapt to salt stress and maintain growth, would help in the selection of stress tolerant cultivars (Jin et al., 2007).

According to a survey, more than 800 million hectares of land throughout the world are salt affected (Anonymous, 2008). The impact of salt stress has been correlated with some morphological and physiological traits such as reduction in fresh and dry weight (Chartzoulakis & Klapaki, 2000). In fact, salinity affects plant metabolism by disturbing their physiological and biochemical processes of plants due to ionic and osmotic imbalances which slows down plant growth and productivity (Munns, 2005). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, or a combination of these factors (Ashraf & Harris, 2004). Studies on plant tolerance to salt stress cover many aspects of the influences of salinity on plant behaviour, including alterations at the morphological, physiological and molecular levels. Recently, investigations have focused on biotechnology, transgenic plants, improvement of breeding, screening methodologies and modification of the genetic structure of existing crops aiming at enhanced adaptation to salinity conditions (Mahdava et al., 2006). Zan et al. (2007), studying physiological and ecological characteristics of plants with seawater irrigation, reported that salinity stress results in decrease in tissue water, total soluble sugars and glucose. Mustafa (1995) suggested that in *A. Vera*, 0.1% salinity has resulted in an increase in growth parameters while 0.4% salinity reduced growth parameters. Additionally, he reported the highest amount of carbohydrate compounds was obtained with 0.4% salinity. The aim of this study was to examine the effect of salinity and traits related to growth, and phytochemical compounds in gel and leaf of *A. Vera* L. plant.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse facilities of Imam-Khomeini Higher Education Center in Karaj, Iran in spring of 2015.

In-vitro plantlets of *Aloe Vera* were transplanted into 30 cm diameter pots containing cocopeat and perlite under greenhouse conditions. The experimental plants were irrigated with a nutrient solution containing different levels of NaCl including 0 (as control), 50, 100, 150, 200 and 250 mM, with consequent EC including 7.7, 13.9, 18.7, 22.8, 25.7 and 1.3 µs, in a Randomized Complete Design (RCD) with three replications. After the trial period of six months, the treated plants in each replication were assessed for morphological and physiological characteristics as follow:

Morphological measurements

The measurements include bush height, number of leaves, leaf length, leaf weight, leaf gel weight, root length and weight.

Determination of total soluble sugars

The amount of total soluble sugars was estimated using Anthrone reagents as discussed by Thimmaiah (2004). One hundred mg sample was placed in a boiling

tube and hydrolysed with 5 ml 2.5 N HCl in a water bath for three hours before it was neutralised with solid sodium carbonate. The volume was made to 100 ml followed by centrifuge at 5000 rpm for 10 min. The supernatant was collected and one ml sample was taken for analysis. Four ml Anthrone reagent was added to aliquot and heated for one minute in the water bath (70°C). The sample was then rapidly cooled and the change of green to dark green colour was read at 630 nm compared to the blank.

Determination of total phenol

Total phenols content was measured using the Folin- Ciocalteau reagent (McDonald et al. 2001). The extract sample (0.5 ml) was mixed with 0.5 ml Folin-Ciocltеau reagent, and then 4 ml 1 M aqueous Na_2CO_3 was added to the mixture. The mixture was allowed to stand for 15 minutes, and phenols were measured using colorimetric method at 765 nm using UV visible spectrophotometer. Total values were expressed in terms of Gallic acid equivalent and total phenol contents were calculated as Gallic acid from a calibration curve (Shui & Leong, 2002).

Statistical Analysis

Data for each parameter was subjected to one-way analysis of variance (ANOVA) and significant differences between treatment means and simple correlation coefficient of traits were determined by Duncan's multiple range test (DMRT) in a RCD, using the SPSS software package (version 16). Microsoft Office Excel (version, 2007) was applied to draw the diagrams.

RESULTS AND DISCUSSION

Morphological traits

The results indicate that some morphological characteristics including bush weight, leaf weight, leaf gel weight, root length and root weight ($p<0.05$) as well as leaf length ($p<0.01$) were significantly affected by salinity stress, while bush height and number of leaves were not affected (Table 1).

Table 1
Analysis of variance for the effect of salinity stress on morphological traits in Aloe Vera

Changes sources	Df	EC	M.EC	Busch weight (g)	Busch hight (cm)	Leaf number	Leaf length (cm)	Leaf weight (g)	Leaf gel weight (g)	Root length(cm)	Root weight (g)
Treatment	5	433.12**	228.79**	4495.91**	3.99ns	1.17ns	25.76*	214.79**	65.85**	162.14**	39.49**
Error	24	1.81	1.61	765.45	18.03	1.83	9.35	11.61	0.65	18.20	3.24
CV		7.1	7.4	19.3	14.1	16.7	12.8	19.7	14.3	17.8	15.1

Table 2
Mean comparison of the effect of salinity stress on morphological traits in Aloe Vera

