Ilmu Pertanian (Agricultural Science)
http://journal.ugm.ac.id/jip
Vol. 7 No. 3 December, 2022: 178–187| DOI: doi.org/10.22146/ipas.78646

Effect of short and long period of salinity stress on physiological responses and biochemical markers of *Aloe vera* L.

Mandana Mirbakhsh[1]* and Seyedeh Sara Sohrabi Sedeh[2]

[1]Department of Agronomy, Purdue University
West Lafayette. IN. 47907, USA.
[2]Department of Plant physiology, Alzahra University
Vanak Village-street, Tehran, Iran. 1993893973.
*Corresponding author: mmirbakh@purdue.edu

Article Info

Received : 26th October 2022
Revised : 26th November 2022
Accepted: 5th December 2022

Keywords:
Aloe, abiotic stress, biochemical response, stress biomarkers

Abstract

Salinization reduces soil health and quality, drastically limiting plants' growth and crop yield. This comprehensive research aimed to evaluate the impact of sodium chloride increment on growth factors, amount of oxidative stress biomarker (Malondialdehyde), osmotic response (evaluated by both proline and sugars contents), photosynthesis efficiency (expressed with chlorophyll fluorescence measurement) and activity of Malate dehydrogenases (MDHs) as a regulator under abiotic stress tolerantly in Aloe vera in Alzahra University, Tehran. Experiments were conducted in two studies at the following concentration of sodium chloride: 0 (control), 100, 200, and 300 (mM) NaCl for 30 days (short-term treatments) and: 0 (control), 54.7, 109.5, and 164.5 (mM) NaCl for 150 days (long-term treatments). Three replications in a completely randomized design were applied. The results showed that while the fresh weight of belowground biomass declined at higher salinity level (164.5 mM), no significant differences were reported in the short period of salt treatments. A considerable amount of free proline that was accumulated in both short (3.594 $\mu g.g^{-1}$ dw) and long (2.20 $\mu g.g^{-1}$ dw) term studies raised the role of proline in osmoregulation. Our results showed the decline of MDA amount (0.0003 $mmol.g^{-1}FW$) in 54.7 NaCl (mM) may be due to less membrane damage in presence of moderate salinity, and indicated a variety of dependent differences in biochemical markers activity.

INTRODUCTION

Aloe vera L. is a perennial plant with well-known medicinal values and a short growth period originally from Southern Africa but has been indigenous to dry tropical and subtropical regions (Hekmatpou et al., 2019). Although more than 250 species of Aloe genus have been identified, only a few species of Aloe have been known for commercial importance (Eshun and Qian, 2004). Aloe vera contains several nutritional compounds, such as vitamins (vitamin E, C, A), phenol, lignin, enzymes (peroxidase, amylase, catalase), minerals, and saponins, that are widely used in healthcare and therapeutic products (Maan et al., 2018).

Recently, climate change, global warming, and water stress have directly given rise to a different kind of abiotic stress and put more than 800 million hectares of land in danger and salt-affected (Asseng et al., 2015). Environmental stress including salinity can cause osmotic and nutritional imbalances, which have been correlated to physiological traits like quality reduction and productivity losses (Ma et al., 2020). Low osmotic potential of soil solution is related to deleterious effects of saline stress on plant metabolisms (Ashraf &Harris, 2004). In general, salt stress retards plant growth and yield, especially in arid regions, which have become the most disturbing problem in the world (Olfati, 2012).

How to cite: Mirbakhsh, M. and Sedeh, S.S.S. (2022). Effect of short and long period of salinity stress on physiological responses and biochemical markers of *Aloe vera* L.. *Ilmu Pertanian (Agricultural Science)*, 7(3), pp. 178–187.

ISSN 0126-4214 (print) ISSN 2527-7162 (online)

In recent decades, many studies have investigated impacts of salinity levels on crop quality and showed enough watering in late autumn to early spring, especially in rainfed conditions, increased plants' production (Mirbakhsh and Hosseinzadeh, 2013). Aloe vera is no exception and is known as an important alternative crop for industrial and pharmaceutical application in sodic lands due to its tolerance potential as a xerophyte (Rahi et al., 2013). The impact of salinity on Aloe yield, cell formation, and expression of genes is well studied. In this regard, Sahu et al. (2011) recorded a decline in growth and biomass of two Aloe species under saline stress integrated with high soil pH, and the effect of salinity stress on Aloe's yield potential and the amount of carbohydrate was reported by Moghbeli et al. (2012).

In contrast, the level of NaCl tolerance of Aloe vera and its impacts on the leaf photosynthetic machinery and chlorophyll content are incomplete. Moreover, the oxidative status of growing plants and more specifically capabilities of the tissues for producing reducing energy under the stressful condition of saline stress need more investigation.

In this study, physiological responses of Aloe vera was studied in zero, low, medium, and high NaCl treatments, while we kept pH unchanged at 6.5 to avoid further effects. The mechanism of Malate dehydrogenase (ME)/NADP-ME activity as a mediator signaling event to oxidative burst was studied. This study aimed to investigate association of biochemical markers, such as lipid peroxidation and free proline alternation, to determine their role in Aloe vera salt tolerance and also delineate physiological processes and mechanisms underlying these responses to salinity stress.

