Physiological response of henna (*Lawsonia inermis* L.) to salicylic acid and salinity

Hassan Farahbakhsh a,b, Amin Pasandi Pour a,c and Narges Reiahid d

aFaculty of Agriculture, Department of Agronomy and Plant Breeding, Shahid Bahonar University of Kerman, Kerman, Iran; bResearch and Technology Institute of Plant Production, Shahid Bahonar University of Kerman, Kerman, Iran; cYoung Research Society, Shahid Bahonar University of Kerman, Kerman, Iran; dFaculty of Agriculture, Department of Agronomy, Sari University, Sari, Iran

**ABSTRACT**

Henna (*Lawsonia inermis* L.) is naturally cultivated from north-east Africa to India as a medicinal-industrial plant. The objective of this study was to evaluate the possible role of salicylic acid (SA) for mitigating the salinity stress. For this purpose, we evaluated the effect of three concentrations of SA (0, 40 and 80 μM) and salinity (0, −3 and −6 bar) on photosynthetic pigments, protein content, catalase (CAT, EC 1.11.1.6) activity, electrolyte leakage and leaf relative water content (RWC). The experiment was carried out with a factorial arrangement based on complete randomized design in triplicates at University of Kerman, Iran. The results revealed that salinity caused a significant decrease in photosynthetic pigments, protein content, RWC and quantum yield of henna. By increase in salinity levels from 0 to −6 bar, the mean values of mentioned traits were reduced. CAT activity, electrolyte leakage, $F_o$ and $F_m$ were elevated significantly with increasing the salinity concentration. Application of SA under salinity stress increased the photosynthetic pigments, protein content, CAT activity, leaf RWCS and quantum yield, while it decreased electrolyte leakage, $F_o$ and $F_m$. It can be concluded that SA alleviated the stress generated by NaCl possibly through the ameliorated antioxidant defense system.

**1. Introduction**

Worldwide industrial demand for the medicinal plant resources is rising due to buoyancy in the herbal sector engaged in production of medicinal herbs and health care formulations such as herbal based cosmetic products and herbal nutritional supplements (Ved & Goraya, 2007).

*Lawsonia inermis* L. is an important medicinal-industrial plant that popularly known as henna. It grows in warm and arid regions. No reliable figures are available on the global and regional scale in the production and area under cultivation. The major exporters of henna are India, Pakistan, Iran, Sudan and Egypt. The major importers are the Islamic countries of the Near East and North Africa (Farooqi & Sreeramu, 2004). The dried leaf-yield in the first year of cultivation is around 200 kg ha$^{-1}$ while in the years after, the yields normally range from 1000 to 1500 kg ha$^{-1}$ with three cropping per year (Farooqi & Sreeramu, 2004). The dye which is derived from green leaves of henna is used for decorating the body with intricate designs and the principle coloring matter is lawsone, 2-hydroxy-1, 4-naphthoquinone (Prosen et al., 2005). Literature show henna possess nootropic (Iyer et al., 1998), analgesic, antipyretic, anti-inflammatory (Alia et al., 1995), antibacterial (Vinoth et al., 2013) and anti-immunomodulatory activities (Mikheil et al., 2004) along with other properties. The natural constituents of henna are essential oils, 1,4-naphthoquinone, tannins, gallic acid, flavonoids, lipids, sugars, triacontyltridecanoate, mannitol, xanthones, coumarins (5-alkyloxy 7-hydroxy-coumarin), 2–3% resins, 5–10% tannic ingredients and up to 2% lawsone (2-hydroxy-1,4-naphthoquinone). A major portion of lawsone is glycosidic bound, which is cleaved by enzymatic hydrolysis of the glycosidichennosids and auto oxidation of a glucons.

Salinity is one of the most important limiting factors for agricultural development around the world (Abdel Latef, 2010). Unlike drought, salinity stress is an intricate phenomenon that includes osmotic stress, specific ion effect, nutrient deficiency, etc., thereby affecting various physiological and biochemical mechanisms associated with plant growth and development. Salinity disrupts cellular processes through several mechanisms such as inducing
osmotic stress by limiting water absorption and ionic stress as a result of high concentrations of toxic salt ions (Kohler et al., 2009).

Plant cells evolve an antioxidative defense system to protect cellular structures from oxidative damage. The importance of cellular antioxidant machinery for protection against various environmental stresses has been well documented (Gill et al., 2011). Salt stress may affect the operation of photosynthetic mechanism, namely photosystem II (PSII). Measurements of chlorophyll fluorescence, like maximal PSII photochemical efficiency ($F_v/F_m$), make it possible to evaluate the plant’s photosynthetic performance and the extent of its tolerance to salt stress (Maxwell & Johnson, 2000).

