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*Original paper*

# Salicylic acid-mediated salt stress tolerance by mitigation of the oxidative effects in Moringa

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## ABSTRACT

Salinity stress amelioration in Moringa was investigated on five weeks old plants by treatment with salicylic acid (SA) in a pot culture experiment. Salt stressed plants showed a reduction in growth and decreased carotenoid content, while ion leakage and lipid peroxidation increased significantly. To avoid oxidative stress due to salt stress, plants increased significantly the activities of studied antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX)]. A significant increase in the activities of these enzymes was observed, resulted in amelioration of growth inhibition when stressed plants were subjected for exogenous application of SA. The role of esterases in support of antioxidant systems was discussed. Increase of SOD or POX activities under salt stress or SA treatment was not due to expression of new isoenzyme forms but because of overexpression of the turned on iso-loci. SA induced activation of the antioxidant system resulted in a significant increase of carotenoids content but H2O2 concentration, lipid peroxidation and ions leakage significantly decreased. These results indicate that SA mediated increase plant content of ROS scavengers and membrane stability leading to promoting more stress tolerance in Moringa.

**KEYWORDS**

**Salt stress,**
**Salicylic acid,**
**Izoenzymes,**
**ROS,**
**Stress markers,**
**Moringa.**

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## INTRODUCTION

Moringa (*Moringa oleifera* L.) is a fast grown tree belongs to the family Moringaceae. Tree leaves, fruits, flowers, immature pods and seeds are highly nutritious as human food or animal feeds. In addition, moringa is used as green manure to improve soil fertility (Davis, 2000). Also, Moringa is used as a food supplement and water treatment. The medical evidences of Moringa as nutritional, therapeutic, and prophylactic plant materials was reviewed (Fahey 2005).

Salinity is one of the environmental conditions which restrict the plant production, especially in arid and semi-arid regions. Under these conditions, few economical plant species such as Moringa can be grown commercially (Qadir *et al*., 2014).

Physiological aspects such as seed germination, growth, photosynthesis and pigment content, carbohydrate and protein fractions, mineral composition and antioxidants are altered under salinity stress (Faheed *et al*., 2005; Shobbar *et al*. 2010; Faheed 2012; Jini & Joseph 2017).

In plants, stress conditions produce and accumulate reactive oxygen species (ROS) leading to oxidative stress, they cause damage to cellular macromolecules including membranes, proteins and nucleic acids (Mittler 2002). Leakage of ions and peroxidation due to salt stress are two key indicators reflecting the degree of membrane

injury (Yang *et al*. 2003; Tripathi *et al*. 2017). On the other side, stressed plants possess defense mechanisms by increasing the antioxidant systems to control the elevated concentrations of ROS. When the level of ROS exceeds the defense mechanisms, stressed plants will be adversely affected. It is carried out by increase of non-enzymatic antioxidant component such as carotenoids (Miyake & Asada 1994; Ramel *et al*. 2012) and antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) and others (Nakano & Asada 1981; Hassanein 1999; Faheed 2012; Torabi *et al*. 2015; Sharma & Laxmi 2015). Esterase such as feruloylesterases (EST; EC 3.1.1.73) catalyzes the release of ferulic acid from cell wall polysaccharides. Ferulic acid has higher potential of radical scavenging (Huang *et al*. 2013). Then, esterases mediates scavenging potential of ROS by liberate of ferulic acid from where is bonded. In addition, ferulic acid increased the expression of CAT, APX and POX, and protected the stressed plants by reducing toxic levels of H2O2 (Sharma & Laxmi 2015). Various agents such as jasmonic acid, ferulic acid and salicylic acid mediate the acclimation of plants to environmental stress (Senaratna *et al*. 2000; Faheed 2012; Huang *et al*. 2013; Jini & Joseph 2017).

Exogenous application of SA increased membrane stability by decreasing malondialdehyde content (MDA) (Kazemi *et al*. 2011; Faheed 2012). In addition, SA was found to support the antioxidant scavengers in plants such as SOD, POX, CAT, APX and esterases (Horvath *et al*. 2007; Hayat *et al*. 2010; Liu et al. 2014; Abdul Qados 2015; Ma *et al*. 2017).

Exogenous application of SA results in increase of compatible osmotic solutes and/or activities of antioxidant enzymes (Pirasteh-Anosheh *et al*. 2014; Faheed 2012; Abdul Qados 2015; Ma *et al*. 2017). Except moringa plant, application of SA to improve growth under stress conditions was examined in different plant species (Javaheri *et al*. 2012; Bagheri 2014), but further studies using different plant species and stress conditions are needed to describe how SA mediates the recovery of plants from salinity damage. Consequently, the aim of this work was to investigate how salicylic acid mediates increase of ROS scavengers and membrane stability to mitigate the injuries of salt stress in Moringa.