MATERIALS AND METHODS

Plantation of Aloe vera

The study was conducted in a greenhouse in the Plant Physiology Department, Alzahra University, Tehran, Iran (35.7683° N, 51.3926° E). Plants were obtained from the local nursery in North of Tehran, uniform in size, health, color, and an average number of 8 leaves. Plants were put in plastic pots with free drainage. Each pot had a 4.7 inch height and 5.1 inch width and contained an ultrafine texture of clay, silt, and sand. The plants in the pots were kept for 120 days with day/night temperatures of 25°C /21°C and 16 h photoperiod with an approximate humidity of 55% to get accustomed to the greenhouse situation before conducting the research with different treatments. The pots were settled in rows, and irrigation was done every three days.

NaCl treatment

Plants were divided into two separate groups for short (30 days) and long (150 days) period of salinity treatments with three replications arranged in completely randomized design. Plants of each group were then separated into four groups marked as control (0 mM), low (100 mM), medium (200 mM), and high (300 mM) of NaCl for short-term treatments and control (0 mM), low (54.7mM), medium (109.5 mM), and high (164.2 mM) of NaCl for the long-term treatments. In both treatments, plants were treated every three days and only uniform eight-month-old plants were chosen for long-period experiment and five-months-old plants were used for short-period treatments right 7 days after the last treatment.

Morphological observations and growth analysis

The fresh weight of above and belowground plant biomass of all treatments was measured after 150 days of long-term saline treatments with eight-month-plants and 30 days of short-term saline treatments with five-month-plants. Fresh samples were dried for 48 h at 80°C to obtain dry matter. After cooling, the dry samples were weighed and dry matter content was calculated.

Proline concentration

Free proline content was estimated after 150 days of long-term saline treatments with eight-month-plants and 30 days of short-term saline treatments with five-month-plants, according to the method of Bates et al. (1973). 0.1 g of dry matter content of each sample was homogenized using a pestle and mortar in 5 mL of 3% Sulphosalicylic acid. Glacial acetic acid (2 mL) and 2 mL of ninhydrin reagent were added to 2 mL of extract in test tube. The mixture of reaction was boiled in a water bath at 100°C for 30 min. 4 mL of toluene was added after cooling the reaction mixture. The chromosphere was read at 520 nm; it contained toluene and was separated, and the absorbance was measured on UV-visible spectrophotometer. Proline concentration was measured using calibration curve, and mg proline per g fresh weight of tissue [$\mu g.g^{-1}$ (DW)] was used for expression.

Peroxidation of lipid

By observing the expression of MDA content and a slight modification in the process, the level of lipid production was determined according to the method of Heath and Packer (1968). After removing the gel, 2.5 g of leaf tissue was homogenized in 10% (w/v) trichloroacetic acid (TCA; 10 mL) using 10 min of 10,000 rpm. An equal volume of 10% TCA solution containing 0.5% 2-thiobarbituric acid was added to the supernatant. Incubation was done at 95°C for 30 min. The homogenized samples were cooled quicky by using ice-bath and centrifuged at 10,000 rpm for 15 min. At 532 nm, the absorbance was measured and corrected for nonspecific absorbance at 600 nm. The concentration of MDA was calculated using extinction coefficient as 155 $mM^{-1}.cm^{-1}$. The MDA content was measured as µmol g^{-1} FW.

Extraction of protein and total malate dehydrogenase assay

Proteins were extracted and measured; liquid N_2 was used to frizz 2 g of tissue, and the frozen sample was grounded. Sodium phosphate buffer (0.2 M, pH = 6.8) was used to homogenize samples. Bradford method was used to determine total protein content of samples. Standard curve was made by Bovine serum albumin (1 mg/mL). Protein of each sample (50 µg) was used for loading. The protein concentration was determined using a curve of calibration for protein as µM g^{-1} FW (fresh weight). The spectrophotometer was used to measure the absorbance at 520 nm. According to Davis (1964), polyacrylamide gel electrophoresis (PAGE) was performed to study isozymes in tissues. Total malate dehydrogenase (NAD^+-MDH, EC 1.1.1.37)'s activity was assayed in samples of long-term treatments. Briefly, according to Jonathan and Wendel (1990), 0.05 M Tris HCl (pH 8.5) containing 25 mg EDTA ,25 mg NBT, 10 mg malic acid, 25 mg NAD, 10 mg malic acid and 3 mg PMS was used in 100 ml for soaking the gel. 0.05 M Tris HCl (pH 8.5) was prepared by dissolving 0.605 g Tris in 50 mL distilled water. HCl was used to adjust pH to 8.5. Then, the solution was completed to 100 mL with distilled water. The solution was dialyzed in cold for 3 h against the extraction buffer. The vertical electrophoretic vats were used to place gels at 4–6°C, with a potential difference of 1.0 V mm^{-1}. Bands were revealed for the enzyme systems malate dehydrogenase (MDH), described by Alfenas (1991). Visual analysis of electrophoresis gels was used to interpret the intensity of each electrophoretic band.

