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# Water Stress Alleviation of Roselle Plant by Silicon Treatment Through Some Physiological and Biochemical Responses

**E. F. Ali[1,2*] and F. A. S. Hassan[1,3]**

*[1]Biology Department, Faculty of Science, Taif University, Saudi Arabia.*
*[2]Floriculture Department, Faculty of Agriculture, Assuit University, Egypt.*
*[3]Horticulture Department, Faculty of Agriculture, Tanta University, Egypt.*

### *Authors' contributions*

*This work was carried out in collaboration between both authors. Author EFA did the experiments of germination and cultivation Roselle seeds, treatments application, collecting the experimental results such the plant growth characters, yield components, nutrient elements determination, physiological activities as well as sharing in the statistical analysis of the experimental data. Author FASH shared in all activities during the implémentation of the experiment, i.e. sharing in germination and cultivation Roselle seeds, treatments application, collecting the experimental results such the plant growth characters, yield components, nutrient elements determination, physiological activities as well as the statistical analysis of the experimental data. Both authors read and approved the final manuscript.*

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## ABSTRACT

A research trial was planned to find out the effect of water deficit and/or silicon (Si) foliar application on the growth, yield, sepal quality and some physiological characters of roselle plants. Under water stress treatment, the growth parameters (plant height, branch number, dry weight and leaf area) sepal yield and relative water content (RWC) were significantly reduced; however Si treatment ameliorated the negative effects of water stress and improved the growth even with non-stressed plants. The contents of N, P, K, Ca and Mg were significantly decreased in water stressed plants

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*Corresponding author: E-mail: esmatfarouk@yahoo.com;*

relative to plants grown under higher water levels. Si application increased the contents of N, P, K and Mg, while Ca content was decreased due to Si treatment in stressed or non-stressed plants. Water stress significantly decreased the total soluble solids and pH value however; Si application substantially alleviated this reduction. In contrast, the anthocyanin content was significantly increased due to water stress treatment. Otherwise, Si application increased anthocyanin content in stressed or non-stressed plants. Water stress significantly decreased the chlorophyll content and membrane stability index (MSI) compared with non-stressed plants. However, Si application had positive effects in this respect and alleviated the adverse effects of water stress. The total soluble sugars and proline content were significantly increased under water stress conditions and/or Si application. Water stress treatment promoted the activities of CAT, SOD and POD enzymes in roselle leaves however; Si application increased the activities of those enzymes except POD that decreased.

*Keywords: Water stress; silicon; anthocyanin; chlorophyll; proline; antioxidant enzymes.*

## 1. INTRODUCTION

The main challenge facing the researchers in arid and semi-arid regions is how to improve the plant production per unit of water because of the limited water resources. Moreover, the frequency and severity of drought will increase in several regions around the world due to climatic changes [1]. Therefore, drought is considered one of the main reasons that challenges sustainable development and causes severe plant yield reductions. Otherwise, water resources need to be used efficiently because of the increasing competition of the limited water resources between domestic, industrial and agricultural consumptions [2].

Water stress adversely affects the plant growth and hence causes a reduction in stem elongation, leaf expansion, growth rate and stomatal conductance [3]. Photosynthesis is one of the first physiological processes affected by drought [4]. The growth, yield, chlorophyll content, RWC, nitrogen, potassium and phosphorus contents of basil and rosemary plants were decreased under water stress in contrast to proline and total carbohydrate content which were increased [5,6,7].

In order to facilitate the adaptations of plants to limiting environmental conditions including water stress, several physiological, biochemical, and molecular processes are motivated [8,9]. Cornic [10] reported that plants diminish the reduction of water under stress through stomatal closure and restriction of $CO_2$ diffusion into the leaves which resulted in photosynthetic rate reduction. Díaz-Lópeza et al. [11] illustrated that the reduction in both growth and stomatal conductance is involved in the plant ability to alleviate water stress. The reduction in growth and yield of medicinal and aromatic plants under water stress was occurred by lessening the absorption of photosynthetically active radiation and its efficiency in dry matter production, consequently reducing the harvest index [12]. Photosynthetic pigments cracking or loss is a negative consequence of water deficit; however, it has been considered as an adaptive feature in plants grown under water stress [13]. Munne-Bosch and Alegre [5] suggested that the induction in antioxidant enzymes is involved in prevention of plant tissues damage in rosemary under water stress.

Silicon (Si) is considered a non-essential element for the majority of plants. It has been reported that Si is the second abundant mineral element in the earth’s crust after oxygen [14,15]. Despite Si has not yet been listed among the essential elements for higher plants, its beneficial effects on plant growth and development have been proved especially under biotic and abiotic stresses [16,17,18]. In this regard, Sanglard et al. [19] reported that Si is able to mitigate various abiotic stresses including water stress. Si has been found to mediate drought stress tolerance in different plants, such as sunflower (*Helianthus annuus* L.) [20], cotton (*Gossypium spp*.) [21] and canola (*Brassica napus*, L.) [22]. Even in poor accumulator plants, Si has several benefits such as structural rigidity of cell walls even before biotic and abiotic stresses exposure [23].