Salicylic acid or orthohydroxy benzoic acid is ubiquitously distributed in the whole plant kingdom. In early 1960s, it was suggested that salicylic acid is synthesized in plants from cinnamic acid by two possible pathways. One pathway involves the decarboxylation of the side chain of cinnamic acid to form benzoic acid, which in turn undergoes a 2-hydroxylation to form salicylic acid (Hayat et al., 2010). The other pathway proposed for the biosynthesis of salicylic acid involves a 2-hydroxylation of cinnamic acid to o-coumaric acid which is then decarboxylated to salicylic acid (Hayat et al., 2010).

A high level of salinity induces serious metabolic perturbations in plants, as it generates ROS (reactive oxygen species) which disturb the cellular redox system in favor of oxidized forms, thereby creating an oxidative stress that may damage DNA, inactivate enzymes and cause lipid peroxidation (Smirnoff, 1993). Damaging effects of salinity were alleviated by exogenous application of SA in Arabidopsis seedlings (Borsani et al., 2001). Kaydan et al. (2007) observed that presowing soaking treatment of seeds with SA positively affected the osmotic potential and contents of photosynthetic pigments (chlorophyll a, b and carotenoids) in wheat (Triticum aestivum) seedlings, under both saline and nonsaline conditions.

With considering the fact that the under severe stress conditions antioxidant capacity may not be sufficient to minimize the harmful effect of oxidative injury, we set this experiment to evaluate SA effect for reducing harmful effect of oxidative injury, and also determine the possible role of exogenous application of SA to induce salt tolerance in L. inermis L. and optimize the most effective concentration of SA for future investigations.

2. Materials and methods

2.1. Plant material and stress treatments

A factorial experiment based on complete randomized design with three replicates was carried out at the glasshouse of agriculture faculty of Shahid Bahonar University of Kerman, Iran. Henna (L. inermis L.) seeds were surface-sterilized with a 3% sodium hypochlorite solution, rinsed in distilled water for three times and dried before the experiment. Afterward, the seeds were sown in pots containing peat and placed in the glasshouse condition (14 h photoperiod, 60% relative humidity and 25/22 °C day/night). The seedlings were irrigated with tap water during the first 5 days and then with a Hoagland solution, pH 6.2–6.5. When the plants had 5–6 leaves, they were transferred to hydroponic culture for 3 days. These plants were treated with different concentrations of salicylic acid (SA) (0, 40 and 80 μM) for 48 h and then again transferred to Hoagland solution for 24 h before being exposed to salinity stress (0, −3 and −6 bar which was caused by 0, 65 and 134 mM NaCl, respectively). After 48 h in this condition, the plant materials were harvested and stored in −80 °C for measuring photosynthetic pigments, protein content and CAT activity.

2.2. Electrolyte leakage determination

Fifteen discs of fresh leaf (.25 cm² of each leaf) were rinsed three times (2–3 min) with deionized water and then floated on 10 mL of deionized water (Campos et al., 2003). The electrolyte leakage in the solution was measured after 22 h of floating at room temperature (EC$_1$) using a conductivity meter (Elmetron, C−C 505). Total conductivity was obtained after keeping the flasks in an oven (90 °C) for 2 h (EC$_2$). Results were expressed as relative electrolyte conductivity (REC).

$$REC = \left(\frac{EC_1}{EC_2}\right) \times 100$$

(1)

2.3. Relative water content determination

Relative water content (RWC) was determined using fresh leaf discs of 2 cm². After weighting, the leaf discs were immersed in deionized water for 24 h and excess water wiped with tissue paper. Saturated leaf weights were recorded and the dry masses measured after drying at 70 °C for 48 h. The following equation was used to calculate RWC:

$$RWC = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgor weight} - \text{dry weight}} \times 100$$

(2)

2.4. Measurement of chlorophyll and carotenoid content

Samples (100 mg fresh leaves) were homogenized in chilled 80% (v/v) acetone and centrifuged at 11,200×g for 10 min at 4 °C. Absorbance of the acetone extracts was measured at 663, 645 and 470 nm. The contents of chlorophyll a, b, a+b and carotenoid were calculated as described by Lichtenthaler (1987).
2.5. Preparation of enzyme extract

Fresh leaf tissues (100 mg sample⁻¹) were placed into liquid nitrogen and then homogenized with a prechilled mortar and pestle under ice-cold conditions in 4 ml 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA (ethylenediamine tetraacetic acid). The homogenates was centrifuged at 25,200×g, at 4°C for 20 min (Mishra et al., 1993). The supernatant was stored at −20°C and used for the assay of enzyme activity.

2.6. Determination of protein content

Total soluble protein was measured according to Bradford (1976). Hundred microliter of enzyme extract was added to 5 ml of protein reagent and after 25 min the absorbance was read at 595 nm with spectrophotometer (SPUV-26, SCO-TECH).