Assay of polysaccharides

Phenol/sulfuric acid reagent was used to measure polysaccharide contents of the samples (Dubois et al., 1956). 0.1 g of dry content of samples were homogenized in 80% ethanol by 10 min centrifugation at 5000 rpm. 30 min water bath incubation was used for the final solution of 5 ml of anthrone reagent and 1 ml of supernatant. Absorbance was measured at 620 nm for the estimation of glucose. 1 ml of resorcinol reagent was added to 2 ml of extract to estimate carbohydrate fructose after 10 min of incubation and measuring the absorbance at 520 nm. Zucrose and starch's content were estimated according to Ramachandra Reddy et al. (2004). Additionally, glucose (80 µg/mL) was used with 0 to 80 µg/mL range concentrations for standard curve. At λ = 485 nm, polysaccharide contents of samples were measured by a spectrophotometer.

Photosynthetic pigment measurements

0.1 g of Fresh plant samples were used to extract carotenoids (mg g^{-1}) and Chlorophylls (Chl b and a), then 1.5 ml acetone (80%; v/v) was used to complete extraction and quantified spectrophotometrically. Absorbances were measured at 646.8, 663.2, and 470 nm by UV-visible spectrophotometer (WPA model S2100). Equation 1 was used to calculate the amounts of photosynthetic pigments (mg/gfw), where the volume of acetone (mL) is V and the fresh weight of the shoot (g) is W (Lichtenthaler, 1994). Acetone 80% was used as a control.

Chla = $[12.25A_{663.2} - 2.79A_{646.8}]$ (V/1000W)

Chlb = $[12.21A_{646.8} - 5.1A_{663.2}]$ (V/1000W)

ChlT = Chlb + Chla

Caratenoid = $[(1000A_{470} - 1.80Chla - 85.2Chlb)/198]$ (V/1000W)

Statistical analysis

Each treatment had three replications, and mean values of each treatment were taken from measurements, then SE of the means were calculated. Statistical analysis was performed using one-way analysis of variance (ANOVA), and Tukey's test was used to determine the significant differences between means of treatments ($p<0.05$) using the Statistical Package for Social Sciences (SPSS) for windows (version 13.0).

RESULTS AND DISCUSSION

Growth analysis

The growth parameters of Aloe vera plants under short-term and long-term salinity stress showed significant differences between fresh and dry weight at p<0.05 under different NaCl treatments. In general, the results showed that short period stress was more suitable for the plants but increasing the duration of the stress caused growth reduction, especially in the fresh weight of belowground biomass. In short-term salinity treatments and aboveground biomass observation, the highest fresh weight of aboveground biomass (89.09 g) was shown in 0 mM of NaCl, and the lowest (51.25 g) was in 100 mM of NaCl but it did not have a significant difference with other two treatments. For dry matter, the highest (1.40 g) was reported in 0 mM of NaCl and the lowest (0.62 g) in 100 mM of NaCl (Table 1). The highest fresh weight of root (belowground biomass) (3.20 g) was reported in 0 mM of NaCl, while 200 mM NaCl treatment showed the lowest (2.40 g) (Table1).

In long-term salinity treatments, the significant difference was shown between 0 and 54.7 mM NaCl concentration at P<0.05. Moreover, the fresh weight of below ground biomass showed significant difference between 0 and 109.5 mM NaCl at P<0.01 and at P<0.05 between 0–164.2 mM NaCl and 54.7-09.5 of NaCl. The aboveground measurement showed the highest fresh weight (138.04 g) in 0mM NaCl and the lowest (92.04 g) in 54.7 mM NaCl (Table1).

Proline content

The proline content from short-term salt treatments showed the highest (3.594 $\mu g.g^{-1}$ dw) in 300 mM NaCl and the lowest (2.601 $\mu g.g^{-1}$ dw) in 0 mM NaCl. The data did not show any significant differences at P<0.05 for 100 and 200 mM NaCl (Figure 1A). The long-term treatments showed the highest proline content (2.20 $\mu g.g^{-1}$ dw) under 164.2 mM and the lowest (1.38 $\mu g.g^{-1}$ dw) in control. The result showed significant differences between 0 and 109.5 mM and also between 0 and 164.2 mM NaCl (Figure 1B). The results indicated that salinity stress caused greater increase in proline content in short-term treatments.

Lipid peroxidation

The results indicated that lipid peroxidation was significantly affected by salinity stress, and substantial increase in MDA content was observed. Yazici et al. (2007) reported no accumulation of MDA within short period of treatments in contrast to sever damages, destroy of cell membrane, and lower activity of enzymes under long-term treatment. Lipid peroxidation was investigated just under long-term salinity to report the specific results (Table 2) in our study. Under long-term treatment, the significant differences were reported between 0 and 54.7 mM of NaCl, 0 and 109.5 mM, and between 54.7 and 164.2 mM of NaCl concentrations. However, MDA concentration was declined in 5 $ds.m^{-1}$ of NaCl treatment that may be due to less membrane damage in presence of moderate salinity. The study showed the significant differences between 54.7 mM and the control and between control and under greater

Table 1. Fresh and dry weights of Aloe's shoot (aboveground biomass) and root (belowground biomass) in both short-term and long-term salinity treatments (mean±SE)