Silicon has numerous positive effects on different plants like enhancing the growth, decreasing transpiration and motivating photosynthesis and therefore alleviating the adverse effects of abiotic and biotic stresses [24,25,26,27,28,29]. Si is improved the resistance of plants undergoes abiotic stresses through counteraction of oxidative stress [15]. Silicon application of floricultural plants grown in soilless substrates has been found to improve growth, quality and resistance to biotic and abiotic stresses [30,31,32]. In zinnia, the amelioration effect of Si on water stress was accompanied by stimulation of plant growth [33].

Si treatment increased RWC and chlorophyll content while decreased malondialdehyde content which resulted in membranes degradation reduction [34]. Water stress significantly reduced the growth, leaf area, chlorophyll content, RWC and photosynthetic rate, however Si treatment minimized the water stress induced decreases in abovementioned parameters [35]. The efficacy of Si in improving plant water stress resistance in different plants has been reported [1,36] and this improvement may be ascribed to control the stomatal conductance or decrease evaporation and consequently reducing transportation [37]. Additionally, proline accumulation, some osmotic solutes and inorganic ions due to Si treatment decreased osmotic potential [38] restricting water stress induced oxidative damage through motivating enzymatic and non-enzymatic antioxidant machinery [1,39,40]. Si application also has a beneficial effect in enhancing the strength and rigidity of plant tissues [41]. Under water stress, the RWC, Chlorophyll content, stomatal conductance and photosynthetic rate in Si treated plants were higher than those without Si application [42,43].

Hence, these positive effects of Si generated interest for its application with various horticultural plants. Roselle (*Hibiscus sabdariffa*, L.) is a subtropical medicinal plant belonging to Malvaceae family and the calyx extraction is of a great therapeutic action for curing heart and nerve diseases, high blood pressure and calcified arteries [44]. The calyces have vitamin C beside two types of anthocyanin one of them is hibiscin (delphinidin) and the other is gossyptin (cyanidin) and hence are suitable for use as natural food coloring agents [45,46].

Although several studies about roselle cultivation techniques have been realized, the physiological responses to water stress are scarce [47]. Recently, several studies proved that Si treatment alleviates the water stress on different plants; however the mechanisms for silicon-mediated water stress tolerance remain not fully understood to date [19,35]. Furthermore, to our knowledge, studies on water stress alleviation by Si in plants have mostly been restricted to high-Si-accumulating monocotyledons. Studies on Si non-accumulating dicots are relatively few and rare on *Hibiscus sabdariffa*, L. plant in particular. For the great importance of medicinal and aromatic plants, the aim of this study was to investigate the water stress alleviation of roselle plant by Si treatment through some physiological and biochemical responses.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials and Treatments

This experiment was conducted at a private farm in Taif region, Saudi Arabia during two successive seasons (2016 and 2017 seasons) to investigate the alleviatory effects of Si treatment on water stress in roselle (*Hibiscus sabdarrifa* L) plants.

#### 2.1.1 Plant materials

Roselle seeds were sown directly in holes on the top of the ridge 70 cm apart in plots (1.5 x 1.5 $m^2$) including two rows and each row contained six hills at 25 cm apart. Four seeds were placed in each hill and one month later thinning was done leaving one seedling/hill. All other agriculture practices were conducted when required. The experiment was laid out in a complete randomized block (split-plot design) with four replicates.

The physical and chemical characteristics of the soil used in this study were (sand, 77.06%, silt 8.43% and clay 14.51%) and chemical properties were (pH, 7.96, EC, 2.13 $dSm^{-1}$, OM, 0.17%, 0.83%, $Na^+$, 3.28 ($meqL^{-1}$)**,** $Ca^{+2}$, 43.14 ($meqL^{-1}$), Total $CaCO_3$, $SO_4^{-2}$, 49.18 ($meqL^{-1}$), $Cl^-$, 0.62 ($meqL^{-1}$), $HCO_3$, 2.09 ($meqL^{-1}$), total $N^+$, $PO_4^{-3}$, $K^+$ were 0.25, 0.049 and 0.058%, respectively).

#### 2.1.2 The experimental treatments

*2.1.2.1 Drought (water deficit)*

The roselle plants received water through drip irrigation system, after one month from sowing, the water deficit treatments was applied thereafter as follows; 2, 4 and 6 L/water every week. Pipe lines from (about 16 mm diameter) put in the lateral side. The amounts of water were added in the different treatments, expressed through drippers (2L/h) to give such amounts of water which we need. Exactly the amount of water added can be tuned using a stop watch.

#### *2.1.2.2 Silicon treatments*

Three foliar applications of silicon treatments were evaluated in this study; 0, 2 and 4 mM. Si was supplied over the entire course of the experiment as monosilicic acid (which was prepared by passing potassium silicate through cation-exchange resin). The silicon treatments were done after one month from sowing and repeated four times, three weeks intervals. The spraying was going until whole vegetative growth was completely wet and run off. The Si levels used in this experiment were chosen based on environmental relevance [48] as well as on previous studies in which Si concentration resulted in improved growth of plants [49,50].