## MATERIALS AND METHODS

### Growth conditions

In this work, Moringa seeds were obtained from CASP "Central Administration for Seed Production, 8 Gamaa El Kahira St, Giza, Egypt". Seeds were surface sterilized and divided into two groups. One half of these Moringa seeds were soaked for 5 h in 0.5 mM SA, while the other half of seeds was soaked for the same time in deionized water (control). For seed germination and plant growth, ten Moringa seeds were sown in plastic pots. Three weeks old plants were sprayed with or without 0.5 mM SA. Plants were harvested after further two weeks and subjected to the intended analysis. Plants were germinated and grow using plastic pots supplemented with 1700 gm air-dried soil. The field capacity of the used soil was determined (24.45%). Consequently, soil in pots was daily adjusted to full field capacity using one tenth strength of Hoagland's solution (Hewitt 1963).

### Determination of some growth parameters

Growth parameters including length, fresh weight (FW) and dry weight (DW) were determined/ seedling. Determination of DW was estimated after drying the plant material to constant weight at 65°C for 48 h in drying oven. Then, fresh weight and dry weight of the same sample was used to estimate water content.

### Determination of carotenoids

Carotenoids of moringa plants subjected to different concentrations of NaCl with or without SA treatments were measured in fresh leaf samples, a week before the harvest time. Moringa leaf samples (0.250 g) were homogenized in acetone (85% v/v). Carotenoids were calculated according to Lichtenthaler (1987).

## Determination of membrane permeability

Membrane permeability of the excised leaves of Moringa plants was estimated according to Yan *et al*. (1996). Briefly, parts from the middle of the leaves (0.5 gm) were cut, transferred into glass beaker containing 10 ml of deionized water and incubated at 30°C for 20 h in the dark with shaking. Using a conductivity meter (Mi 170 Bench Meter EC/ TDS/ NaCl/ Temp.), the electrolyte leakage (EC) was measured. After boiling for 2 min, samples conductivities were measured again and percentages of EC was calculated (% EC = EC1/EC2 x 100, EC1 and EC2 are the measured electrolyte conductivities before and after boiling, respectively).

## Determination of malondialdehyde

In terms of malondialdehyde (MDA) content, the level of lipid peroxidation was determined under the applied experimental conditions (Hernandez and Almansa 2002). Plant leaf (1000 mg) was homogenized in 5 ml of 5% trichloroacetic acid (TCA) under chill conditions; the obtained homogenate was centrifuged at 15000 g for 20 min at 4oC. Mixture of 1 mL supernatant, 3 mL of 0.5% thiobarbituric acid (TBA) and 20% TCA was prepared and heated at 95°C for 30 min. Then, mixture was quickly cooled on ice. The mixture was centrifuged at 10,000 g for 10 min and transferred to measure its absorbance at 532 nm. Non-specific absorption at 600 nm was subtracted. Finally, MDA equivalent was calculated (Zhang, 1992): MDA (μmol/g FW) = ((A532- A600)/ 155) ×103).

## Protein extraction

For determination of enzyme activity under the applied conditions, Moringa leaf tissue (0.5 gm) was homogenized in a mixture of 3 ml of 50 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA and 1% polyvinylpolypyrrolidone.

The homogenate was centrifuged at 15000 g for 15 min and the obtained supernatant was stored at -20 °C. All protein extraction steps were carried out at 4 °C. Enzyme activities were estimated using Appota 1600 UV/Vis spectrophotometer (Nakano & Asada 1981).

## Determination of superoxide dismutase

Protein extract of plants grown under the applied conditions was used to determine superoxide dismutase (EC. 1.15.1.1) activity according to Beauchamp and Fridovich (1971). In this concern, mixture of 3 ml containing 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 mM nitrobluetetrazolium (NBT), and 0.0044% (w/v) riboflavin were used. The photoreduction of NBT resulted in the formation of purple formazan and it was measured at 560 nm. Activity of SOD was expressed as the amount of SOD that inhibits the nitrobluetetrazolium photoreduction (Extinction factor (E) = 10.3 mM $cm^{-1}$). Then, enzyme activity was expressed as mM of NBT that reduced $min^{-1}$ $g^{-1}$ FW.