Study	NaCl (mM)	Aboveground fresh weight (g)		Aboveground dry weight (g)		Root fresh weight(g)		Root dry weight (g)	
Short-term	0	89.09±2.08	a	1.40±0.015	a	3.20±0.020	a	1.40±0.010	a
	100	51.25±0.01	b	0.62±0.200	b	2.40±0.018	a	0.67±0.012	a
	200	61.08±0.01	b	1.18±0.017	a	2.45±0.030	a	0.30±0.010	a
	300	60.05±0.012	b	1.20±0.021	a	3.08±0.011	a	0.34±0.007	a
Long-term	0	138.04±0.01	b	2.85±0.01	a	2.87±0.010	a	0.345±0.01	a
	54.7	92.04±1.97	a	3.37±0.30	ab	4.77±0.400	ab	0.722±0.27	ab
	109.5	115.09±21.6	ab	4.71±0.40	c	6.93±1.070	c	1.705±0.001	c
	164.2	133.53±18.9	ab	4.14±0.40	bc	5.31±1.120	bc	0.751±0.17	b

Remarks: Means and standard errors followed by the same letters are not significantly different according to Tukey's test (p<0.05) in short-term NaCl treatment. Means and standard errors followed by the same letters are not significantly different according to Tukey's test (p<0.05) in long-term NaCl treatment.

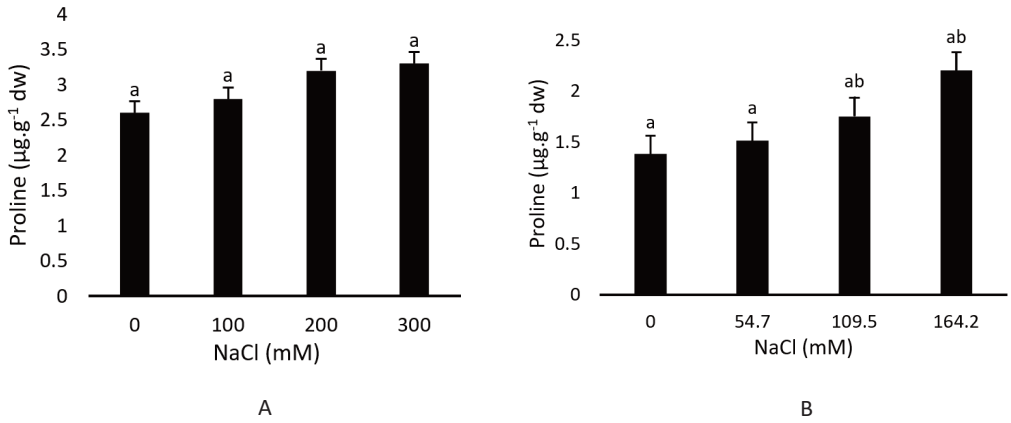


Figure 1. The effect of short-term and long-term salinity treatments on proline content (µg.g^{-1} dw); A. Proline content in short-term salinity treatments; B. Proline content in long-term salinity treatments

Table 2. Fresh and dry weights of Aloe's shoot (aboveground biomass) and root (belowground biomass) in both short-term and long-term salinity treatments (mean±SE)

Study	NaCl (mM)	Sugar (mg.g^{1} DW)	MDA (µg.g^{-1} w)
Short-term	0	1.21±0.02 ab	(Not investigated)
	100	0.98±0.01 a	
	200	1.24±0.01 ab	
	300	1.32±0.05 b	
Long-term	0	2.08±0.01 ab	0.00052±0.001 b
	54.7	2.02±0.03 ab	0.00030±0.002 a
	109.5	2.5±0.100 b	0.00098±0.001 c
	164.2	1.48±0.05 a	0.00096±0.002 c

Remarks: Means and standard errors followed by the same letters are not significantly different according to Tukey's test ($p<0.05$).

NaCl concentration (Table 2).

Protein content and total Malate dehydrogenase determination

The result showed the substantial differences between the total protein content under long and short-term studies. In short period treatment the highest amount of protein (0.36 g^{-1}DW) was at 300 mM NaCl, which had significant difference with the other three treatments at $P<0.05$ (Figure 2A). However The highest amount (93.7 mg.g^{-1} FW) was at 164.2 mM NaCl and the lowest (36.91 mg.g^{-1} FW) at 109.5 mM of sodium chloride (Figure 2B).

The Malate dehydrogenase electrogram of *Aloe vera* plant from four sodium chloride concentrations in long-term treatments showed the highest intensity in 54.7 mM of NaCl. Isoform with Rm=0.41 was shown in all of the treatments except control (Fig 3).

Polysaccharide content

The results of short-term treatments showed the significant differences between 100 and 300 mM of NaCl at $P<0.05$ (Table 2). Salt treatments induced noticeable variations in sugar contents in both parts of each of treatment under investigation. In short-term treatments, the highest amount of polysaccharide (1.32 mg.g^{1} DW) was shown in 300 mM. A significant increase in sugar content (2.5 mg.g^{1} DW) was observed at 109.5 mM of Sodium Chloride in long-term treatment (Table 2).

