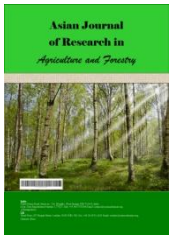

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Alleviation of Salt-Induced Adverse Effects on Gas Exchange, Photosynthetic Pigments Content and Chloroplast Ultrastructure in *Gerbera Jamesonii* L. by Exogenous Salicylic Acid Application

Kapila Kumara[1*], A. D. Ampitiyawatta[1], Adithya Padmaperuma[2], Chalinda Beneragama [2] and Xia Yi Ping[3]

[1]Department of Export Agriculture, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka.
[2]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.
[3]Department of Horticulture, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China.

Authors' contributions

This work was carried out in collaboration among all authors. Author KK designed the study, conducted the study, collected data, wrote the protocol, and wrote the first draft of the manuscript. Author ADA managed the literature survey and statistical analysis of the study. Author AP Prepared the tables, figures, graphs, reference list and formatted them. Author CB contributed to data analysis and interpretation of results. Author XYP fine-tuned the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effects of exogenously applied salicylic acid (SA) on gas exchange characteristics, photosynthetic pigments and chloroplast ultrastructure were investigated in gerbera at their reproductive stage under salt-stressed conditions.
Methodology: A pot experiment was conducted under glasshouse conditions at the Zhejiang University, Hangzhou, China, (30° N/120° E) between February 2008 and March 2009.Plants,

**Corresponding author: E-mail: gdonkk@agri.sab.ac.lk, gdonkk@yahoo.com;*

pretreated with foliar applications of 0, 0.5, and 1.0 mmoldm^{-3} SA at the onset of flower initiation were irrigated with 100 mmoldm^{-3}NaCl$_{(aq)}$ for two weeks, starting after three days from the SA pretreatment. Control did not receive either NaCl or SA.Photosynthetic rate, gas exchange, photosynthetic pigments content and chloroplast ultrastructure were investigated against treatments. All data were subjected to analysis of variance (ANOVA) and Generalized Linear Model (GLM) using SAS statistical software. Pearson's correlation test was carried out to study the relationships among the parameters. The means were compared using Duncan's multiple range test (DMRT). For all the tests, $P< .05$ was considered statistically significant.

Results: Salt stress adversely affected the gas exchange characteristics, photosynthetic pigment contents and chloroplast ultrastructure. SA application significantly increased the net photosynthesis, stomatal conductivity, intra-cellular CO_2 content and transpiration rate but decreased the stomatal limitation, compared to those of untreated salt-stressed plants. Further, the enhanced photosynthetic pigment contents and notably undamaged chloroplast ultrastructure were evident of the ameliorative effects of SA on photosynthetic system under salt stress. Of the two concentrations tested, 0.5 mmoldm^{-3} SA concentration seemed to have greater effect throughout the experiment showing no significant variation from control in some attributes (chlorophyll contents and chloroplast ultrastructure).

Conclusion: Responses of plants pretreated with SA spraying and significant correlation among them plausibly suggest SA-induced enhancement of photosynthetic system as another target for conferring salt tolerance in crop plants.

Keywords: Gerbera; salt stress; salicylic acid; photosynthetic system; salt tolerance.

1. INTRODUCTION

Salinity is one of the most relentless abiotic stress conditions, which, causes a substantial loss in both quantitative and qualitative aspects of yield [1]. The increasing salinity in arable lands is considered a serious global issue. It is projected to result in a 50% land loss by the middle of the 21st century due to salinization [2-4]. The scarcity of high-quality irrigation water may aggravate the problems. As a consequence, catering to the demands for the agricultural supplies of the ever-increasing world population has become a great challenge [5].