2.7. Assay of catalase activity

Catalase activity was determined by consumption of H₂O₂ using the method of Dhindsa et al. (1981). The reaction mixture contained 50 mM potassium phosphate buffer with pH 7.0, 15 mM H₂O₂ and enzyme extract. The consumption of H₂O₂ was spectrophotometrically monitored at 240 nm (e = .28 mM⁻¹ cm⁻¹). The enzyme activity was expressed in terms of unit mg⁻¹ protein. One unit of CAT is the amount of enzyme that decomposes 1 mM H₂O₂ in 1 min.

2.8. Chlorophyll florescence

The chlorophyll fluorescence was measured with the saturation pulse method. A leaf was adapted to dark condition for at least 10–15 min prior to the measurement and starting the test. Then, F₀, or minimal fluorescence, was measured without actinic light and the application of a saturating pulse (about 8000 μmol m⁻² s⁻¹ for .6–1 s), raised the fluorescence to a maximum value (Fₘ). This measurement allows the determination of the maximum quantum efficiency of photosystem II (PSII), given as Fᵥ/Fₘ. This parameter is called ‘intrinsic quantum yield’ (Kitajima & Butler, 1975), calculated as follows:

\[ F_v/F_m = (F_m - F_0)/F_m \]  (3)

2.9. Statistical analysis

Analysis of variance (ANOVA) and correlation analysis were performed on the data using SAS (ver. 9.2, 2010). The means were evaluated with the LSD test, and the differences were considered significant if P values were ≤.05. Stepwise multiple regression analyses were performed using dry matter as dependent variable and other parameters as independent variables. Since the variables are not in the same unit of measures, a standardized regression coefficient, beta (β), was used. The β coefficients values show the direction either positive or negative and the contribution of the independent variable relative to the other independent variables in explaining the variation of the dependent variable.

3. Results

3.1. RWC

RWC was significantly affected by both salinity and SA application as indicated in Table 1. The changes in RWC under different salinity levels followed a linear relationship (Table 1). This parameter was decreased with increasing salinity levels so that the lowest value was gained at −6 bar osmotic potential (Table 2). The regression relationship between RWC and SA was quadratic (Table 1). It was noted that there was a significant increase in RWC with application of 40 μM SA whilst the highest concentration (80 μM) had a negative effect on the trait in comparison with control (0 μM SA) (Table 2). Interaction of SA and salinity stress showed that in all salinity levels the concentration of 40 μM SA was more effective than the other levels for alleviating the harmful effects of salinity; the results are shown in Table 2.

Based on correlation results, there was a positive correlation between RWC with dry matter under normal and salt stress (−3 and −6 bar) conditions (Tables 3–5). The Pearson correlation coefficients were significant just under moderate (−3 bar) and severe (−6 bar) salt stress conditions. Also, the correlation between RWC and quantum efficiency of photosystem II (Fᵥ/Fₘ) was positive and significant for all conditions (Tables 3–5).

3.2. Relative electrolyte conductivity (REC)

This trait was significantly affected by the treatments and their interaction (Table 1). There was a linear relationship between REC and salinity levels (Table 1). The highest and lowest amounts of REC was obtained in −6 bar and control treatments, respectively (Table 2). In fact, increment in osmotic potential (from 0 to −6 bar) resulted in increased electrolyte leakage. The effect of SA treatment on the trait was significant at 1% probability level (Table 1). A significant quadratic regression was obtained between REC and SA (Table 1). Minimum of this trait belonged to concentration of 40 μM SA, while the maximum value of REC was obtained from SA level of 80 μM (Table 2). The response of this trait varied at different levels of salinity with different concentrations of SA (Table 2). The concentration of 40 μM of SA had the lowest REC value under normal and stress condition.
Table 1. Variance analysis of measured traits in Henna (L. inermis L.) in response to salicylic acid and salinity stress.

| S.O.V        | df  | RWC  | REC  | Chl a | Chl b | Chl a+b | Car  | Protein | CAT  | Dry matter | F₀    | Fₘ    | Fₒ/Fₘ  |
|--------------|-----|------|------|-------|-------|---------|------|---------|------|------------|-------|-------|---------|
| Salinity     | 2   | 1589.56** | .2929** | .719** | .4737** | 2.3573** | .04205** | 25.284** | .07727** | 20.6512** | 14.402** | 67.080** | .0028** |
| Linear       | 1   | 3172.5*** | .568** | 1.377*** | .929** | 4.570** | .084** | 50.003** | .157** | 40.95** | 28.560** | 132.956** | .0057** |
| Quadratic    | 1   | 6.538ns | .0170** | .060** | .0181** | .144** | .0004ns | .564* | .0014ns | .352** | 244.9** | 1204** | .0001** |
| SA           | 2   | 467.039** | .0160** | .0589** | .0607** | .2386** | .01825** | 13.352** | .05378** | .8125** | 848.48** | 4488.4** | .0015** |
| Linear       | 1   | 113.75** | .057** | .070** | .256** | .1022** | 4.307** | .1073** | .261** | 648** | 3930** | .0011** |
| Quadratic    | 1   | 820.32** | .050** | .060** | .220** | .0262** | 22.399** | .00002ns | 1.363** | 1048** | 5046** | .0002** |
| Salinity × SA| 4   | 54.898** | .0053** | .0158** | .01625** | .05811** | .00198** | 2.5589** | .0037** | .0854** | 34.148** | 166.88** | .00013* |
| Error        | 18  | 2.94  | 2.91  | 2.44  | 4.18  | 2.96  | 3.86  | 3.35  | 2.79  | 1.62  | .93  | .35  | .23  |