Chlorophyll and Carotenoid Contents

The effects of short and long-term treatments of sodium chloride on chlorophyll are shown in Table 3. The results showed that the impact of salinity stress on chlorophyll a, chlorophyll b and total chlorophyll

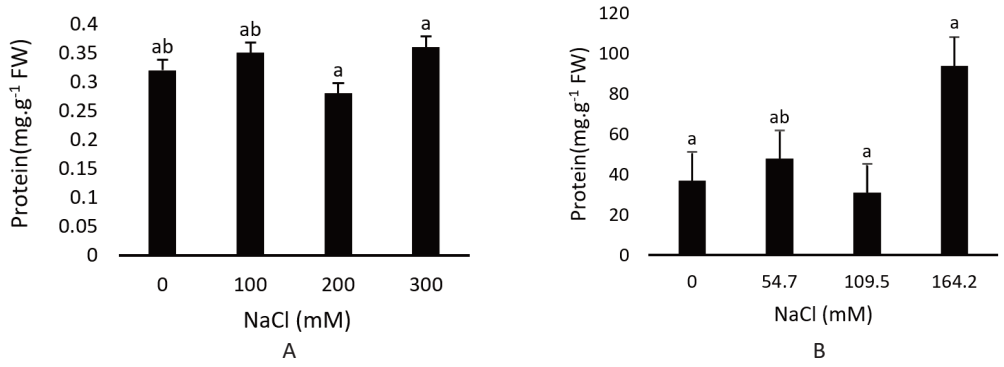
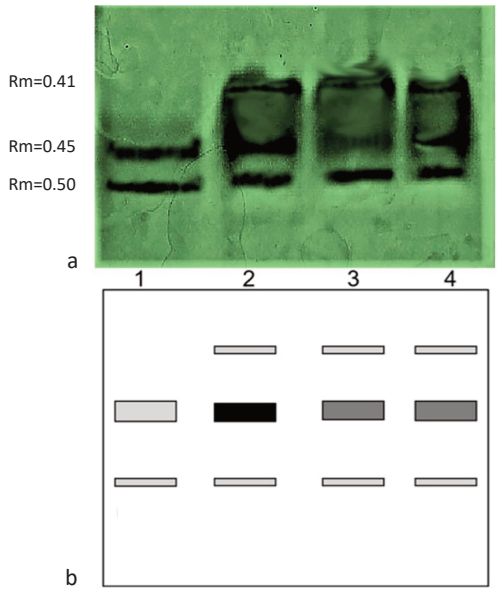


Figure 2. The effect of protein in short-term and long-term salinity treatments on proline content (mg.g^{-1} FW); A. Proline content in short-term salinity treatments; B. Proline content in long-term salinity treatments



NaCl / Rm	0	54.7	109.5	164.2
0.41		++	+	+
0.45	+	+++	++	++
0.50	++	++	++	+

c

Figure 3. (a) Malate dehydrogenase electropherogram showing isoform with Rm=0.41 in all sodium chloride concentration except the control (0 NaCl); (b) showing the location of all isoforms under four NaCl treatments; 1= 0 NaCl (control), 2= 54.7 NaCl, 3= 109.5 NaCl, 4= 164.2 NaCl; (c) showing the intensity level of malate dehydrogenase expression with +, which shows the higher intensity in isoform with Rm=0.45 under 54.7 mM sodium chloride

was significant. The highest amount of chlorophyll a (0.2 mg/gfw) in both parts of each of treatment under study was observed in short-term salinity treatments with the application of 200 (mM) NaCl. Respectively, among four sodium chloride treatments in long-term treatments, the highest (0.078 mg/gfw) was obtained with the application of 164.2 (mM) sodium chloride. Chlorophyll b content did not show significant difference under different salinity treatments, while the content of total chlorophyll and carotenoids were significantly different between different salt concentrations.

Physiological parameters and biochemical markers of plant resistance to abiotic stress are helpful to delineate the mechanisms underlying the responses of plants to abiotic trigger (Negrao et al., 2016). Plants avoid excessive salt accumulation on their tissues by extracting and preserving water and nutrient from saline soil (Zorb et al., 2019; Kaleem et al., 2018). Several studies reported plant growth reduction under salinity stress (Taibi et al., 2016). Our observation in this investigation indicated that Aloe did not show early responses leading to a decline in growth (above and below ground) in a short period of sodium chloride treatment (30 days) (Table 1). However, during long-term exposure to salinity (150 days), the plant experienced a significant decrease in the weight of belowground biomass (Table 1). The negative response of the plant to increasing salinity stress (more than 30 days) indicates that Aloe is unable to tolerate long period of salinity stress, while a short period of salt treatment is suitable

Table 3. The amount of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Chlt), and carotenoids were measured as (mg/gfw) and shown for both short and long studies in the result