## Determination of peroxidase activity

Peroxidase (POX-EC 1.11.1.7) activity was estimated in 50 μL of the prepared enzyme extract using 3 mL of reaction mixture. It contains 40 mM phosphate buffer (pH 6.5), 0.1 mM EDTA, 25 mM guaiacol and 15 mM $H_2O_2$. Oxidation of guaiacol (E = 26.6 mM cm-1) was measured at 470 nm. The enzyme activity was estimated in terms of μmol of guaiacol that oxidized $min^{-1}$ $g^{-1}$ FW at 25 ± 2 °C (Zhang 1992; MacAdam *et al*. 1992).

## Determination of catalase activity

Catalase (CAT- EC 1.11.1.6) activity was estimated depending on previously described protocol of Aebi (1984). The reaction mixture (3 mL) was prepared to contain 50 mM phosphate buffer (pH 7.0), 0.1μM EDTA, 25 mM $H_2O_2$ and 50μL enzyme extract. The decrease in the concentration of $H_2O_2$ was measured at 240 nm and quantified by its molar extinction coefficient (36 $M^{-1}$ $cm^{-1}$). Results were expressed as μmol $H_2O_2$ $min^{-1}$ $g^{-1}$ FW.

## Determination of ascorbate peroxidase activity

Ascorbate (APX-EC 1.11.1.11) activity was investigated by estimating the rate of ascorbate oxidation (extinction coefficient: 2.8 $mm^{-1}$ $cm^{-1}$). Reaction mixture (3 ml) containing 50 mm

phosphate buffer (pH 7.0), 15 mM H2O2, 0.1 mm EDTA, 0.5 mm sodium ascorbate, and 50 µl enzyme extract was prepared. The enzyme activity was expressed as mM of ascorbate oxidized $mim^{-1}$ $g^{-1}$ FW at 25 ± 2 °C.

### Detection of isoenzyme patterns

Three isoenzymes (SOD, POX and EST) were visualized to study their expression under salinity stress with or without SA treatments using previously prepared protein extract. Slab gels (7.5 % polyacrylamide) were loaded by protein samples and electrophoretic runs were carried out at 24 mA for 6 h at 10 °C. Run buffer containing 0.025 M Tris and 0.129 M glycine buffer pH 8.9 was used. Bands of SOD, POX and EST were visualized according to the methods of Beauchamp & Fridovich (1971), Siegel & Galston (1967) and Brewer (1970), respectively.

### Statistical analysis

In this work, all experiments were designed as completely randomized with three replications. The obtained data were statistically analyzed by ANOVA.

## RESULTS

Germination percentage, plant height, fresh and dry weights and water content of moringa plants decreased significantly upon exposure to salt stress especially at relatively high salinity levels compared to control plants (Table 1). Reduction in the previous determined parameters was significantly improved when moringa plants were treated by 0.5 mM SA, especially at high salinity levels.

The carotenoids content of stressed moringa plants showed a highly significant decrease under all salinity levels (Table 2). Treatment of the salt stressed moringa plants with SA resulted in insignificant decrease in the carotenoids content at relatively low NaCl concentration (50 mM NaCl). On the other hand, exogenous application of SA caused a highly significant increase ($P < 0.01$) in the carotenoids content when the concentration of NaCl was increased more than 50 mM (100 to 200 mM).

The changes in stress markers such as membrane permeability, lipid peroxidation as well as concentration of H2O2 of moringa plants were studied (Fig. 1a). A significant increase in membrane permeability of excised moringa leaves was observed when the concentration of NaCl was increased more than 50 mM. Exogenous application of SA showed a significant decrease ($P < 0.05$) in membrane permeability.

It is well known that MDA is the key product of membrane-lipid peroxidation. The values of MDA in Moringa plants showed highly significant increase at moderate and high salinity levels compared to control plants. Exogenous application of SA alleviated significantly the drastic increase in MDA values ($P<0.05$) as compared with the corresponding salinized level (Fig. 1b).

Concentration of $H_2O_2$ showed highly significant increase in shoots of moringa plants under the influence of moderate and high levels of salinity.

Under these conditions, SA-treated plants showed a highly significant decrease ($P< 0.01$) in $H_2O_2$ concentration compared to SA untreated-stressed plants (Fig. 1c).

The activity of SOD, POX and APX increased with the increase in NaCl concentration (Fig. 2). On the other side, CAT activity showed a general decrease at salinity stress treatments except at 100 mM NaCl, where a significant increase in CAT activity was detected compared to that of control plants. In comparison with stressed- SA- untreated plants, exogenous application of SA resulted in significant increase ($P< 0.05$) in SOD, POX, CAT and APX activities.