Notes: S.O.V: Source of variation, SA: Salicylic acid, CV: Coefficient variation, df: Degree of freedom, RWC: Relative water content, REC: Relative electrolyte conductivity, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Car: Carotenoid, CAT: Catalase, F₀: Minimum florescence yield, Fₘ: Maximum florescence yield, Fₒ/Fₘ: quantum efficiency of photosystem II. **, * and ns significant at 1 and 5% probability levels and nonsignificant, respectively.

Table 2. Mean Comparisons of measured traits in Henna (L. inermis L.) in response to salicylic acid and salinity stress.

| Salinity | SA | RWC (%) | REC | Chl a (mg g⁻¹ FW) | Chl b (mg g⁻¹ FW) | Chl a+b (mg g⁻¹ FW) | Car (mg g⁻¹ FW) | Protein (mg g⁻¹ protein) | Dry matter (g) | F₀ | Fₘ | Fₒ/Fₘ |
|----------|----|----------|-----|------------------|------------------|-------------------|----------------|--------------------------|----------------|----|-----|--------|
| Control  | Control | 87.86a | .48g | 2.09a | 1.15a | 2.337a | .687a | 11.32a | .113f | 5.9451b | 204.6h | 1237h | .834a |
|          | 40 μM  | 88.59a | .46g | 1.98b | 1.04b | 3.02b | .677ab | 10.88a | .17e | 6.2153b | 204.3h | 1235.3h | .834a |
|          | 80 μM  | 82.80b | .51f | 1.87c | .867c | 2.737c | .627cd | 8.637c | .24d | 6.0152b | 222g | 1281.6g | .826b |
|          | −3 bar | 68.45c | .72d | 1.58e | .727d | 2.31e | .59de | 7.393d | .13f | 3.8551e | 243.3e | 1324.3e | .816d |
|          | 40 μM  | 83.16b | .66e | 1.68d | .82c | 2.5d | .647bc | 9.88b | .26d | 4.7992c | 233f | 1303.3f | .821c |
|          | 80 μM  | 64.68d | .76c | 1.55e | .66e | 2.21e | .557e | 7.642d | .36b | 4.2695d | 255d | 1341.6d | .809e |
|          | −6 bar | 57.92e | .84b | 1.42f | .54f | 1.95f | .517f | 6.35e | .306c | 2.7696c | 291.3b | 1421b | .794g |
|          | 40 μM  | 70.01c | .79c | 1.55e | .64e | 2.19e | .597d | 8.637c | .363b | 3.3472f | 280.3c | 1401c | .799f |
|          | 80 μM  | 51.67f | .89a | 1.32g | .51f | 1.83f | .467g | 5.85f | .41a | 3.0088g | 298.3a | 1447.6a | .793g |

Notes: SA: Salicylic acid, RWC: Relative water content, REC: Relative electrolyte conductivity, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Car: Carotenoid, CAT: Catalase, F₀: Minimum florescence yield, Fₘ: Maximum florescence yield, Fₒ/Fₘ: quantum efficiency of photosystem II. The means with the same letters are not significantly different (LSD).
Pearson’s correlation analysis showed that correlation coefficients between REC with photosynthetic pigments, protein content, catalase activity and quantum efficiency of photosystem II were negative and nonsignificant under normal condition (Table 3).

### 3.3. Photosynthetic pigments

The content of chlorophyll (Chl) a, b, a+b and carotenoid were significantly affected by both salinity and SA treatments (Table 1). All photosynthetic pigments measured had a linear relationship with salinity changes (Table 1). Maximum value of Chl a was recorded due to no stress condition while increase in osmotic potential led to a significant reduction in the trait (Table 2). The highest and lowest content of Chl a belonged to concentrations of 40 and 80 μM SA, respectively (Table 2). Interaction of salinity and SA (Table 2) indicated that application of SA under no salinity condition, caused a significant decrease in the content of Chl a, indicating the highest content of Chl a was gained from 0 μM SA (control). Photosynthetic pigments content as affected by SA varied under tested salinity conditions. The highest value of the Chl a belonged to 40 μM SA treatment. Similar results were found for Chl b, a+b and Carotenoid.