Salt stress is closely associated with the low osmotic potential of the soil solution (water stress), specific ion effect (salt stress), and nutrient imbalances or a combination of these factors [1] which can adversely affect plant morphology, cellular structures and major physiological processes such as growth, protein synthesis, nutrient uptake, lipid metabolism, and photosynthesis [6]. Reduction in photosynthesis of horticultural crops grown under saline conditions can be attributed to the declined stomatal or mesophyll conductance [7], oxidative damage leading to biochemical limitations [8], destruction of chloroplast ultrastructure [9,10] and reduced photosynthetic pigment contents [11]. Thus waning growth of plants due to salt stress is explicable by the suppression of photosynthesis, as the plant growth is well related to the rate of photosynthesis [12-14].

Enhancing the stress tolerance in plants has major implications in agriculture [15]. Some plants respond to exogenous application of organic compounds and develop a tolerance to abiotic stresses which in turn significantly contribute to increased crop production under salt stress [16,17]. Salicylic acid (SA) protects plants against some abiotic stresses such as salinity through regulation of physiological processes [18]. It has been suggested as a signal transducer or messenger under stress conditions [19]. Ameliorative effects of SA have shown promise in inducing salt tolerance in numerous plants including horticultural crops [20]. Numerous authors have reported the possible ameliorative effects of exogenously applied SA on redox homeostasis [21], photosynthetic rate, stomatal conductance and transpiration [22], membrane functioning and water relations [23] under abiotic stress conditions. These functions may also, possess a key role in plant tolerance to salinity stress. However, the physiological and biochemical basis for this phenomenon and the mechanism of signal regulation in the resistance induced by SA are yet to be understood [15,24].

Gerbera (*Gerbera jamesonii* L.) which belongs to the family Asteraceae, holds the fifth position in the international cut flower trade [25]. However,

salinity has prevented it from reaching its maximum genetic potential and limited the productivity world-wide. Thus, strengthening the plant by exogenous application of SA to withstand the extreme salinity conditions will be a groundbreaking finding in the cut flower industry.

The objective of the present study was to investigate the effect of SA in ameliorating important attributes related to photosynthesis in gerbera under salt stress conditions.

2. MATERIALS AND METHODS

2.1 Plant Materials and Growth Conditions

The experiment was conducted in a glasshouse at Zhejiang University, Hangzhou, China, (30° N/120° E). Three months old micro-propagated *Gerbera jamesonii* L 'Amaretto' were planted in 4 L plastic pots filled with a potting medium containing perlite and peat moss in 1: 2 v/v ratio. Plants were grown under natural light conditions. Average maximum and minimum ambient temperatures during the study period were 24 ±3 ℃ and 16 ±3 ℃, respectively. Plants were manually irrigated with a standard nutrient solution recommended for gerbera in the Netherlands [26]. The electrical conductivity (EC) of the nutrient solution was maintained between 1.8 to 2.0 dSm-1 while pH was maintained around 5.8.

2.2 Salicylic Acid Application and Salt Stress Treatment

Preparation of salicylic acid solutions for the treatments was done by dissolving salicylic acid (SA; Sigma®) in absolute ethanol and then mixing in distilled water (ethanol: water, 1:1000 v/v; [27]) which contained 0.02% Tween 20 (polyoxyethylenesorbitanmonolaurate) [22]. Two solutions were made in which the final SA concentrations were maintained at 0.5 mmoldm-3 and 1.0 mmoldm-3 which we named as Spray 1 and 2, respectively. Spray 3 for negative treatments was prepared using the same method but excluding SA. These solutions were used in SA pretreatments.

Plants were pretreated by spraying the respective spray solutions on both adaxial and abaxial surfaces until it was dripping. To start with the pretreatments, plants were divided into four groups with the same number of plants in each. Two groups of plants were treated by spray solution 1 and 2 separately while the other two groups of plants were treated by the spray solution 3. These treatments were repeated on two consecutive days.

Salt treatments were commenced two days after the final salicylic acid application. It was done manually by irrigating the plants with a 100 mmoldm-3 sodium chloride solution. All the groups except for one group of plants out of two groups which were treated with spray solution 3 received the same salt treatment. Salt treatment was continued throughout the study duration with a two days interval.

Pots receiving the corresponding treatments were arranged in a randomized complete block design with three replications.