**Table (1)** Effect of different concentrations of NaCl (0, 50, 100, 150, 200, 250 mM) on growth parameters of Moringa plants treated with or without 0.5 mM SA.

| | NaCl (mM) | Germination (%) | Shoot length (cm) | Root Length (cm) | Fresh Weight of Shoot (g plant$^{-1}$) | Dry Weight of Shoot (g plant$^{-1}$) | Water content /Shoot (%) | Fresh Weight of Root (g plant$^{-1}$) | Dry Weight of Root (g plant$^{-1}$) | Water content /Root (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| $H_2O$ | Control (0.0) | 94.44 | 18.30±1.01 | 10.40±1.39 | 1.64±0.09 | 0.54±0.07 | 67.07 | 0.51±0.03 | 0.136±0.03 | 73.55 |
| | 50 | 92.22 | 16.20±0.65** | 9.33±1.70 | 1.51±0.08* | 0.40±0.03* | 73.11 | 0.45±0.04 | 0.14±0.026 | 69.34 |
| | 100 | 76.66 | 14.03±0.20** | 8.43±0.51* | 1.39±0.07** | 0.31±0.02** | 77.75 | 0.34±0.04** | 0.097±0.01* | 71.83 |
| | 150 | 64.44 | 10.10±0.55** | 8.80±0.91* | 0.96±0.03** | 0.27±0.04** | 71.18 | 0.28±0.04** | 0.095±0.005* | 66.63 |
| | 200 | 46.44 | 8.70±0.72** | 5.73±0.64** | 0.69±0.04** | 0.26±0.06** | 62.68 | 0.23±0.04** | 0.076±0.011** | 66.96 |
| | 250 | 36.66 | 6.23±0.70** | 3.33±0.90** | 0.55±0.07** | 0.19±0.02** | 64.36 | 0.14±0.03** | 0.054±0.009** | 63.41 |
| SA | 0.0 | 92.22 | 17.80±0.36 | 11.06±0.96 | 1.63±0.05 | 0.52±0.09 | 68.16 | 0.52±0.03 | 0.131±0.043 | 74.68 |
| | 50 | 90 | 15.73±0.77** | 11.63±1.15 | 1.54±0.06 | 0.49±0.04 | 67.97 | 0.46±0.04 | 0.110±0.019 | 76.36 |
| | 100 | 66.67 | 14.33±0.80** | 9.10±1.24* | 1.40±0.03** | 0.30±0.09** | 78.20 | 0.4±0.07** | 0.101±0.016* | 74.75 |
| | 150 | 58.88 | 10.86±0.35** | 6.73±0.23** | 1.16±0.10** | 0.29±0.07** | 75.07 | 0.30±0.02** | 0.092±0.007* | 69.73 |
| | 200 | 41.11 | 9.16±0.60** | 5.56±0.70** | 0.92±0.05** | 0.27±0.04** | 70.04 | 0.27±0.03** | 0.096±0.014* | 65.06 |
| | 250 | 31.11 | 7.73±0.86** | 5.50±0.91** | 0.68±0.06** | 0.23±0.02** | 66.18 | 0.24±0.03** | 0.073±0.01** | 70.41 |
| LSD at 5 % | | | 0.86 | 1.30 | 0.05 | 0.038 | - | 0.031 | 0.006 | - |
| LSD at 1 % | | | 1.12 | 1.94 | 0.14 | 0.19 | - | 0.12 | 0.009 | - |

**Table (2)** Effect of different concentrations of NaCl (0, 50, 100, 150, 200, 250 mM) on carotenoids content of moringa plants treated with or without 0.5 mM SA

| | NaCl conc. (mM) | Carotenoid (mg/g FW) |
|---|---|---|
| $H_2O$ | 0 | 0.38±0.011 |
| | 50 | 0.32±0.003** |
| | 100 | 0.29±0.008** |
| | 150 | 0.19±0.011** |
| | 200 | 0.17±0.013** |
| | 250 | 0.11±0.006** |
| SA | 0 | 0.37±0.004 |
| | 50 | 0.34±0.004 |
| | 100 | 0.33±0.008** |
| | 150 | 0.24±0.011** |
| | 200 | 0.19±0.009** |
| | 250 | 0.13±0.010** |
| LSD at 5% | | 0.012 |
| LSD at 1% | | 0.048 |

Values are means of three replicates ± standard deviation (SD)
Statistical significance of differences compared to control: *significant at $P<0.05$; **significant at $P<0.01$

Under the applied conditions, SOD of salt stressed Moringa plants treated with or without SA was visualized (Fig. 3). Both SA treated or untreated plants expressed five isoenzyme forms (SOD-1, SOD-2, SOD-3, SOD-4 and SOD-5). Plants subjected to moderate or relatively high NaCl concentrations (150, 200 and 250 mM NaCl) expressed isoenzyme form SOD-6. The staining intensity of isoenzyme form SOD-1, SOD-2 and SOD-4 increased with the increase of NaCl concentrations. Comparison between SOD patterns of SA treated plants with SA untreated plants indicated that untreated-stressed plants showed slightly low staining intensity.