Based on Pearson’s correlation analysis, there was positive and significant correlation between Chl a with quantum efficiency, $r = .789$ (Table 3), $r = .821$ (Table 4) and $r = .679$ (Table 5).

### 3.4. Protein content

Salinity and SA treatments affected protein content significantly ($p < .01$). Protein content decreased with increasing salinity (Table 2), based on the obtained significant linear regression (Table 1). The variations of protein content under different SA levels were explained with a quadratic regression (Table 1). The highest and lowest protein content was obtained from concentrations of 40 and 80 μM of SA, respectively (Table 2). Salinity stress × SA indicated that 40 μM SA lead to producing the highest protein content under normal and stress conditions (Table 2).

The Pearson correlation between protein content and dry matter under normal condition is about .061, which indicates that there is a very small positive correlation between the variables (Table 3). This coefficient increased under moderate and severe salinity stress (−3 and −6 bar) to .919 and .812, respectively (Table 4 and 5).

### 3.5. CAT activity

A significant linear regression was established between CAT activity and salinity (Table 1, Figure 1(a)). Increasing
### Table 4. Pearson correlation coefficients of Henna (*L. inermis* L.) parameters under moderate salt stress (−3 bar) condition.

|          | Mean   | Std Dev | Dry matter | RWC    | REC     | Chla    | Chlb    | Chla+b  | Car     | Protein | CAT     | \( F_0 \) | \( F_m \) | \( F_{v/F_m} \) |
|----------|--------|---------|------------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------------|
| Dry matter | 4.307  | .417    | 1          |        |         |         |         |         |         |         |         |         |         |         |              |
| RWC      | 72.09  | 8.705   | .763*      | 1      |         |         |         |         |         |         |         |         |         |         |              |
| REC      | .72    | .044    | −.565      | −.857**| 1       |         |         |         |         |         |         |         |         |         |              |
| Chla     | 1.603  | .068    | .700*      | .852** | −.580   |         |         |         |         |         |         |         |         |         |              |
| Chlb     | .735   | .071    | .622       | .924** | −.803** | .924**  | 1       |         |         |         |         |         |         |         |              |
| Chla+b   | 2.338  | .137    | .671*      | .904** | −.705*  | .982**  | .984**  | 1       |         |         |         |         |         |         |              |
| Car      | .597   | .045    | .608       | .842** | −.596   | .989**  | .948**  | .984**  | 1       |         |         |         |         |         |              |
| Protein  | 8.305  | 1.217   | .919**     | .934** | −.741*  | .871**  | .851**  | .826**  | 1       |         |         |         |         |         |              |
| CAT      | .252   | .103    | .522       | −.123  | .285    | −.103   | −.320   | −.217   | −.233   | .179    | 1       |         |         |         |              |
| \( F_0 \) | 243.7  | 9.627   | .515       | −.890** | .885**  | −.828** | −.964** | −.913** | −.866*  | −.783*  | .443    | 1       |         |         |              |
| \( F_m \) | 1323   | 16.83   | −.922      | −.912** | .907**  | −.834** | −.962** | −.919** | −.861** | −.821** | .359    | .986**  | 1       |         |              |
| \( F_{v/F_m} \) | .815   | .004    | .480       | .875** | −.863** | .821**  | .958**  | .906**  | .863**  | .763*   | −.472   | −.997** | −.972** | 1         |              |

Notes: Std Dev: Standard deviation, RWC: Relative water content, REC: Relative electrolyte conductivity, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Car: Carotenoid, CAT: Catalase, \( F_0 \): Minimum fluorescence yield, \( F_m \): Maximum fluorescence yield, \( F_{v/F_m} \): Quantum efficiency of photosystem II. ** and * significant at 1 and 5% probability levels, respectively.

### Table 5. Pearson correlation coefficients of Henna (*L. inermis* L.) parameters under severe salt stress (−6 bar) condition.