2.3 Photosynthetic Rate and Gas Exchange Measurements

Photosynthetic pigment content analysis was commenced three days after the first salt treatment and carried out at six-day intervals. Starting on the same day, gas exchange measurements were taken five times at three-day intervals.

Net photosynthetic rate (*Pn*), leaf stomatal conductance to water vapor (*Gs*), intercellular partial pressure of CO2 (*Ci*), and transpiration rate (*Tr*) of the youngest fully expanded leaves were measured using a portable photosynthesis system (LI-6400, LI-Cor Inc., USA) with a red-blue light source. Gas exchange characteristics were measured from 1100 h to 1500 h (Chinese local time GMT+8) maintaining the following conditions in the leaf chamber: the photosynthetic photon flux density, block temperature, CO2 concentration and photosynthetically active radiation was 1000 µmolm−2s−1, 27 ℃, 450 µmolmol−1, and 500 $\mu molm^{-2}s^{-1}$, respectively. The value of stomatal limitation (*Ls*) was calculated using the following method: *Ls* = (1−Ci)/Ca (Ca is the CO2 concentration in the atmosphere) [22].

2.4 Determination of Photosynthetic Pigments Content

Pigments of the leaf samples (0.5 g) were extracted using acetone (90% v/v), filtered and made up to a final volume of 50 ml. The absorbance was recorded at 470, 645 and 663 nm using a spectrophotometer (UV-2250, Shimadzu®, Japan). Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*tChl*) and

carotenoid (*Car*) contents were calculated using the formula suggested by [28].

2.5 Chloroplast Ultrastructure

Sampling to the chloroplast ultrastructure determination was done nine days after the first salt treatment. Fully expanded, uppermost leaves of the plants were taken to carry out the investigation.

To prepare the leaves for transmission electron microscopic (TEM) imaging, leaves were fixed for 4 h at room temperature with 2.5% glutaraldehyde in a phosphate buffer (pH 7.0). Then, they were post-fixed in 1% OsO4 in the same buffer for 1 h. Samples were dehydrated in a graded series of ethanol, and absolute acetone. Later, the specimens were placed in a 1:1 absolute acetone and the final Spurr resin mixture for 1 h at room temperature. Then, they were transferred in to a 1:3 absolute acetone and final resin mixture for 3 h. Ultimately, the specimens were transferred to the final Spurr resin mixture and kept for 12 hours. Ultrathin sections of prepared samples were placed in capsules that contained embedding medium and heated at 70 ℃ for about 9 h. These sections were stained by uranyl acetate and alkaline lead citrate for 15 min, respectively and observed under TEM (H-7650, Hitachi®, Japan). Photographs were taken from more than three random sites in three segments representing each sample and representative pictures of mesophyll cells were presented.

2.6 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) [29] and Generalized Linear Model (GLM) using SAS statistical software (SAS Institute, Cary, NC, USA). Pearson's correlation test [30] was carried out to study the relationships among the parameters. The probability levels of significance were denoted as * at 0.05, ** at 0.01, and *** at 0.001. The means were compared using Duncan's multiple range test (DMRT) [31]. For all the tests, $P<.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Photosynthetic Rate and Gas Exchange Measurements

In general, SA-pretreated plants performed well compared to the salt-stressed untreated plants, throughout the experimental period ($P<.01$) in all tested gas exchange attributes: net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), intercellular CO2 concentration (*Ci*), transpiration rate (*Tr*) and stomatal limitation (*Ls*) except for *Ls* and *Ci* of plants pretreated with 1.0 mmoldm-3 SA. Plants pretreated with 1.0 mmoldm-3 of SA did not show a significant difference compared to salt-stressed untreated plants in *Ci* and *Ls* towards the later part of the study period (Fig. 1).

Except for the slight rise in *Pn* of SA-pretreated plants on day nine, *Pn* of salt-stressed plants tend to decline with time in general (Fig. 1a). *Pn* of the SA-pretreated plants was significantly higher than that of the salt-stressed plants that were not treated with SA. Salt-stressed plants pretreated with 0.5 mmoldm-3 SA (Spray 1) showed the highest Pn among the stressed plants whereas the rate was even higher than that of control on day three and nine.