## 2.2 Data Recorded

### 2.2.1 Growth characters

After three weeks from the end of $K_2SiO_3$ treatment: Plant height (cm), number of branches/plant, herb dry weight as well as leaf area ($cm^2$) were measured. For leaf area determination, blade area was measured using digital image analysis according to Matthew et al. [51] method. Leaf blade digital image was created in digital format using a Hewlett- Packard scanner (Hewlett Packard, Cupertino, ca), image was scanned at dot/inch (100 dpi), the blade area was measured using public domain software.

### 2.2.2 Relative water content (RWC)

The following relationship as described by Weatherley [52] was used for leaf RWC determination and calculation:

$(W_{fresh}-W_{dry}) / (W_{turgid}-W_{dry}) \times 100$, where $W_{fresh}$ is the sample fresh weight, $W_{turgid}$ is the sample turgid weight after saturating with distilled water for 24 h at 4℃, and $W_{dry}$ is the oven-dry (70℃ for 48 h) weight of the sample.

### 2.2.3 Nutritional characters measurements (Macronutrients determination)

Leaf dry samples (0.5 g) were digested for leaf nutrient determination using sulphuric and perchloric acids method [53, 54]; nitrogen (nitrogen percentage in leaves was determined using the micro-Kjeldhl method as described by Nelson and Sommers [55], while phosphorus percentage was determined colorimetrically (spectrophotometer; Pharmacia, LKB-Novaspec II) using the stannous chloride phosphomolibdic-sulforic acid system and measured at 660 nm wave length according to Jackson [56], potassium (percentage was determined by flame photometer as described by Jackson [54], calcium and magnesium were spectrophotometrically determined as described by A.O.A.C. [57].

### 2.2.4 Total anthocyanin

Total anthocyanin content in the sepals was determined by using the method of Fuleki and Francis [58] and developed by Du and Francis [59].

### 2.2.5 pH values

pH values of sepals were determined according to Diab [60].

### 2.2.6 Total soluble solids (TSS)

Total soluble solids were determined by using the method described by Diab [60].

### 2.2.7 Physiological parameters measurements

Physiological characteristics such as chlorophyll content, stomatal conductance, membrane stability index as well as total soluble sugars were determined in leaves three weeks after the end of $K_2SiO_3$ treatments as follow:

#### *2.2.7.1 Chlorophyll content*

Leaf samples from the middle part of plant stem were isolated randomly for chlorophyll determination. Extraction in acetone was repeated until all pigments extracted. The absorbance of extracts was determined using spectrophotometer, chlorophyll content was determined according to Sadasivam and Manickam [61], chlorophyll content was calculated as ($mgg^{-1}$ FW) and the following equations was used to calculate chlorophyll a and chlorophyll b, then total chlorophyll was calculated:

Chlorophyll (a) = $10.3\ E_{663} - 0.918\ E_{644}$ = µg/ml.
Chlorophyll (b) = $19.3\ E_{644} - 3.87\ E_{663}$ = µg/ml.

#### *2.2.7.2 Stomatal conductance*

Stomatal conductance (mol $H_2O\ m^{-2}s^{-1}$) was determined in roselle leaves using Delta T AP4 leaf porometer, UK.

#### *2.2.7.3 Membrane stability index (MSI)*

MSI was determined by the method of Sairam et al. [62]. Two leaf samples (0.2 g) were taken and placed in 20 mL of double distilled water in two different 50 mL flasks. The first one was kept at 40℃ for 30 min while the second one was kept at 100℃ in boiling water bath for 15 min. The electric conductivity of the first ($C_1$) and second ($C_2$) samples was measured with a conductivity meter. The leakage of ions was expressed as the membrane stability index according to the following formula, MSI = $[1- (C_1/C_2)]$ X 100.

#### *2.2.7.4 Total soluble sugars*

Total soluble sugars were determined in leaf samples according to the method of Dubois et al. [63].

#### *2.2.7.5 Proline content*

For free proline determination, the method described by Bates et al. [64] was used. Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3% sulfosalicylic acid at 4℃. Then, the obtained extract was filtered with Whatman No. 2. Mixture of 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid was mixed in a test tube and incubated at 100℃ for 1 h. The reaction was terminated on ice, and the reaction mixture then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as µmol $g^{-1}$ FW.

#### *2.2.7.6 Antioxidant enzyme activity*

Antioxidant enzymes activity was determined as the method previously described by Hassan and Mahfouz [65]. The resulting supernatant was used as an enzyme extract to determine superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities. Soluble protein contents of the enzyme extract were assayed according to the method of Bradford [66].

SOD (EC 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). SOD activity was expressed as SOD units $min^{-1}$ $mg^{-1}$ protein. One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50% as described by Giannopolitis and Ries [67] by measuring the absorbance at 560 nm by a spectrophotometer.

CAT (EC1.11.1.6) activity was spectrophotometrically estimated by method of Clairbone [68], following the disappearance of $H_2O_2$ at 240 nm. The level of enzyme activity was expressed as µmol $min^{-1}$ $mg^{-1}$ protein.