|          | Mean   | Std Dev | Dry matter | RWC    | REC     | Chla    | Chlb    | Chla+b  | Car     | Protein | CAT     | \( F_0 \) | \( F_m \) | \( F_{v/F_m} \) |
|----------|--------|---------|------------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------------|
| Dry matter | 3.041  | .258    | 1          |        |         |         |         |         |         |         |         |         |         |         |              |
| RWC      | 59.86  | 8.184   | .698*      | 1      |         |         |         |         |         |         |         |         |         |         |              |
| REC      | .84    | .046    | −.513      | −.882**| 1       |         |         |         |         |         |         |         |         |         |              |
| Chla     | 1.426  | .103    | .648       | .993** | −.849** | 1       |         |         |         |         |         |         |         |         |              |
| Chlb     | .563   | .064    | .724*      | .961** | −.720*  | .973**  | 1       |         |         |         |         |         |         |         |              |
| Chla+b   | 1.990  | .167    | .681*      | .987** | −.805** | .996**  | .989**  | 1       |         |         |         |         |         |         |              |
| Car      | .526   | .058    | .649       | .999** | −.863** | .998**  | .968**  | .993**  | 1       |         |         |         |         |         |              |
| Protein  | 6.945  | 1.309   | .812**     | .936** | −.907** | .897**  | .851**  | .885**  | .901**  | 1       |         |         |         |         |              |
| CAT      | .360   | .049    | .339       | −.282  | .340    | −.345   | −.219   | −.299   | −.320   | −.126   | 1       |         |         |         |              |
| \( F_0 \) | 290    | 8.200   | −.660      | −.937** | .921**  | −.918** | −.849** | −.897** | −.914** | −.957** | .360    | 1       |         |         |              |
| \( F_m \) | 1423   | 21.188  | −.442      | −.939** | .878**  | −.949** | −.880** | −.928** | −.955** | −.813** | .427    | .867**  | 1       |         |              |
| \( F_{v/F_m} \) | .796   | .003    | .716**     | .737*  | −.759*  | .697**  | .643    | .681*   | .685   | .879*   | −.217   | −.900** | −.565  | 1         |              |

Notes: Std Dev: Standard deviation, RWC: Relative water content, REC: Relative electrolyte conductivity, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Car: Carotenoid, CAT: Catalase, \( F_0 \): Minimum fluorescence yield, \( F_m \): Maximum fluorescence yield, \( F_{v/F_m} \): Quantum efficiency of photosystem II. ** and * significant at 1 and 5% probability levels, respectively.
salinity levels caused a remarkable increase in CAT activity. The highest and lowest CAT activity was recorded for −6 bar and control, respectively (Table 2). SA had a significant effect on CAT activity of henna (Table 1). The concentration of 40 and 80 μM SA caused an increment in the trait compared to control (Table 2, Figure 1(b)). The interaction of salinity and SA showed that the treatment of henna with SA led to an increase in CAT activity. Moreover, plants treated with 80 μM SA had the highest activity of CAT under normal and stress conditions (Table 2).

A low Pearson correlation coefficient was obtained between CAT activity and dry matter under normal condition, $r = .176, p = .650$ (Table 3). Also, there was a moderate positive correlation between CAT activity and dry matter under salt stress (−3 and −6 bar) conditions, $r = .522, p = .148$ (Table 4), $r = .339, p = .371$ (Table 5).

### 3.6. Dry matter

Dry matter as an important index of plant growth or biomass yield was significantly affected by the interactions of salinity and salicylic acid (Table 1). The relationship of dry matter with the levels of osmotic potentials (Figure 2(a)) and SA concentrations (Figure 2(b)) was linear and quadratic, respectively. Reduced dry matter caused by salinity was improved by applying salicylic acid treatments (Table 2). Ameliorating effect of salicylic acid on dry matter was not progressive with increasing salicylic acid level and even it was slightly decreased.

There was a positive correlation between dry matter and quantum efficiency under normal ($r = .242, p = .529$) and severe salinity stress ($r = .716, p = .029$) conditions (Tables 3 and 5).

### 3.7. Chlorophyll florescence

The minimum chlorophyll florescence was significantly affected by salinity levels (Table 1). There was a linear relationship between $F_v/F_m$ and salinity (Table 1, Figure 3(a)). Minimum value of $F_v$ was recorded for no stress conditions while increase in salinity level led to a significant increase in this trait (Table 2). The regression relationship between quantum yield ($F_v/F_m$) and SA was quadratic (Table 1, Figure 3(b)). Considering the SA treatments, the highest and lowest value of $F_v$ belonged to concentrations of 40 and 80 μM SA, respectively (Table 2). Interaction effect of

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Relationship of CAT activity with the osmotic potential (a) and SA concentrations (b) in Henna (*L. inermis* L.).

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Relationship of dry matter with the osmotic potential (a) and SA concentrations (b) in Henna (*L. inermis* L.).
4. Discussion

Herbal medicines are in great demand in the developed and developing countries for primary health care because of their wide biological and medicinal effectiveness, higher safety margins and lesser costs (Padma, 2005). Plants have developed various combating mechanisms to cope with the deleterious effects of salinity stress. Salicylic acid (SA) is an important signal molecule which is biosynthesized by two pathways in plants (Hayat et al., 2010). In the present study, effect of exogenous application of salicylic acid was evaluated to determine the most effective concentration of this compound that can alleviate the salinity stress effects in henna seedlings. Based on the result, increasing salinity levels reduced the RWC, protein content, photosynthetic pigments and quantum yield, while REC and CAT activity were increased. RWC in leaves is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues (Flower & Ludlow, 1986). The results showed that RWC was significantly decreased by salt stress compared to the control treatment. The decrease in RWC under salinity stress has been reported previously (Karlidag et al., 2011; Srivastava et al., 1998; Zhang et al., 2006). This decrease could be attributed to root systems, which are not able to compensate for water loss by transpiration through a reduction of the absorbing surface (Sreenivasulu et al., 2000).