Salt stress, caused a reduction in *Gs* throughout the experiment (nearly 65% reduction compared to the control) (Fig. 1b). Pretreatment with SA significantly elevated the Gs over the untreated salt-stressed plants. *Gs* of the plants pretreated with 0.5 mmoldm-3 SA solution did not vary significantly during the initial nine days. After the ninth day, a marked reduction in *Gs* was observed in all stressed plants, while making the difference of the values between SA-pretreated and untreated considerably small towards the latter part of the study. Interestingly, the effect of salt stress and SA pretreatment on *Pn* was mirrored in *Gs*.

Intercellular CO_2 concentration in SA-pretreated plants remained similar to that of the control until day nine and was followed by a slight decline afterward (Fig. 1c). Salt-stressed untreated plants showed the lowest *Ci* throughout the experiment with pronounced reductions of 40% and 50% compared to the control on day 3 and 6, respectively. The pattern of the effect of SA pretreatment on *Ci* was more or less similar to that of *Pn* and *Gs*.

In general, SA-pretreated plants maintained a higher *Tr* than that of the untreated salt-stressed plants. Moreover, the *Tr* of SA-pretreated plants was greater than that of the control, on the first and last sampling days (Fig. 1d). The *Tr* of the salt-stressed plants declined until day nine (day six in untreated salt-stressed plants) followed by a progressive increase reaching a level where the variation is very less by day fifteen of salt stress.