POD (EC 1.11.1.7) activity was tested according to Shanon et al. [69]. Sodium acetate buffer (0.1M) and 0.5% guaiacol was added to the enzyme extract. The reaction was started with 0.1% $H_2O_2$. The rate of change in absorbance was spectrophotometrically measured at 470 nm and the level of enzyme activity was expressed as µ mol $min^{-1}$ $mg^{-1}$ protein.

### 2.3 Statistical Analysis

The results of this study were combined for the two experiments (*n=8*) and analysis of variance (ANOVA) was performed. SPSS 13.3 program was used for data analyzing to compare means by Duncan multiple range test at *P=0.05* level.

## 3. RESULTS

### 3.1 Growth Characteristics

The growth parameters of roselle were investigated under different water treatments and in the presence of Si as well. The plant height, branch number, dry weight and leaf area were significantly reduced under water stress treatment (2L/week). However, the inhibiting effects of water stress on growth were ameliorated by Si application especially with higher level (4 mM). Moreover, the abovementioned growth characters were increased with increasing the water level applied. Similarly, Si treatment also improved the growth even with non-stressed plants (Table 1).

### 3.2 Sepal Yield/Plant

The fresh and dry weights of sepal/plant of roselle as affected by water levels and Si treatments were presented in Table (2). The obtained data revealed that water stress significantly reduced the sepal yield relative to 4 or 6L water/week treatments. Considering the yield of stressed plants (2L/week) equal 100%, the increment in dry sepal yield was 30.97 and 63.61% when plants received 4 and 6 L/week, respectively. Si application significantly improved the sepal yield in stressed or non-stressed plants however, the impact of Si treatment was clearly observed when applied under water stress and higher Si level was superior to lower one. Considering the dry sepal yields of plants grown under 2, 4 and 6 L/week without Si application equal 100%, the increment in sepal yield was 15.87, 9.95 and 7.81% when Si at 4 mM was applied, respectively.

### 3.3 RWC and Stomatal Conductance

As shown in Table (2), water stress remarkably reduced RWC compared with higher water levels. In addition, increasing Si level resulted in a significant increase in RWC of stressed or non-stressed roselle plants. Under water stress the RWC was 71.12% while it was 74.29 and 76.57% in Si treated plants. The same trend was recorded with the water treatments of four and six L/week. Since Si treated plants maintained a higher water statues than the untreated plants, it improves the drought resistance. The stomatal conductance of water stressed plants was significantly lower relative to non-stressed plants. Si application increased the stomatal conductance under any water treatment and the effect of Si in this concern was more pronounced when applied under water stress.

### 3.4 Nutrient Elements

The content of some nutrient elements in roselle leaves was shown in Table (3). Data obtained clearly indicate that the contents of N, P, K, Ca and Mg were significantly decreased in water stressed plants. However plants grown under higher water levels, their contents of the previous elements were remarkably increased. Si application increased the contents of N, P, K and Mg, while Ca content decreased due to Si treatment in stressed as well as in non-stressed plants.

**Table 1. Plant height, branch number, dry weight and leaf area parameters in Roselle plants grown under different levels of irrigation and silicon application**

| Water treatments (L/week) | Si treatments (mM) | Plant height (cm) | Branch number/plant | Dry weight (g/100 g FW) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|
| | 0 | 84.73h | 3.65e | 15.34e | 5.63e |
| 2 | 2 | 88.36g | 3.98e | 16.18e | 5.94de |
| | 4 | 95.14f | 4.12de | 17.82d | 6.19cd |
| | 0 | 94.16f | 3.75e | 17.45d | 6.04cd |
| 4 | 2 | 102.19d | 4.24d | 18.92c | 6.32c |
| | 4 | 108.02c | 5.42c | 19.64bc | 6.84b |
| | 0 | 98.64e | 6.54b | 20.16b | 6.76b |
| 6 | 2 | 110.58b | 7.75a | 21.76ab | 6.98a |
| | 4 | 116.46a | 7.89a | 22.54a | 7.26a |

*Means followed by different letters in each column were significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8)*

**Table 2. Fresh and dry sepal yield/plant, RWC and stomatal conductance in Roselle plants grown under different levels of irrigation and silicon application**

| Water treatments (L/week) | Si treatments (mM) | Fresh sepal yield/plant (g) | Dry sepal yield/plant (g) | RWC (%) | Stomatal conductance (mol $H_2O$ $m^{-2}s^{-1}$) |
|---|---|---|---|---|---|
| | 0 | 70.52i | 7.75i | 71.12g | 0.214e |
| 2 | 2 | 79.18h | 8.19h | 74.29f | 0.232e |
| | 4 | 85.45g | 8.98g | 76.57e | 0.237e |
| | 0 | 97.64f | 10.15f | 76.48e | 0.315d |
| 4 | 2 | 103.38e | 10.57e | 79.56d | 0.345c |
| | 4 | 110.15d | 11.16d | 83.15c | 0.356c |
| | 0 | 112.36c | 12.68c | 82.92c | 0.377b |
| 6 | 2 | 123.81b | 13.15b | 86.58b | 0.382b |
| | 4 | 138.19a | 13.67a | 89.14a | 0.398a |