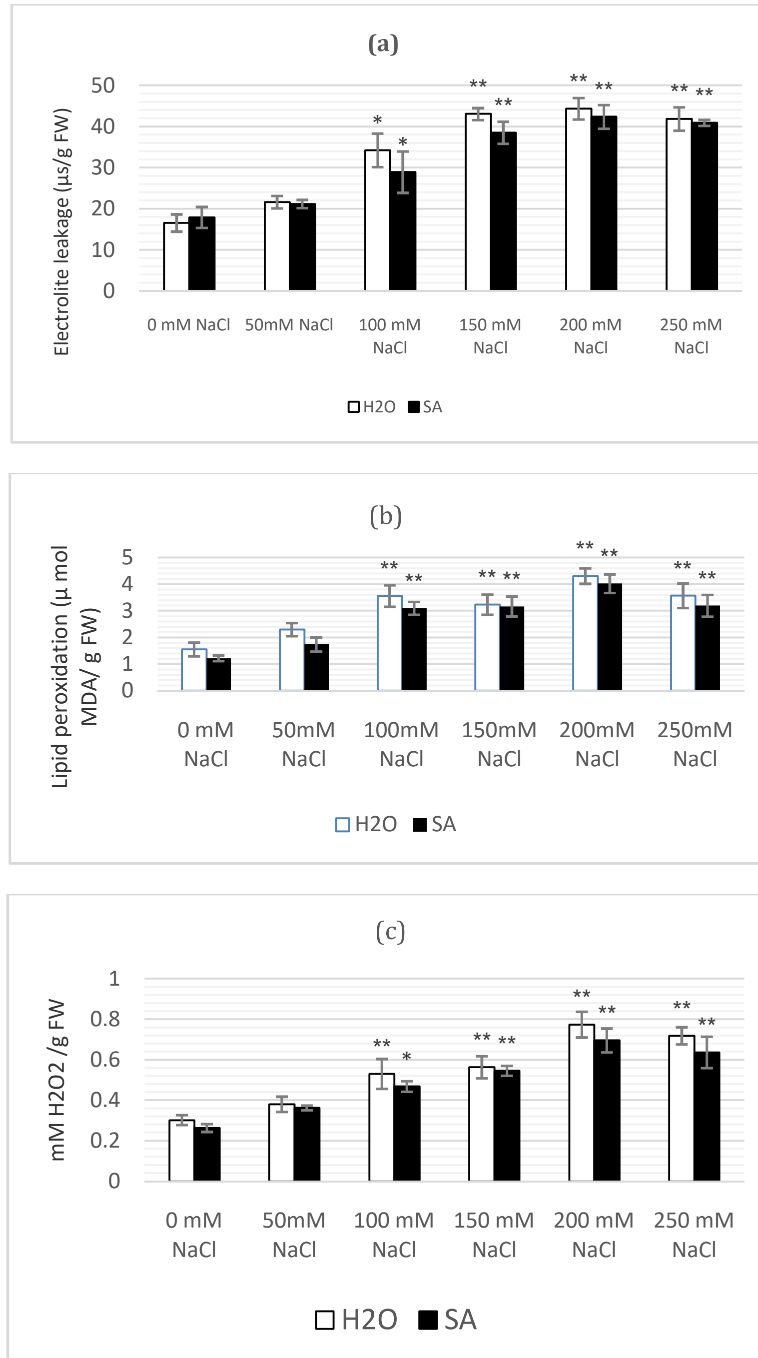


**Fig. (1)** Effect of (0, 50, 100, 150, 200, 250) mM NaCl, on (a) electrolyte leakage, (b) lipid peroxidation, (c) hydrogen peroxide production of the shoots of Moringa plants treated with or without 0.5 mM SA.

Moringa shoots expressed nine different peroxidase isoenzyme forms (Fig. 4). Generally, staining intensity of all POX isoenzyme forms increased with the increase of NaCl concentrations with or without SA treatments. Comparing between peroxidase patterns of SA untreated (lanes 1-6) plants with treated plants (lanes 7-12) indicated that SA treated plants expressed slight increase in staining intensity of POX isozyme forms.

A total of ten different EST isoenzyme forms were detected (Fig. 5). SA untreated plants expressed isoenzyme form EST-4 at salinity levels of 150 and 200 mM NaCl, while the same isoenzyme form was detected in SA treated plants subjected to 100, 150, 200 and 250 mM NaCl levels.