Treatment NaClmMolart	Busch weight(g)	Busch hight(cm)	Leaf number	Leaf length (cm)	Leaf weight (g)	Leaf gel weight (g)	Root length (cm)	Root weight (g)
50	152.6ab	30.2a	8.0a	25.9a	21.7b	5.8b	23.2ab	16.8a
100	144.1b	31.4a	8.4a	22.4ab	12.8c	4.7c	21.0ab	12.0b
150	155.9ab	30.1a	8.6a	23.6ab	14.9c	5.8b	17.9bc	12.2b
200	118.0bc	28.9a	8.0a	20.5c	11.2c	2.4d	10.1d	10.8bc
250	102.3c	29.6a	7.2a	24.4a	14.6c	2.4d	13.0cd	8.1c
Control	186.9a	30.8a	8.2a	26.6a	28.5a	12.3a	24.4a	12.2b

Table 2 shows that the bush weight appeared highest in control plants and lowest when irrigated with 250 mM salt. Except root weight, other traits appeared to be also the highest in control. The highest root weight was obtained in 50 mg/lit salt treatment. In fact, salinity negatively affected most traits except number of leaves and bush height, but they did not correspond to the same pattern. As had been expected, the higher the salinity stress, the less the value of these traits, so that the lowest value referred to conditions between 200 and 250 mM.

Effect of salinity stress on biochemical traits

The results showed that salinity stress affect biochemical traits of *Aloe Vera* gel and leaf, such as total phenol compounds, total soluble sugars and their components including sucrose, glucose, and fructose, significantly ($p<0.01$) (Table 3). The phenol compounds were lower in leaves produced under stress conditions, so that the lowest content referred to leaves of plants exposed to 250 mM of NaCl and untreated plants, control plants, induced the highest content. However, the phenol content in gel was lower as the lower concentration of salt was applied and in high level of stress, minor changes were observed during treatments. The effect of salinity in soluble sugars of leaf and gel are shown in Table 4. Soluble sugars showed different pattern in plants, so that 150 and 250 mM of salt caused highest and lowest values respectively, and the value of control treatment appeared to be less than the value caused with 150 mM NaCl. Glucose content also followed approximately the same pattern as soluble sugars. In addition, sucrose content in gel was the highest in 150 mM salt treatment, but in leaf, the stress resulted in decreasing the content compared with control treatment. On the other hand, salinity stress led to an increase in fructose content in gel which was highest in plants treated with 100 mM salt, while in leaf, it decreased (see Figure 8). Although there is no similar pattern for the effect of salinity stress on carbohydrate compounds, as Table 4 indicate, total soluble sugars and their components appeared to have an increase with a certain level of the stress, particularly in gel, in which 150 mM NaCl was mostly

found to be the level so that the higher and lower levels of salt resulted in negative effect on the carbohydrate compounds production in the plant. In terms of sucrose and fructose contents of leaves, stress at any level caused lower values compared with *r* control. In terms of total phenol compounds in the leaf, a decrease in the content caused by the stress was observed. However, this effect did not follow any certain pattern. In fact, some times, the content in low level of stress turned out to be lower compared with severe stress conditions. Accordingly, the highest decreasing effect on yield of total phenol in gel appeared by the lowest level of stress. In addition, a descending trend of phenol content in gel occurred as the level of stress reduced. For both leaf and gel, control plants contained the highest amount of phenol. The increase in total soluble sugars and their components under certain levels of stress, particularly in gel, indicates that stress on its own does not necessarily determine the content of such compounds, but the level of stress can be regarded as the main determinant of that. Moreover, content of soluble sugar components can vary, especially at150 mM NaCl stress level.

Table 3
Analysis of variance for the effect of salinity stress on biochemical traits in Aloe Vera

Changes sources	Df	Phenols leaf gel (mg/ gDW)	Phenols leaf (mg/gDW	Total sugar leaf gel (mg/ gDW)	Total sugar Leaf (mg/gDW)	Sucrose leaf gel (mg/ gDW)	Sucrose leaf (mg/ gDW)	Glucose leaf gel (mg/gDW)	Glucose leaf(mg/ gDW)	Frouctose leaf gel(mg/ gDW)	Frouctose leaf (mg/gDW)
Treatment	5	2386.9*	121305.1**	1.022**	1.036**	1272.1**	3673.3**	1012255.5**	28199.4**	205104.4**	142908.5**
Error	24	625.4	3421.8	0.027	0.013	3.7	8.6	1638.8	227.7	2986.6	1119.7
CV		10.3	7.2	17.1	7.6	6.5	3.4	6.6	7.1	15.1	7.1

Table 4
Mean comparison of the effect of salinity stress on biochemical traits in Aloe Vera