Study	NaCl (mM)	Chla (mg/gfw)	Chlb (mg/gfw)	Chlt (mg/gfw)	Carotenoids (mg/gfw)
Short-term	0	0.12±0.02 a	0.0087±0.015 a	0.1400±0.02 a	0.062±0.020 a
	100	0.15±0.01 a	0.0100±0.020 a	0.1600±0.013 a	0.080±0.012 a
	200	0.20±0.02 a	0.0120±0.070 a	0.2000±0.007 a	0.100±0.010 a
	300	0.11±0.012 a	0.1100±0.012 a	0.0013±0.011 a	0.063±0.007 a
Long-term	0	0.059±0.020 ab	0.012±0.0018 b	0.070±0.012 a	0.036±0.011 a
	54.7	0.075±0.012 ab	0.009±0.009 b	0.083±0.010 ab	0.046±0.004 ab
	109.5	0.058±0.007 a	0.015±0.003 b	0.071±0.004 a	0.040±0.005 ab
	164.2	0.078±0.011 b	0.017±0.0015 b	0.111±0.020 b	0.059±0.006 b

Remarks: Means and standard errors followed by the same letters are not significantly different according to Tukey's test ($p<0.05$).

for this plant (Chaves et al., 2009; Olfati et al., 2012).

One of the common physiological responses of plants to biotic and abiotic stresses is the accumulation of proline, which is also a part of plants' developmental program (Verbruggen and Hermans, 2008). In the present study, a significant increase in the amount of free proline was found in both long-term (164.2 mM NaCl) and short-term (300 mM NaCl) (Fig 1) treatments. In response to salinity, a large amount of proline may accumulate and play important role in osmoregulation. By preserving osmoregulation and detoxifying reactive oxygen species (ROS), proline protects plants from stress, which maintains membrane integrity and stabilizes enzymes and other proteins (Liang et al., 2013).

Exposure of plants to saline stress results in lipid peroxidation, measured as malondialdehyde content, as an indicator of oxidative damage (Del Rio et al., 2005). The effect of increasing NaCl was studied on lipid peroxidation in the long-term treatments, showing a significant increase of MDA in greater salt concentrations (109.5 mM and 164.2 mM) (Figure 3). The increase in lipid peroxidation under long-term treatments may be due to the disability of antioxidants to scavenge reactive oxygen species as the result of salt stress (Abogadallah, 2010).

The effect of salt stress on protein content and activity of Malate dehydrogenase were investigated on the leaves of *Aloe Vera* L. The amount of protein differs greatly between the short-term and long-term treatments. The results of long-term treatments showed a significant decline in protein content due to greater salt concentration (Figure 2b). The results agreed with Caplan et al. (1990) and Chen et al. (2007), reporting the negative impact of long-term stress on protein synthesis due to potassium ions removal by plant roots and physiological imbalance. Dehydrogenase activity has been studied with the regulatory mechanism in plant metabolism and catalyzing the oxidation and reduction of the substrate (Takahashi-Iniguez et al., 2016). Malate dehydrogenase supports redox cycling in cells and has a diversified role in plant cell metabolism by generating reducing power for multiple biosynthetic processes (Suzuki et al., 2012). Malate dehydrogenases exist in various isoforms that catalyze the interconversion of oxaloacetic acid and malate (Gieti, 2000). Although four isoforms were investigated in different treatments of sodium chloride, the isoform with Rm=0.41 was the only one that has not been seen in control. (Figure 3). This behavior of the enzyme reflects the significant role of this isoform in osmotic regulation and the possible association of Malate dehydrogenase with salt tolerance in Aloe Vera.

Polysaccharides with multiple forms play an important role in assembly and adaptability of cell wall components to abiotic stress (Vago et al., 2021). The hydroxyl groups in polysaccharide allows to reserved water and offer plants to be more resistant in physiological stress (Parida and Das, 2005; Franz, 1979). Thereby, increment of reducing sugars at high level is one of the protective mechanisms in plants and a good parameter to estimate the tolerantly of the plants under stress (Pérez-López et al., 2010). Our results showed significant increases in the accumulation of polysaccharides in both parts of treatments under study, which is a good indicator for Aloe to withstand saline soil (Table 2).

One of the main processes that are affected by

stresses is photosynthesis (Morales et al., 2020). Long-term salinity stress leads to a greater accumulation of chloride in the root, which makes more ions such as Na^+ to be accumulated and negatively affects photosynthetic components, thereby reducing enzyme activities and pigment synthesis (Ma et al., 2020). In the results of our study, the total amount of chlorophyll and carotenoids declined in a long-term treatments. Low photosynthetic pigment contents may decrease photosynthetic potential and decline plant production. The amount of chlorophyll b did not show significant differences between the four concentrations of each treatment, while the amount of chlorophyll a and carotenoid was greatly affected (Table 3). Our results are in agreement with Donglar et al. (2010), Kafi et al. (2010), and Rahdari et al. (2012) who reported a significant increase in chlorophyll a, b, and carotenoid content under salinity stress. Hence, chlorophyll has been proposed as one of the biochemical indicators of salt tolerance (Ma et al., 2012). The results indicate that Aloe can tolerate short-term saline stress but long-term exposure to salinity is not suitable for this plant.