(a)

SOD µM NBT/g FW

12 10 8 6 4 2 0

0 mM NaCl | 50mM NaCl | 100 mM NaCl | 150 mM NaCl | 200 mM NaCl | 250 mM NaCl

H2O SA

(b)

POX (mM tetraguaiacol/g FW)

6 5 4 3 2 1 0

0 mM NaCl | 50mM NaCl | 100 mM NaCl | 150 mM NaCl | 200 mM NaCl | 250 mM NaCl

H2O SA

(c)

CAT(MM H2O2/G FW)

1.0 0.8 0.6 0.4 0.2 0.0

0 mM NaCl | 50mM NaCl | 100 mM NaCl | 150 mM NaCl | 200 mM NaCl | 250 mM NaCl

H2O SA

(d)

APX (mM Ascorbate/g FW)

15 10 5 0

0 mM NaCl | 50mM NaCl | 100 mM NaCl | 150 mM NaCl | 200 mM NaCl | 250 mM NaCl

H2O SA

**Figure (2)** Effect of (0, 50, 100, 150, 200, 250) mM NaCl, on activity of (a) SOD, (b) POX, (c) CAT, (d) APX of the shoots of moringa plants treated with or without 0.5 mM SA.

Values are means of three replicates ± standard deviation (SD)
Statistical significance of differences compared to SA untreated plants: *significant at P<0.05; **significant at P<0.01.

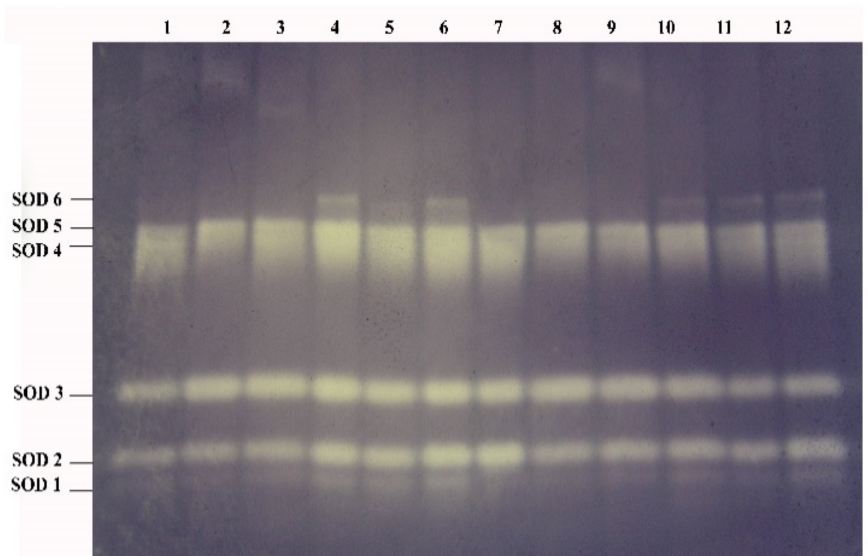


**Figure (3)** Native gel electrophoresis of SOD isoenzyme pattern of moringa shoots grown for five weeks in soil under salinity stress, SA treated plants (lanes 1 to 6), SA untreated plants (lanes 7 to 12); 0 (lanes 1, 7), 50 (lanes 2, 8), 100 (lanes 3, 9), 150 (lanes 4, 10), 200 (lanes 5, 11), 250 (lanes 6, 12) mM NaCl respectively.