*Means followed by different letters in each column were significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8)*

**Table 3. Macronutrients content of Roselle leaves grown under different levels of irrigation and silicon application**

| Water treatments (L/week) | Si treatments (mM) | Macronutrients (mg $g^{-1}$ DW) N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| | 0 | 15.82e | 2.12e | 14.74e | 8.23de | 3.96f |
| 2 | 2 | 16.74e | 2.63de | 14.93e | 8.05e | 4.17e |
| | 4 | 18.21c | 2.91d | 15.25d | 7.86f | 4.23e |
| | 0 | 17.93cd | 2.83d | 15.12d | 8.54c | 4.19e |
| 4 | 2 | 18.65c | 3.14c | 15.64c | 8.35cd | 4.52cd |
| | 4 | 19.24b | 3.45ab | 16.46b | 8.12de | 4.67bc |
| | 0 | 19.82b | 3.26bc | 15.95c | 9.34a | 4.36de |
| 6 | 2 | 20.65ab | 3.57a | 16.73b | 9.02b | 4.72b |
| | 4 | 21.36a | 3.37ab | 18.26a | 8.91b | 5.08a |

*Means followed by different letters in each column were significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8)*

### 3.5 Anthocyanin Content, Total Soluble Solids and pH Value of Sepals

The characteristics of sepal quality were investigated by measuring anthocyanin content, total soluble solids and pH value in sepal juice due to water and/or Si treatments (Table 4). The total soluble solids and pH value were significantly decreased as a result of water stress treatments relative to higher water levels. However, Si application substantially alleviated this reduction and significantly increased those values under water stress. The anthocyanin content, in contrast, was significantly increased as a result of water stress treatment. Meanwhile, this parameter was significantly decreased when plants grown under higher water levels. However, Si application increased anthocyanin content in stressed or non-stressed plants.

### 3.6 Chlorophyll Content and Total Soluble Sugars

Water stress significantly decreased the chlorophyll content compared with non-stressed plants. However, Si application under water stress kept the chlorophyll content higher than it was in untreated plants (Table 5). Moreover, when Si was applied under higher water levels, it also improved the chlorophyll content. On the other hand, the total soluble sugars were significantly increased under water stress conditions and/or Si application. Therefore, the highest total soluble sugars (13.42%) were obtained by applying the treatment of Si at 4 mM under water stress (2L/week).

### 3.7 Membrane Stability Index (MSI) and Proline Content

It is clear from data presented in Table (5) that MSI was significantly decreased due to water stress however plants grown under higher water levels maintained their membrane stability. Si treatment had positive effect in this respect and alleviated the negative effects of water stress. In non-stressed plants Si application improved MSI as well. The proline content in rossele leaves significantly accumulated under water stress compared with higher water levels treatments. Proline content also increased as a result of Si application. The accumulation of proline was more pronounced in Si-treated plants under water stress.

### 3.8 Antioxidant Enzyme Activities

The activities of CAT, SOD and POD enzymes in roselle leaves were significantly increased due to water stress treatments. However, Si application increased the activities of CAT and SOD while it decreased POD activity (Fig 1). Plants received 2L water/week resulted in the highest activities of three enzymes unlike well-watered plants which recorded the minimum values in this respect. Increasing Si level from 2 to 4 mM increased the antioxidant enzyme activities investigated except POD under any water level but the values are still higher under 2L/week relative to 4 or 6L/week water treatments.

## 4. DISCUSSION

In current study, the vegetative growth parameters of roselle plants were significantly

**Table 4. Anthocyanin content, pH value and total soluble solids of Roselle juice extracted from plants grown under different levels of irrigation and silicon application**

| Water treatments (L/week) | Si treatments (mM) | Anthocyanin content (mgg$^{-1}$ DW) | pH value | TSS (%) |
|---|---|---|---|---|
| 2 | 0 | 21.19d | 3.65e | 29.87g |
| | 2 | 23.15b | 3.71de | 32.58f |
| | 4 | 25.08a | 3.82d | 34.26e |
| 4 | 0 | 20.76e | 3.97cd | 33.15f |
| | 2 | 22.07c | 4.02c | 36.57d |
| | 4 | 23.34b | 4.11bc | 38.88c |
| 6 | 0 | 19.43f | 4.24ab | 37.16d |
| | 2 | 20.18e | 4.26a | 42.25b |
| | 4 | 21.77d | 4.38a | 46.41a |

*Means followed by different letters in each column were significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8)*

**Table 5. Chlorophyll content, total soluble sugars, MSI and proline content of roselle leaves grown under different levels of irrigation and silicon application**

| Water treatments (L/week) | Si treatments (mM) | Chlorophyll content (mgg$^{-1}$ FW) | Total soluble sugars (%) | MSI (%) | Proline (µmol g$^{-1}$ FW) |
|---|---|---|---|---|---|
| 2 | 0 | 1.13b | 12.56c | 72.65f | 1.86b |
| | 2 | 1.19f | 13.09b | 74.18d | 1.89ab |
| | 4 | 1.26d | 13.42a | 75.66e | 1.94a |
| 4 | 0 | 1.16g | 10.72f | 74.72e | 1.74d |
| | 2 | 1.23e | 11.24e | 78.15c | 1.76d |
| | 4 | 1.31c | 11.81d | 81.65b | 1.80c |
| 6 | 0 | 1.24de | 9.79h | 78.22c | 1.57f |
| | 2 | 1.36b | 10.36g | 81.54b | 1.62e |
| | 4 | 1.45a | 10.97f | 83.77a | 1.66e |