CONCLUSIONS

The results reflected different physiological responses of Aloe vera to salinity that depend on salt concentration as well as time extent of subjecting to salinity. Biochemical markers of plant resistance to salinity are useful to determine the impact of physiological processes and mechanisms underlying the responses observed. The interaction of salinity with total chlorophyll contents indicates that this plant can withstand moderate salt stress in a short period. Increases in proline content besides polysaccharides by increasing the salinity to maintain osmotic pressure, membrane integrity, and water retention make Aloe more resistant to physiological stress and known as a viable plant in soil salinity. In our experiment, the greater amounts of proteins under saline conditions not only plays a key role in osmoregulation but also may accumulate nitrogen. The greater activity of antioxidant enzymes has a key role in enhancing tolerance and protects plants against oxidative reactions. In the current study, we figured out that MDH activity increased when plants faced long period of salinity stress compared to control. However, MDH activity was affected differently under different sodium chloride concentrations, indicating a variety of dependent difference in MDH activity.

REFERENCES

Abogadallah, G. (2010). Antioxidative defense under salt stress. *Plant Signal Behav.*, 5(4), pp. 369–374.

Alfenas, A.C., Peters, I., Brune, W., and Passador, G.C. (1991). Eletroforese de proteínas e isoenzimas de fungos e essęncias florestais. Viçosa, Universidade Federal de Viçosa. pp. 242.

Ashraf, M. and Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in Plants. *Plant Science*, 166(1), pp. 3–16.

Asseng, S., Ewert, F., Martre, P., Rötter, R.P., Lobell, D.B., Cammarano, D., Kimball, B.A., Ottman, M.J., Wall, G.W., White, J.W., Reynolds, M.P., Alderman, P.D., Prasad, P.V.V., Aggarwal, P.K., Anothai, J., Basso, B., Biernath, C., Challinor, A.J., De Sanctis, G., Doltra, J., Fereres, E., Garcia-Vila, M., Gayler, S., Hoogenboom, G., Hunt, L.A., Izaurralde, R.C., Jabloun, M., Jones, C.D., Kersebaum, K.C., Koehler, A-K., Müller, C., Naresh Kumar, S., Nendel, C., O'Leary, G., Olesen, J.E., Palosuo, T., Priesack, E., Eyshi Rezaei, E., Ruane, A.C., Semenov, M.A., Shcherbak, I., Stockle, C., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Thorburn, P.J., Waha, K., Wang, E., Wallach, D., Wolf, J., Zhao, Z., and Zhu, Y. (2015). Rising temperatures reduce global wheat production. *Nat. Clim. Change*, 5, pp. 143–147.

Bates, L.S., Waldren, R.P., and Teare, I.D (1973) Rapid determination of free proline for water studies. *Plant Soil*, 39, pp. 205–208.

Caplan, A.B., Dekeyser, C.R., and Van Montago, M. (1990). Salinity and drought stress in rice. In: Sangwan, R.S. and Sangwan-Norrell, B. eds. *The Impact of Biotechnology in Agriculture.* Netherland: Kluwer Academic Publishers the Netherlands, pp. 391–402.

Chen, Z., Cuin, T.A., Zhou, M., Twomey, A., Naidu, B.P., and Shabala, S. (2007). Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *Journal of Experimental Botany,* 58(15–16), pp. 4245–4255.

Del Rio, D., Stewart, A.J., and Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism, and Cardiovascular,* 15, pp. 316–328.

Davis, B.J. (1964). Disc electrophoresis. II. Method and application to human serum proteins. Ann. *N.Y. Acad. Sci.*, 121, 404–427.

Doganlar, Z.B., Demir, K., Basak, H., and Gul, I. (2010). Effects of salt stress on pigment and total soluble protein contents of the three different Tomato cultivars. *Afr.J. Agri.,* 5(15), pp. 2056–2065.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.,* 28, pp. 350–356.

Eshun, K., and Qian, H. (2004). Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries–a review. *Crit Rev Food Sci Nutr,* 44 , pp. 91–96.

Gietl, C. (1992). MDH isoenzymes: cellular localization and role in the flow of metabolites between the cytoplasm and cell organelles, *Biochem. Biophys. Acta*,1100, pp. 217–234.

Heath, R. L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys,* 125, 189–198.

Hekmatpou, D., Mehrabi, F., Rahzani, K., and Aminiyan, A. (2019). The effect of aloe vera clinical trials on prevention and healing of skin wound: a systematic review Iran. *J. Med. Sci.,* 44, pp. 1,

Jonathan F.W., and Weeden N.F. (1990). Visualization and interpretation of plant isozymes. In: Soltis D.E., Soltis P.S., editors. *Isozymes in Plant Biology*. London, UK: Chpman and Hall, pp. 5–45.

Kafi, M., Bagheri, A., Nabaati, J., Mehrjerdi, M.Z., masoumi, A. (2010). The effect of salt stress on some physiological parameters of 11 Pea genotypes. Science of greenhouse culture, pp. 55–69.

Kaleem, F., Shabir, G., Aslam, K., Rasul, S., Manzoor, H., Shah, S.M., and Khan, A.R. (2018) An overview of the genetics of plant response to salt stress: Present status and the way forward. *Applied Biochemistry and Biotechnology,* pp. 1–29.

Liang, X., Zhang, L., Natarajan, S., and Becker, D. (2013). Proline mechanism of stress survival. *Antioxid Redox Signal,* 19(9), pp. 998–1011.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids-pigments of photosynthetic biomembranes. *Method Enzymol.* 148, pp. 350–382.