In this research, SA treatments alleviated the deleterious effects of salinity on henna plant. These results are in agreement with those obtained by other authors, showing that SA plays an important role in the plant sensitivity to different types of abiotic stress (Rao & Davis, 1999). SA pretreatment caused the RWC increase under drought stress (Table 2). These results were in agreement with the findings of Singh and Usha (2003). Increasing of RWC may be related to the role of SA in accumulation of compatible osmolytes in plants subjected to drought stress.

3.8. Regression analysis

The stepwise regression analysis between dry matter as dependent variable and other measured parameters as independent variables under normal and salinity stress (−3 and −6 bar) conditions was done. The results showed that no variable met the significance level for entry into the model under normal condition. The most important variables that affected the dry matter of henna under moderate salt stress (−3 bar) condition were protein content and CAT activity (Table 6). This showed that the mentioned traits explained 97% of dry matter variability under moderate salt stress (−3 bar) condition. Under severe salt stress (−6 bar) condition, protein content, REC and F_o were entered into the model and explained 97.5% of the dry matter variability (Table 7). Based on the obtained results protein content had the maximum effect on dry matter under moderate and severe salinity stress with the values of 82.4 and 61.2% adjusted R^2, respectively.

Figure 3. Relationship of F_v/F_m with the osmotic potential (a) and SA concentrations (b) in Henna (L. inermis L.).
Therefore, photosynthesis as a major controlling factor for plant growth and yield might have been increased due to SA application. The results obtained in this research showed a significant decrease in protein content which is in general in agreement with Chen et al. (2007) who found that sodium chloride reduced soluble protein content. The observed increase in the protein content due to SA application was likely the result of the increase of protein synthesis or less protein degradation (Peixoto et al., 2006). To support the accumulation of proteins due to SA treatment, it was reported that SA results in pronounced increase in total protein content and formation of new proteins in sunflower leaves (Cag et al., 2009).

In order for plants to be able to endure oxidative damage under high/low temperatures, water deficit and salinity conditions, plant must possess efficient antioxidant system. Previous studies showed that salt tolerant cultivars generally have an enhanced or higher constitutive antioxidant enzyme activity under salt stress when compared with the sensitive cultivars. This has been demonstrated in numerous plant species such as Medicago truncatula (Mhadhbi et al., 2011). In this research, CAT activity was higher in plants that were treated with salinity stress. High CAT activity is responsible for the detoxification of accumulates H₂O₂. Ben Amor et al. (2007) reported that H₂O₂ accumulation

| Table 6. The most important variables affecting the dry matter of Henna (L. inermis L.) under moderate salt stress (−3 bar) condition. |
|---|---|---|---|---|---|---|---|
| Model | Model R² | Adjusted R² | B | Std. error | β | t |
| 1 (Constant) | .846 | .824 | 1.687 | .427 | 3.953** |
| Protein | .316 | .051 | .920 | 6.197** |
| 2 (Constant) | .978 | .970 | 1.500 | .177 | 8.453** |
| Protein | .293 | .021 | .853 | 13.823** |
| CAT | 1.492 | .249 | .369 | 5.983** |

Note: CAT: Catalase, Std. error: Standard Error. **significant at 1% probability levels.

| Table 7. The most important variables affecting the dry matter of Henna (L. inermis L.) under severe salt stress (−6 bar) condition. |
|---|---|---|---|---|---|---|---|
| Model | Model R² | Adjusted R² | B | Std. error | β | t |
| 1 (Constant) | .661 | .612 | 1.929 | .427 | 3.953** |
| Protein | .160 | .051 | .920 | 6.197** |
| 2 (Constant) | .947 | .929 | −5.609 | .177 | 8.453** |
| Protein | −3.88 | .021 | .853 | 13.823** |
| REC | 7.051 | .249 | .369 | 5.983** |
| 3 (Constant) | .984 | .975 | −12.017 | 2.033 | −5.911** |
| Protein | 5.837 | .249 | .369 | 5.983** |
| REC | −0.48 | .039 | 2.477 | 12.400** |
| F₀ | 0.023 | .007 | .738 | 3.426** |

Notes: CAT: Catalase, REC: Relative electrolyte conductivity, F₀: Minimum florescence yield, Std. Error: Standard Error. **significant at 1% probability levels.