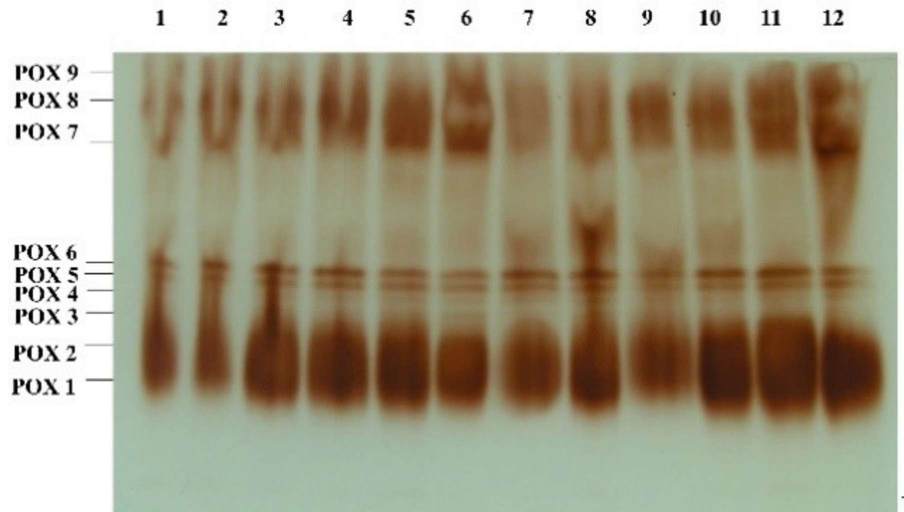


**Figure (4)** Native gel electrophoresis of POX isoenzyme pattern of moringa shoots grown for five weeks in soil under salinity stress, SA untreated plants (lanes 1 to 6), SA treated plants (lanes 7 to 12); 0 (lanes 1, 7), 50 (lanes 2, 8), 100 (lanes 3, 9), 150 (lanes 4, 10), 200 (lanes 5, 11), 250 (lanes 6, 12) mM NaCl respectively.

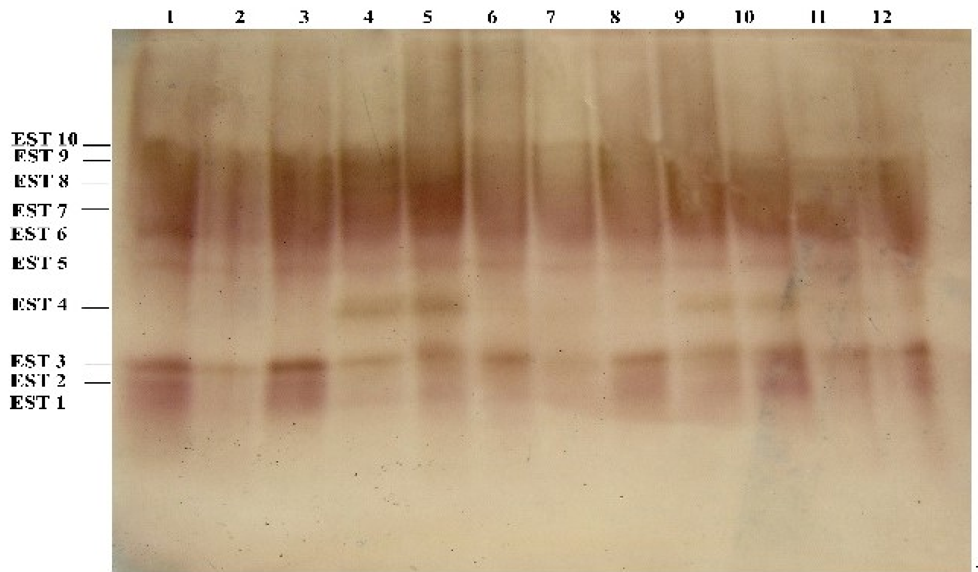


**Figure (5)** Native gel electrophoresis of EST isoenzyme pattern of moringa shoots grown for five weeks in soil under salinity stress, SA untreated plants (lanes 1 to 6), SA treated plants (lanes 7 to 12); 0 (lanes 1, 7), 50 (lanes 2, 8), 100 (lanes 3, 9), 150 (lanes 4, 10), 200 (lanes 5, 11), 250 (lanes 6, 12) mM NaCl respectively.

## DISCUSSION

The obtained results showed that salt stress decreased seed germination and plant growth in Moringa. This reduction may be explained as an adaptive response of Moringa to salt stress (Acosta-Motos *et al*. 2015). The reduction in growth was significantly improved upon treatment of Moringa plants with 0.5 mM SA. In our work, Moringa plants were raised from the seeds pre-soaked in SA, it was potentially alleviate the toxic effects which were generated by NaCl leading to expression of effective tolerance against salinity. Exogenous application of SA increased some physiological concepts; thereby SA improved growth of salt stressed Moringa plants and other plant species (El-Tayeb 2005).

Stress tolerance is positively correlated with increase the efficiency of antioxidative systems including enzymatic and non-enzymatic

components. Carotenoids are effective non-enzymatic antioxidant component which are able to share with other components to scavenge ROS (Miyake & Asada 1994; Ramel *et al*. 2012), but they showed a highly significant decrease under salinity stress in Moringa. This situation could be significantly reversed when Moringa plants were treated with SA.