*Means followed by different letters in each column were significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8)*

lower under water stress while Si application improved those parameters when plants were grown under either water stress or well water conditions (Table 1). It is well documented that water stress firstly decreased the turgor which resulted in a reduction in cell development of leaves and stem and consequently reduced the growth [70]. In this concern, the plant morphology was altered due to water stress including the plant height and shoot growth [47,71,72,73]. Wilkinson and Davies [74] reported that reducing leaf and stem growth rates is one of the important strategies by which the plant avoid the water stress. The reduction of dry weight due to water shortage might be a result of a reduction in chlorophyll content [70] and hence, photosynthesis efficiency, as reported by Khalid [6]. Growth reduction due to water stress has been well documented [1,3,4].

However, the plant dry weights in Si-treated plants was higher than the control, indicating that Si application alleviates the inhibition of leaf area and growth parameters which induced by water stress [74]. Additionally, leaf area of sorghum was increased by Si application under drought stress [75]. It has been observed that leaf area was increased by Si addition [75] and this increment might be associated with active supply of water and increased net photosynthesis as concluded by Hattori et al. [76].

In this study water stress severely reduced the RWC while it remained higher by Si treatment. The previous study of Solatni et al. [77] showed that osmoregulation decreases growth rebuttal to drought stress and the growth changes might be ascribed to turgor pressure adjustment. Decreasing RWC in current study could be explained by the reduction of root water absorption and its move out to the upper parts through transpiration pull. Drought stress significantly decreased dry weight and RWC of sorghum plants, however, the inhibiting effects of drought stress on growth were substantially alleviated by Si application [35].

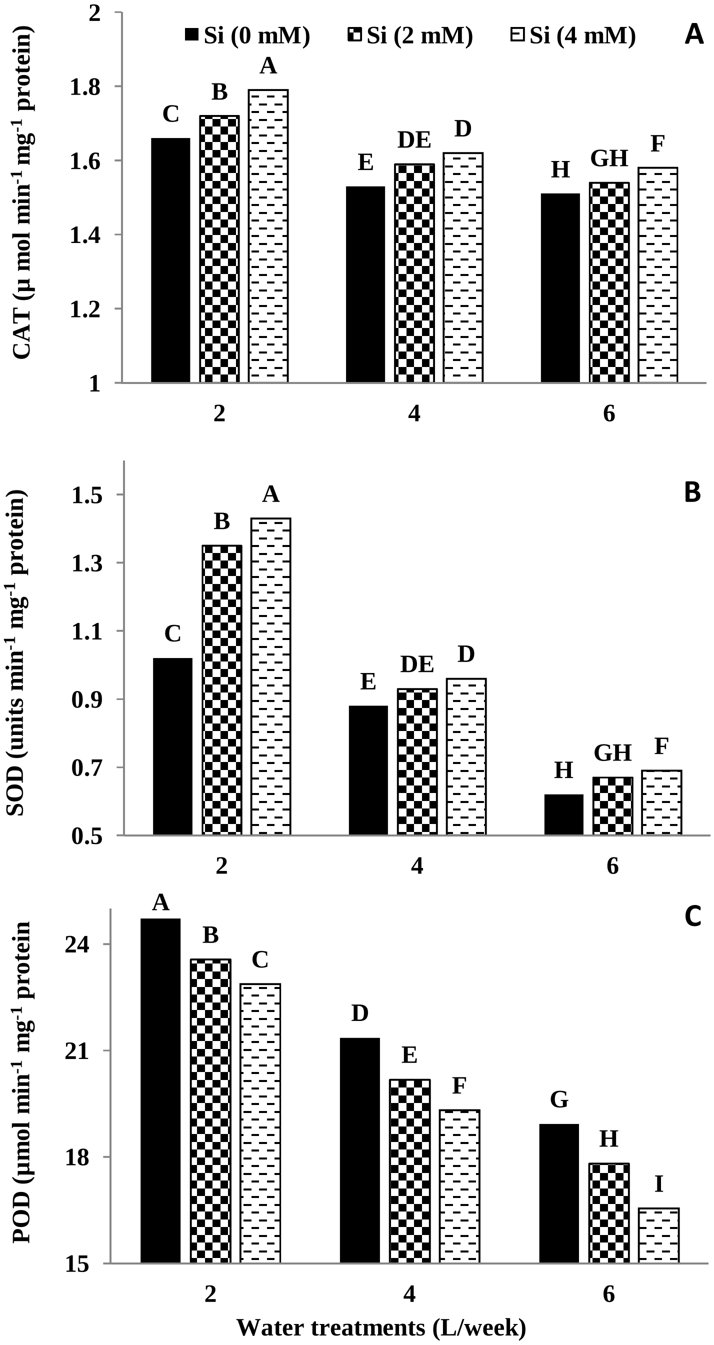

**Fig. 1. Antioxidant enzyme activities; A: CAT, B: SOD and C: POD in roselle leaves grown under different levels of irrigation and silicon application (Bars with different letters are significantly different according to Duncan's multiple range tests, P≤0.05 level (n=8))**