Elevated REC by increase in salt concentration indicates that an increase in membrane permeability or loss of membrane stability might lead to enhanced solute leakage. Increase in electrolyte leakage has often been used as an indicator of membrane damage during stress. An explanation for the release of electrolytes is that the release is mainly due to cells that have died. Electrolytes can leave a cell only when the plasma membrane has lost selective permeability activity. This probably does not occur unless the cell is dead and this phenomenon occurs usually in stress condition such as salinity. In the present experiment, SA decreased REC. SA apparently resulted in a better detoxification of reactive oxygen compounds and maintenance of membrane integrity, during high salt condition.

Salinity stress caused a decrease in photosynthetic pigments. Chl content has been suggested as one of the parameters of salt tolerance in plants and decrease in Chl and carotenoid content due to salinity has already been reported (Gang et al., 2010; Pasandi Pour et al., 2013; Srivastava et al., 1998). In our study, a protective effect of SA against the damage induced by salt stress was found. Results indicated that 40 μM of SA was more effective compared to other concentrations. This concentration of SA enhanced the photosynthetic pigments that can lead to an improvement in the photosynthetic rate and in turn optimum growth of henna plants under salinity stress. Exogenously applied SA increased the photosynthetic rate in soybean (Glycine max) (Khan & Singh, 2008). Therefore, photosynthesis as a major controlling factor for plant growth and yield might have been increased due to SA application.

The results obtained in this research showed a significant decrease in protein content which is in general in agreement with Chen et al. (2007) who found that sodium chloride reduced soluble protein content. The observed increase in the protein content due to SA application was likely the result of the increase of protein synthesis or less protein degradation (Peixoto et al., 2006). To support the accumulation of proteins due to SA treatment, it was reported that SA results in pronounced increase in total protein content and formation of new proteins in sunflower leaves (Cag et al., 2009).

In order for plants to be able to endure oxidative damage under high/low temperatures, water deficit and salinity conditions, plant must possess efficient antioxidant system. Previous studies showed that salt tolerant cultivars generally have an enhanced or higher constitutive antioxidant enzyme activity under salt stress when compared with the sensitive cultivars. This has been demonstrated in numerous plant species such as Medicago truncatula (Mhadhbi et al., 2011). In this research, CAT activity was higher in plants that were treated with salinity stress. High CAT activity is responsible for the detoxification of accumulates H₂O₂. Ben Amor et al. (2007) reported that H₂O₂ accumulation
under salinity stress was related to a decrease in CAT activity. SA not only has an effect on the catalase gene expression, but may also cause a direct inhibition of CAT activity in many plant species (Bowler et al., 1992) however; the results are not always consistent. Similar observations were also made in tomato plants raised from the seeds soaked in salicylic acid and was presumed to be due to the enhanced activation of some enzymes and to the accumulation of osmolytes (Szepesi et al., 2005).

\( F_{v}/F_{m} \) is a sensitive indicator of the potential photosynthetic activity of healthy as well as stressed plants. In this experiment, \( F_{v}/F_{m} \) was highest at low salt level and lowest at high salt level. These results agree with Gang et al. (2010) findings in castor bean (Ricinus communis). A decrease in the quantum yield of leaves with increasing salinity level can be attributed to an inhibition of electron flow at oxidizing site of PS II (Congming & Vonshak, 2002). The result showed that SA application of 40 μM resulted in an increase in \( F_{v}/F_{m} \) which is in agreement with Khan et al. (2013) who reported that application of SA proved most effective in lowering oxidative stress and increased quantum yield efficiency of PS II in wheat (Triticum aestivum L.).

Salinity stress reduces the ability of plant to take up water, and this leads to slower growth. In general, reduction of photosynthesis and plant dry mass with increasing salinity can be attributed to the efficiency of root system in limiting the transport of ions to shoots (Munns et al., 2006). Increases in dry matter of salt stressed plants in response to SA may be related to the induction of protective role of membranes that increases the tolerance of plant to damage. It is reported that SA treated maize plants showed higher dry mass as compared to those of untreated seedlings grown under salt stress (Gunes et al., 2007). An enhanced tolerance against salinity stress was observed in wheat (Triticum aestivum L.) seedlings raised from the grains soaked in salicylic acid (Hamada & Al-Hakimi, 2001).

5. Conclusions

It is conclusive that SA induced salinity tolerance in henna seedlings. The role of SA in henna under salt stress might be through regulation of oxidative stress and managing the antioxidants status. The minimized deleterious effect of salt on plant growth of henna may be due to the high activity of CAT. Furthermore, a positive relationship between CAT activity and SA application has been observed, which suggests SA induced changes in the synthetic CAT gene. Finally, as it was stated before very little researches has been done on this medicinal valuable plant, therefore some more research specially in the field condition is suggested.

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