Yotsova, E.K., Dobrikova, A.G., Stefanov, M.A., Kouzmanova, M., and Apostolova, E.L. (2018). Improvement of the rice photosynthetic. *Theoretical and Experimental Plant Physiology,* 30(3).

Ma, Q., Yue, L. J., Zhang, J. L., Wu, G. Q., Bao, A. K., Wang, S. M. (2012). Sodium chloride improves photosynthesis and water status in the succulent xerophyte Zygophyllum xanthoxylum. *Tree Physiol,* 32, 4–13.

Ma Y., Dias M., and Freitas H. (2020). Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. *Front. Plant Sci.,* 13.

Maan, A.A., Nazir, A., Khan, M.K.I., Ahmad, T., Zia, R., Murid, M., and Abrar, M. (2018). The therapeutic properties and applications of Aloe vera: A review. *J. Herb. Med.*, 12, 1–10.

Moghbeli, E., Fathollahi, Salari, H., Ahmadi, G., Saliqehdar, F., Safari A., and Hosseini M. (2012). Effects of salinity stress on growth and yield of *Aloe vera* L. *Journal of Medicinal Plants Research*, 6(16), pp. 3272–3277.

Morales, F., Ancin, M., Fakhet, D., Gamez, A., Seminario, A., Soba, D., Mariem, S., Garriga, M., and Aranjuelo, I. (2020). Photosynthetic Metabolism under Stressful Growth Conditions as a Bases for Crop Breeding and Yield Improvement. *Plants (Basel)*, 9(1), pp. 88.

Mirbakhsh, M., and Hosseinzadeh, N.M. (2013). Investigation of in vitro apocarotinoid gene expression in perianth of saffron (Crocus sativus) Iran. *2nd National Congress on Medicinal Plants, pp.* 15.

Negrao, S., Schmo¨ckel, S., and Tester M. (2016) Evaluating physiological responses of plants to salinity stress. *Annals of Botany,* 119.

Olfati, J., Moqbeli, E., Fathollahi, S., and Estaji, A. (2012). Salinity stress effects changed during *Aloe vera* L. vegetative growth. *Journal of Stress Physiology & Biochemistry,* 8(2), pp. 152–158.

Hare, P.D., Cress, W.A., Staden, J.V. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*., 21, pp. 535–553.

Pérez-López, U., Robredo, A., Lacuesta, M., Muñoz-Rueda, A., and Mena-Petite, A. (2010). Atmospheric CO2 concentration influences the contributions of osmolytes accumulation and cell wall elasticity to salt tolerance in barley cultivars. *J. Plant Physiol.*, 167, 15–22.

Rahdari, P., Tavakoli, S., and Hosseini, S.M. (2012). Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleraceae* L.) leaves. *Stress Physio and Bio. J.* 8(1), pp. 182–193.

Rahi, T.S., Singh, K., and Singh, B (2013). Screening of sodicity tolerance in Aloe vera: An industrial crop for utilization of sodic lands. *Industrial Crops Products,* 44, pp. 528– 533.

Ramachandra, R.A., Viswanatha, C.K., and Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology,* 161, pp. 1189–1202.

Sahu, P.K., Jyoti, N., and Atul, S. (2011). Comparatives performance of Aloe vera and Aloe ferox species under pH along with desiccation stresses. I*nternational Journal Drug Discovery Herbal Research,* 1, pp. 14–17.

Suzuki, N., Koussevitzky, S., Mittler, R. and Miller, G. (2012). ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ.,* 35, pp. 259–270.

Taibi, Kh., Taibi, F., Abderrahim, L., Ennajah, A., Belkhodja, M., and Mulet, J. (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. Elsevier , 105.

Takahashi-Iniguez, T., Aburto-Rodriguez, N., Vilchis-Gonzalez, A., and Flores M. (2016). Function, kinetic properties, crystallization, and regulation of microbial malate dehydrogenase. J *Zhejiang Univ Sci B.,* 17(4), pp. 247–261.

Vago, M., Jaurena, G., Estevez, J., Agueda, M., Zavala, J., and Ciancia, M. (2021). Salt stress on Lotus tenuis triggers cell wall polysaccharide changes affecting their digestibility by ruminants. *Plant Physiology and Biochemistry.*

Verbruggen, N., and Hermans, Ch. (2008). A review; proline accumulation in plants. *Springer-Verlag.*

Yazici, I., Türkan, I., Sekmen, A.H., and Demiral, T. (2007). Salinity Tolerance of Purslane (*Portulaca oleracea* L.) Is Achieved by Enhanced Antioxidative System, Lower Level of Lipid Peroxidation and Proline Accumulation. *Environ.,* 61, pp. 49–57.

Zheng, Q.S., Liu, Z.P., Liu, Y.L., and Liu, L. (2004). Effects of iso-osmotic salt and water stresses on growth and ionic distribution in Aloe vera seedlings. *Chinese Journal Plant Ecology,* 28, pp. 823–827.

Zörb, Ch., Geilfus., Geilfus, Ch., and Dietz K. (2019). A review of salinity and crop yield. *Plant Biology,* 21, pp. 31–38.