Si treatment also resulted in a higher RWC than the control; therefore Si moderates plant water status through enhancing water uptake under drought stress [74]. Moreover, Lux et al. [78] observed that Si treatment maintained the leaf water potential under water deficit which is due the formation of silica-cuticle double layer on the leaf epidermal cells [78]. Si treatment also had an effective role in the root water uptake under water stress conditions [76]. The RWC of wheat was reduced under drought, however Si treated plants maintained higher RWC than those without Si application [79]. These results indicate that Si treatment enhanced water status in roselle under water stress conditions, which are in accordance with the findings of Pei et al. [40]. Such improvement of water status by Si has been previously documented [22,42,76].

In this experiment, the stomatal conductance was reduced when plants grown under water stress in contrast to Si application which increased it and improved the stomatal conductance under stress. In accordance with these results, increased stomatal conductance was observed in plants with Si under stress [1]. Under drought stress Si supply positively influenced stomatal conductance by stabilizing it and making the conductance level to be almost at the same level with the control in both cultivars [80]. Wilkinson and Davies [74] revealed that in order to alleviate water stress, plants have developed multiple mechanisms including stomatal closures to reduce transpiration. Additionally, Zhang et al. [81] recorded an increase in stomatal conductance as a result of Si application, while it was decreased due to water deficit treatment in chestnut plants. Moreover, the drought-induced decrease in stomatal conductance was significantly mitigated by Si treatment [35]. Increasing the stomatal conductance due to Si treatment under drought was previously reported [43,79,82]. The obtained results in this study are different from those obtained by Habibi [22] who found that Si application had no positive effects on stomatal conductance in canola plants.

In current study, water stress significantly reduced the sepal yield of roselle plant while Si treatment alleviated this reduction. These results might be attributed to the effect of Si in plant growth improvement and enhancing the water status under stress as our data indicated. The reduction in sepal yield may be ascribed to the reduction of growth that reflected in decreasing the sepal yield.

In current study, the mineral content of N, P, K, Ca and Mg were decreased in roselle plants subjected to water stress. However, Si application enhanced the contents of those elements except for Ca which was decreased. The negative effect of water stress on nutrient elements has been previously reported in rosemary plant [70]. Decreasing the absorption of nutrient elements under water stress may be attributed to the growth reduction [83]. Si is suggested to maintain the balance of nutrients under water stress and the mechanism for that might be the role of Si mediated activation of $H^+$-ATPase in the cell plasma membrane [84]. Increasing the phosphorus due to Si application under water stress has been previously reported [79] and this increment might encourage ATP synthesis, which is required in the $CO_2$ assimilation cycle [85] and consequently the improvement of photosynthesis under water stress as a result of Si application might be related to the increment in P content. These results suggest that Si may alleviate the water stress in plants through improving the uptake and utilization of nutrients and water.

The current results showed that the juice quality of roselle sepals was influenced by water stress and Si treatments. It has been reported that abiotic stress factors influence growth and secondary metabolite production in higher plants and anthocyanins are reported to accumulate under drought stress [86]. The improvement of anthocyanins has been observed also under UV-radiation stress [87]. Moreover, Shen et al. [1] reported that soybean plants grown under drought or Si treatment have higher anthocyanin accumulation.

Data obtained here showed that the chlorophyll content decreased due to water stress while Si application maintained the chlorophyll content and alleviated this reduction in roselle leaves. The decline in the level of photosynthetic pigments may be attributed to salinity-induced inhibition of chlorophyll biosynthesis [88] that may be caused by nutrient deficiency or imbalance [89]. It has been reported that drought stress decreased the chlorophyll concentration, but Si application kept the chlorophyll concentration higher than untreated plants [35]. However, Si application enhanced the chlorophyll content and also alleviated its reduction caused by water stress in chestnut plants [81]. Moreover, the chlorophyll content of sorghum increased by Si application under drought stress [75].

The prevention of chlorophyll degradation as a result of Si application might be ascribed to the chloroplast membranes protection [90] or deposition of Si in cell walls which resulted in erection of leaves and improved light interception and photosynthesis. Similarly, degradation of chlorophyll can be prevented due to the Si application in nutrient solution as confirmed by

Gonzalo et al. [91]. Otherwise, Hassan et al. [70] recorded a positive correlation between relative water content and leaf chlorophyll and this may gave an explanation concerning the negative effect of water shortage on chlorophyll content. In this study, the dry weight and chlorophyll content were found to be higher in Si-treated plants than control, clarifying that Si treatment reduced the growth inhibition which is induced by drought stress [74]. The improvement of chlorophyll content under water stress has been also observed by Shen et al. [1] in soybean plants and Saud et al. [82] in Kentucky bluegrass plants. Adversely, Alaei [92] and Khayatnezhad et al. [93] reported that water stress increased the chlorophyll content in wheat. They mentioned that this is because the exact effect of deficit irrigation may vary depending on the intensity of the water stress imposed [94].