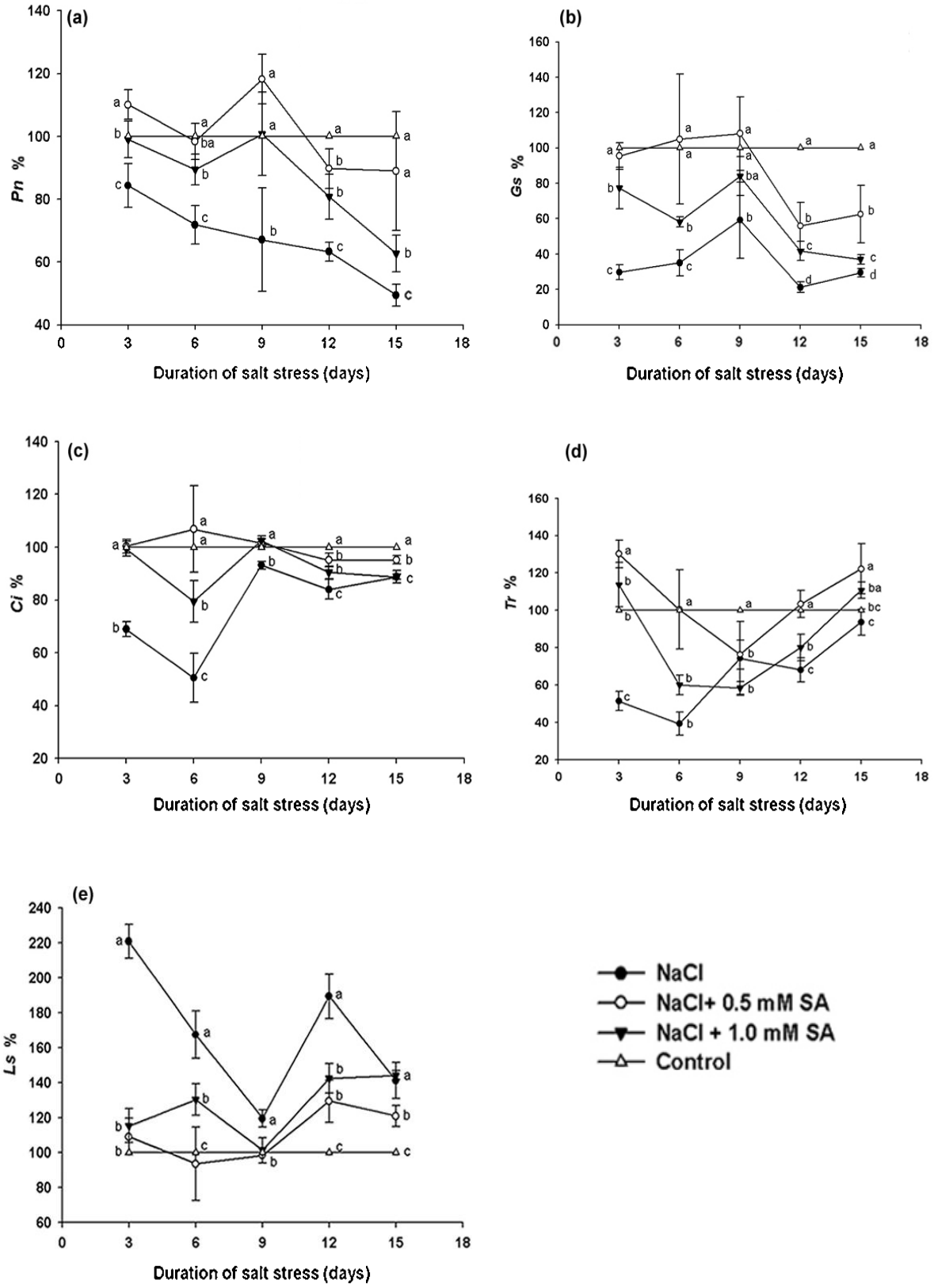

Fig. 1. Percentage variation of net photosynthetic rate (*Pn*) (a), stomatal conductance (*Gs*) (b), intracellular CO_2 content (*Ci*) (c), Transpiration rate (Tr) (d) and stomatal limitation (*Ls*) (e) of untreated salt-stressed and SA-pretreated salt-stressed

The stomatal limitation of untreated salt-stressed plants was remarkably higher than that of the control throughout the experiment (Fig. 1e). However, the pretreatment with SA dramatically reduced the *Ls* in stressed plants, recording a 50% reduction relative to the untreated salt-stressed plants at the first sampling. The effect of SA pretreatment seemed diminishing towards the end of the experimental period showing 29% and 44% greater *Ls* in 0.5 mmoldm-3 and 1.0 mmoldm-3 SA-pretreated plants, respectively compared to the control. Interestingly, the result of *Ls* was noted as nearly the inverse response of *Pn* and *Gs*.

Salt stress adversely affect the photosynthetic traits such as stomatal conductance, intercellular CO_2 concentration, chlorophyll contents, chloroplast structure, transpiration rate, and inhibition of photochemical and carboxylation reactions of leaves [32]. Even though photochemical and carboxylation reactions were not evaluated in the present study, observations made on other attributes substantially explain the response of the photosynthetic system in gerbera plants to salt stress and SA pretreatments.

Salt stress generally causes stomatal closure and hence, decrease the *Gs* and *Ci* of plant leaves. This stomatal closure and subsequent decrease of *Ci* occurs as a result of both osmotic effects and specific ion effects under the salt stress conditions [33]. The results make it clear that soil salinity leads to a suppression of *Gs* in gerbera, which consequently reduces the plants' capability to adequately supply CO_2 into the photosynthetic apparatus. This interdependency is further confirmed by the relationship shown among the patterns of *Pn*, *Gs*, and *Ci* in response to salt stress. The salt-induced inhibition in *Pn*of many plants is associated with the reduction in *Gs* and the inhibition of relevant metabolic phenomena or a combination of both [34]. The role