Diverse abiotic stresses adversely affect the integrity and functions of plasma membrane. Membrane disruptions take place in plants subjected to salinity stress and its degree is measured by the levels of electrolyte leakage (Hayat *et al*. 2012, Barkla & Vera-Estrella 2015; Guo *et al*. 2019). In this work, electrolyte leakages increased significantly under moderate and relatively high NaCl concentrations up to 250 mM. Membrane damage by salt stress induced accumulation of ROS leading to oxidative damage. ROS species can activate either GORK (guard cell outward-rectifying K) or NSCC (nonselective cation channels) channels to enhance poatssium leakage leading to cell death under salt stress (Chakraborty *et al*. 2016; Wu *et al.* 2018). So, prevention of K leakage via ROS-activated NSCC under salt stress is an essential prerequisite for salt tolerance in plants. Application of SA can control K loss via ROS activated NSCC in salt stressed plants (Demidchik *et al*. 2010; Poór *et al*. 2011b). In addition, adversity effects of salinity were moderately ameliorated by the exogenous application of SA as was reported by (Zhang *et al*. 2014).

The obtained data indicated that increase the concentrations of NaCl levels induce extensive lipid peroxidation in moringa plant. Level of lipid peroxidation, was measured as malondialdehyde (MDA) content and it increased under salinity stress which may be due to the oxidative damage of the organelle membranes. Exogenous SA improved the growth parameters of moringa plant under salt stress by promoting more stress tolerance through the enhancement of photosynthetic pigments and maintaining membrane integrity as was reported (El-Tayeb 2005; Gunes *et al*. 2007).

Moringa plants under salt stress showed highly significant increase in $H_2O_2$ at moderate and high levels of salinity, it was significantly reduced by SA treatment; thereby it resulted in additional tolerance against oxidative stress generated by NaCl (Panda & Patra 2007). Both SA and $H_2O_2$ at low concentrations are signaling molecules, resulting in additional stress tolerance against biotic and abiotic stresses (Love *et al*. 2008; Quan *et al*. 2008). Obtained data indicated that exogenous application of SA decreased the concentration of $H_2O_2$ of salt-stressed Moringa plants.

When plants are subjected to environmental stresses, ROS are often elevated leading to disruption of the plant cell metabolism and reduction in plant growth. In Moringa, the activities of a variety of antioxidant enzymes such as SOD, POD, CAT and APX were increased to control ROS damage. These enzymes reduce the elevated concentration of $H_2O_2$, prevent lipid peroxidation and decrease leakage of important nutrients such as K (data not shown). Further increase of these enzymes was detected when stressed plants were treated with SA. Increase the activities of antioxidant enzymes in salt stressed moringa plants due to SA was confirmed in other plant species (Knorzer *et al*. 1999; Zhang *et al*. 2014).

In this work, two of the studied antioxidant enzymes were stained to detect any variation in the expression of iso-loci of these enzymes. Both SA treated and untreated moringa plants expressed five isoenzyme forms of peroxidases but the sixth one (SOD-6) was detected only under moderate or relatively high NaCl concentrations (150, 200 and 250 mM NaCl). Generally, staining intensity of SOD or POX isoezyme forms increased with the increase of NaCl concentrations, further slight increase in staining intensities of some bands of SOD (SOD-2 and ) and POX (POX-1, 2, 5, 6, 7, 8) was detected when salinized plants were treated with SA. The results obtained from determination of enzyme activity (Fig. 2 a & b) were consistent with the results obtained from studying the staining intensity and number of isoenzyme forms

(Fig. 3 & 4). Increase the staining intensities of detected bands gave an indication about increase in the activity of the studied isoenzyme (Khavkin & Zabtrodina 1994). Generally, the detected increase in SOD and POX activities due to salt stress or SA treatment was not because of expression of new isoenzyme form/s but duo to increase of the expression of the switched on iso-loci.

Data of this work confirmed the participation of esterases in stress tolerant mechanisms as was previously reported by Hassanein (1999). In this concern, isoenzyme form number EST-4 was detected only under moderate and relatively high salt stress. In addition, its expression was detected under wide range of NaCl concentrations when plants were treated with SA more than salinized-SA-untreated plants. Esterases can liberate ferulic acid from polysaccharides which form components of lignin in plant cell wall (Scalbert *et al*. 1985; Faulds *et al*. 1998; Faulds *et al*. 2010). Dehydration stress was avoided when ferulic acid was used due to increase the activities of several antioxidant enzymes such as catalase, superoxide dismutase, and quaiacol peroxidase and it associated with increase transcripts of respective genes. Then, increase the activities of these enzymes decreased the contents of malondialdehyde, superoxide radical and hydrogen peroxide. To sustain stress tolerance, ferulic acid increased tissue water content and osmotic solutes such proline and soluble sugars resulting in growth improvement (Lee *et al*. 2013). Our data indicated that the role of esterase during salt stress may be partially due to sustain antioxidant system mediated by ferulic acid.

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