In the present study the effects of water stress and/or Si treatments on total soluble sugars and proline content, as the main osmotic solutes were investigated. The obtained data revealed that total soluble sugars and proline content were increased in plants grown under water stress and/or Si application. In the same line with these results, Yin et al. [35] reported that both the total soluble sugars and proline contents were enhanced under drought stress. In addition, the accumulation of carbohydrates and proline caused by water stress in cucumber show the adjusting role of both osmolytes under stress conditions [80]. It is well known that sugars is involved in osmotic adjustment and might leads to a reduction in the osmotic potential of leaves to maintain turgor and this also considered an adaptive mechanism in crops subjected to water stress [95].

The increment of total soluble sugars due to Si treatment in this study is consistent with the investigation of Sonobe et al. [96] who confirmed that Si mediates the active accumulation of soluble sugars in sorghum, hence reducing the osmotic potential of root, which motivates increased water uptake. On the other hand, in the study of Ouzounidou et al. [80] although both proline and carbohydrates were accumulated under water stress, Si application regulated the proline and carbohydrate production which resulted in considerable reduction in their values to mitigate the stress. Additionally, Si application increased the total soluble sugars, while decreased proline content under water stress in sorghum plants [35].

The results in this study indicated that water stress decreased the MSI in contrast to Si treatment which retained the membrane integrity under stress or non-stress conditions. Therefore, the current results suggest that Si treatment decreases the permeability of plasma membrane and lipid peroxidation in order to maintain membrane integrity under water-stressed roselle. In this regard, Agarie et al. [97] in rice and Ahmed et al. [98] in sorghum observed that the stability of cell membrane was enhanced when rice plants treated by Si under water stress. Similarly, Si application decreased deterioration through maintenance of membrane integrity, reduction of lipid peroxidation, increase in scavenging of reactive oxygen species (ROS) and reduction in lignification [80]. It has been reported that under environmental stresses cell membranes are subject to modifies often associated with the permeability increment and loss of integrity [99]. The membrane deterioration measured by electrolyte leakage has been previously reported in maize [100,101].

Si foliar application of increased the MSI in wheat, under both stress and non-stress conditions while; under water stress more improvement was detected compared with non-stress conditions [102]. It is well known that, the maintenance of membrane stability and integrity is a main factor of water stress tolerance under drought condition [103].

Environmental stresses cause disturbance in plant metabolism and lead to oxidative damages by motivating ROS production. The plant stress resistance was associated with the antioxidant capacity, and the stress damage may be prevented by the increment of antioxidant constituent’s levels [104]. In current study, the activities of CAT, SOD and POD enzymes were increased as a result of water stress treatment. However, when Si was applied the activity of CAT and POD were also increased while a reduction in SOD was observed. Abedi and Pakniyat [105] reported that drought stress improved the antioxidant enzyme activities and plants protect cell systems from the cytotoxic effects of drought accumulated ROS using enzymes, such as SOD, APX, POD, and CAT. Si mediated reduction in POD activity suggests that its ability for $H_2O_2$ scavenging was decreased. When Si was applied, the POD activities reduction might be associated with the increases in CAT activities, which resulted in a lower content of $H_2O_2$, and consequently there was less demand to activate the $H_2O_2$-scavenging enzymes. This is in agreement with a previous report in silicon-applied wheat plants exposed to drought stress [106]. In accordance with our results, Shi et al. [107] found that the activities of SOD, CAT and POD were significantly increased under water stress; however Si treatment further enhanced the activities of SOD and CAT while POD activity was decreased. The increases in activities of SOD, CAT and POD under water stress might be an adaptive response, which facilitated the scavenging of ROS [107]. Similar results were also reported in soybean [108].

It is widely accepted that the function of the antioxidant defense system depends on plant species, and the intensity and duration of the water stress. In this regard, Si application caused a significant increase in the activities of SOD and POD, in contrast to CAT which was decreased [22]. Otherwise, Habibi [22] showed that the activities of SOD and POD were increased by applying Si under water stress in both shoots and roots whereas the activity of CAT was not influenced. In salt stressed cucumber leaves the addition of silicon increased the activity of SOD, whereas CAT activity was not influenced [109]. The motivation of antioxidant system due to Si application under drought stress has been previously documented [20,39,110]. These results suggest that Si may alleviate the water stress in plants through modulating some physiological activities including antioxidant enzymes premonition.

## 5. CONCLUSION

Conclusively, the current results indicated that water stress severely reduced the growth, yield and quality of sepals. Moreover, it diminished the photosynthesis and its associated metabolic activities in roselle. However Si application had ameliorative effects on roselle growth, yield, physiological attributes and antioxidant enzymes under water stress. Counteracting the damaging effects of water stress by Si on those attributes suggests that silicon may be involved in metabolic or physiological activities in roselle plants under drought.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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