of stomatal regulation in controlling the *Pn* and maintaining the water balance of the plants which are growing under stressful environmental conditions have been reported by several authors [35,36]. The reduced *Gs* under salt stress typically promotes a considerable reduction in the transpiration rate and the fact is supported by already published research based on many other crops [37]. A relatively higher level of *Tr* in SA-pretreated plants during the study indicate the influence of SA on *Gs* which in turn promotes *Tr.* In addition, SA pretreatment significantly improved the *Gs* while reducing the *Ls* assuring an efficient supply of CO2 to enhance *Pn*. Significant improvement in gas exchange attributes in salt-stressed gerbera pretreated with SA as observed in the current investigation is parallel to what has been reported in the literature: for instance, root drenching of tomato plants with 0.1 mmoldm-3 SA increased *Pn, Tr*, and *Gs* under saline stress conditions [38]. This view is further supported by the argument that the exogenous application of SA enhances the CO_2 assimilation, thus increase the dry matter content in plants [39,22,40]. A significant positive correlation of *Pn* with *Gs* (r = 0.874**, 0.949***) and *Ci* (r = 0.80**, 0.871**) as well as a negative correlation of *Pn* with Ls (r = _0.754*, _0.821**) were observed in salt-stressed SA-pretreated plants (with 0.5 mmoldm-3 and 1.0 mmoldm-3 SA, respectively). This is in good conformity with the above mentioned information reported in previous literature. Exogenous SA application can reverse the stomatal closure which is induced by abscisic acid [41]. This gives a plausible explanation for the enhancement of gas exchange by reversing the salt-induced stomatal closure with SA-pretreatment observed in the current study. The declining trend of *Pn* and *Gs* against time as the stress treatments continued, may be due to the adverse effects aggravated with the accumulation of salt. The reduced variation between untreated salt-stressed plants and SA-pretreated plants detected in *Gs, Ci, Tr*and *Ls* towards the end of the experiment may be due to the diminution of the ameliorative action of SA on these attributes with time.