Treatment NaCl (mMolar)	Phenols leaf gel (mg/ gDW)	Phenols leaf (mg/ gDW)	Total sugar leaf gel (mg/ gDW)	Total sugar leaf (mg/ gDW)	Sucrose leaf gel (mg/ gDW)	Sucrose leaf (mg/ gDW)	Glucose leaf gel (mg/ gDW)	Glucose leaf (mg/ gDW)	Frouctose leaf gel (mg/ gDW)	Frouctose leaf (mg/ gDW)
50	219.2c	825.8c	0.76c	1.53c	22.4c	68.3d	423.6c	179.3c	541.2b	505.6bc
100	236.0bc	918.8b	0.84c	1.39c	23.2c	95.3b	720.7b	184.1c	652.0a	532.0b
150	233.8bc	791.2c	1.74a	2.19a	52.2a	94.5b	1475.6a	340.6a	477.6b	467.5c
200	243.8b	783.6c	1.09b	1.21d	32.5b	74.2c	475.8c	164.4cd	216.5c	372.2d
250	253.0ab	540.5d	0.38d	0.88e	23.1c	66.3d	269.3e	152.1d	215.3c	235.8e
Control	265.9a	998.5a	0.93bc	1.78b	24.2c	138.4a	323.6d	275.7b	177.4d	741.4a

As discussed earlier, salinity stress negatively affects morphological traits, for which there is no similar pattern. In fact, higher level of stress does not necessarily result in greater decreasing effect and less severe stress does not necessarily have less impact on the traits. However, the more severe the stress, the less rate of growth is observed. As to total phenol compounds, the stress has resulted in decreased content, but no certain association was observed. It could be concluded that only soluble sugars increase with certain levels of stress.

There are several research studies indicating the impact of salinity stress, as an environmental stressing factor, on plant growth (Ashraf & Harris, 2004; Mahdava et al., 2006; Zan et al., 2007). The results of some studies show that morphological and physiological traits such as fresh and dry plant weights are adversely affected by salinity stress (Abdollahi et al, 2011). In fact, deleterious effects of salinity on plant metabolism are due to disorder in physiological and biochemical process caused by ionic and osmotic imbalances, resulting in the reduction of growth and yield (Cha-um & Kirdmanee, 2009). Salinity is reported to affect number of leaves, plant height, root weight, total gel weight, dry root weight of *Aloe Vera* (Moghbeli et al., 2012). The results of similar studies showed that low water potential of soil reduces fresh leaf weight, plant growth rate and leaf yield (Rodriguez-Garcia et al., 2007). Based on the reports, although *Aleo Vera* is relatively tolerant to dry condition, salinity can have deleterious morphological and physiological impacts; the impact of increased salinity is more prominent on the leaf length (Fuentes, 1988). Increased level of salinity stress can alter the content of chlorophyll, soluble carbohydrate, proline and total soluble solid (TSS). Shams et al. (2015) reported that salt could reduce growth and gel yield as well as chlorophyll content of *Aloe Vera*. In addition, higher concentration of sugars, particularly sucrose accumulation, as a consequence of salinity stress, has been broadly reported (Murthy et al., 2013; Zan et al., 2007) but a reduction in glucose content has been reported as well. The reduction of soluble sugars and starch is more common in leaves of trees exposed to long-term salinity stress, thus, no sugar accumulation is observed in such plants (De Oliveira et al., 2009).

CONCLUSION

This study revealed that salinity stress negatively affected a number of growth-related characteristics of *Aloe Vera* plant such as bush weight, leaf length, leaf weight, gel weight as well as root length while traits including bush height and number of leaves were not significantly affected. Although there are different impacts at different levels of salinity, the leaf length showed a significant reduction, but in some levels of salinity, no significant difference was observed compared with control. Furthermore, root length appeared to decrease significantly in salinity stress, but the value was higher than the control in 50 mM NaCl. It could be concluded that

the level of stress can be considered as the determining factor in growth traits.

In addition, the results of study show a significant impact of salinity stress on phyto-biochemical traits. However, the patterns vary according to the type of biochemical compounds as well as extraction sources. As was mentioned above, reduction of total phenol compounds is associated with the level of stress, but the reduction rate depends on the salt concentration as well as the extraction source (leaf or gel), and it cannot be concluded that stress has increasing or decreasing effect on the content. Based on the level of salinity stress, carbohydrate compounds including total soluble sugars, sucrose, glucose and fructose contents were also affected. The highest amount of soluble sugars in leaf and gel, sucrose in gel, glucose in leaf and gel with 150 mM NaCl and fructose in gel with 100 mM NaCl was recorded, although the control produced the highest amount of sucrose and fructose contents in leaf. Furthermore, there were different patterns for the changes of the traits by salinity stress, so that for some traits, high or low level of stress resulted in reduction as the same effect, while for some other traits there was somewhat various changes. In addition, salinity stress appeared to increase soluble sugar content in the plant, particularly in gel.

In terms of measuring biochemical characteristics which play a considerable role in food and pharmaceutical qualities of the plant, salinity stress is not known the main factor in decreased plant yield, but what is more important is the level of stress. It is noteworthy that the ratio of carbohydrate constituents appears to be different with various levels of stress and this is true of other biochemical traits affected by salinity stress. In other words, salinity stress in different levels negatively affect plant growth characteristics, while its effects on phyto-chemical traits as well as trend of changes are different according to the composition, part of plant used for extraction and stress level. Complying with other research reports on the influence of salinity stress on morphological and biochemical traits of *Aloe Vera*, the result of present study shows level of stress is an important rather than stress on its own, to assess its effects on plant growth.

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