3.2 Photosynthetic Pigments Content

All Variations of photosynthetic pigment contents in response to salt stress and SA pretreatment are depicted in Fig. 2. As evidenced by the graphs, two days after the first irrigation with salt, there is no significant difference in the amounts of carotenoid (*Car*), chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) or total chlorophyll (*tChl*: *Chla+b*) among the treatments including the control. But the difference became significant from ninth day onwards. In general, *Car* showed adeclining trend against the time with a more pronounced drop during the initial phase. Salt-stressed plants pretreated with 0.5 $mmoldm^{-3}$ SA contained the highest amount of *Car* throughout the experiment, recording a 25% increment over the salt-stressed untreated plants after fifteen days. Control plants maintained a *Car* content higher than the 1.0 $mmoldm^{-3}$ SA-pretreated plants but lower than that of 0.5 $mmoldm^{-3}$ SA-pretreated plants.

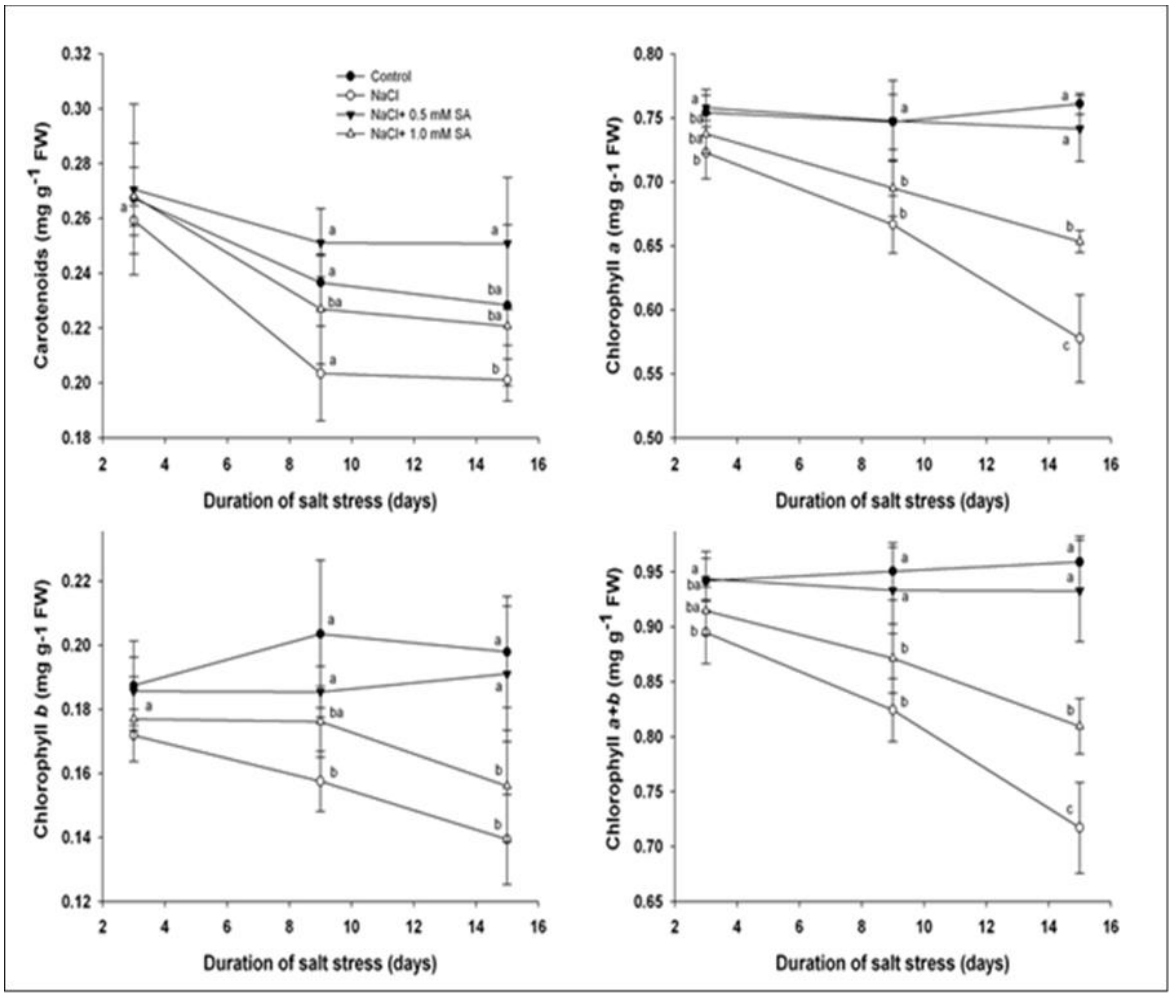

Fig. 2. Variation of carotenoids, chlorophyll a, b and total chlorophyll (Chla+b) contents in leaves of gerbera plants under control, untreated salt-stressed and SA-pretreated salt-stressed conditions over the experimental period. Different letters at data points on the same sampling day indicate significant differences according to $DMRT_{0.05}$. Vertical bars indicate the means ± S.E. (*n* = 3)

Control plants and salt-stressed plants pretreated with 0.5 $mmoldm^{-3}$ SA showed the highest and a similar level of *Chl a*. Untreated salt-stressed plants and 1.0 $mmoldm^{-3}$ SA-pretreated salt-stressed plants decreased the *Chl a* content progressively with time and ended up with 24% and 13% reductions compared to the control, respectively. The response of *Chl b* also exhibited a relatively similar pattern to *Chl a*. Consequently, the *tChl* also resembled a similar pattern. Total chlorophyll content of plants pretreated with 0.5 $mmoldm^{-3}$ SA which did not significantly differ from control, showed a 28% rise over the untreated salt-stressed plants at the end of the experiment. Difference of each pigment content among the treatments, was getting wider towards the end of the experimental period.

According to [35,36], changes in photosynthesis due to exogenous SA application under environmental stress conditions are due to either stomatal or non-stomatal limitations. Reduced chlorophyll content is one of the major reasons for non-stomatal restriction of *Pn* [42]. The reduction in chlorophyll content observed in the salt-stressed plants of the present study, may be due to either a decrease in chlorophyll synthesis or an increase in chlorophyll degradation or a combination of both reasons [43]. In addition, the continued salt treatment may have caused to accumulate a higher amount of salt in the pots over the time, aggravating the above mentioned adverse effects. Corresponding reduction of *Pn* with reduced amount of *tChl* signifies the effect of non-stomatal limitation on *Pn* as the *tChl* is affected by salt stress. Nevertheless, exogenous

application of SA reduced the adverse effects of salt stress on leaf chlorophyll content revealing that these adverse effects of salt stress on chlorophyll content (*Chl a*, *b* and *tChl*) could be fully prevented by 0.5 mmoldm^{-3} SA application, under the given conditions. As supported by many prior research publications, SA is capable of enhancing the chlorophyll content in tomato, barley, maize and cucumber, respectively, when exposed to salt stress [44,45,24,20]. In the present work, positive correlation of*tChl* with *Chl* a (r = 0.957***) and *Chl b* (r = 0.865**) in the plants pretreated with 0.5 mmoldm^{-3} SA as well as with *Chl a* (r = 0.973***), *Chl b* (r = 0.823**) and *Car* (r = 0.858**) in the plants pre-treated with 1.0 mmoldm^{-3} SA demonstrate the ameliorative effect of SA on photosynthetic pigments under salt stress. This is further supported by [46] who reported that foliar SA treatment increased *Chl a*, *b* and *Car* contents leading to an increased rate of photosynthesis in maize subjected to salt stress.

[6] reviewed that salt stress inhibits the chlorophyll and total carotenoid contents in leaves of many crops. It is well documented that *Car* play a protective role against photo-oxidation by dissipating the excessive energy of excitation [47]. Accordingly, the reason for increased amount of leaf carotenoids in SA-pretreated plants in the present investigation, may be an influence of such protective mechanism that counteracts the deleterious effects of oxidative damage resultant from salt stress. It is worth to note that, pretreatment with 0.5 mmoldm^{-3} SA maintained the *Car* content even above that of control and one could expect, therefore, that this dose is more effective as far as the *Car* content and given conditions are concerned. In view of this, [48] reported that one wheat cultivar treated with 0.25 mmoldm^{-3} SA in nutrient solution showed a greater leaf carotenoid content in salt-stressed plants over the plants grown under normal conditions. However, it can be inferred that the reduction of *Car* observed with time irrespective of the treatment, may not be a phenomenon related to cumulative effect of continued salt stress.

3.3 Chloroplast Ultrastructure

Ultrastructure of leaf mesophyll cells was studied with special emphasis on chloroplasts. Disc-shaped chloroplasts of mesophyll cells in control plants possessed the typical ultrastructure with well-developed stromal and granal thylakoids, and intact chloroplast envelope. Starch grains and a few plastoglobuli were also notable (Fig. 3a). Significant changes were observed in untreated salt-stressed plants. Swelling and markedly reduced number of stromal thylakoids, and disintegration of granal thylakoids were the most notable variations that appeared (Fig. 3b). In addition, the number of plastoglobuli was relatively high. Although, some chloroplast envelopes seemed unaffected, it was disrupted occasionally (Fig. 3b, Thicker arrows). Starch grains were hardly observed. Mitochondria did not show any visible damages. In salt-stressed plants pretreated with 0.5 mmoldm^{-3} SA, distinguishable ultrastructural change was not shown and consequently exhibited the typical features as in the case of control plants (Fig. 3c). Salt-stressed plants that were pretreated with 1.0 mmoldm^{-3} SA, exhibited a swelling of thylakoids as well as slight disintegration of grana (Fig. 3d). Nevertheless, the chloroplast envelope remained intact and the number of plastoglobuli was less compared to that in untreated salt-stressed plants. Starch grains were also present. No damage could be noted on mitochondria.

According to the authors' knowledge, this is the first report regarding the effects of SA on salt-induced chloroplast ultrastructural changes. It is reported that excess H_2O_2 and H_2O_2-derived$^{\bullet}$OH are responsible for the deleterious effects of salt stress on chlorophyll content and chloroplast ultrastructure [49]. Chloroplasts are considered to be the most powerful intracellular generator of reactive oxygen species (ROS) [50]. One could expect higher vulnerability of chloroplasts to oxidative damage when plants are exposed to salt stress. The typical phenomenon of the oxidative damage induced by salt stress is prominent swelling of thylakoids, which may finally, cause the disturbance or inhibition of photochemical reactions [51]. In the present study, ultrastructural damages observed in the chloroplasts of untreated salt-stressed plants are in good agreement with the above phenomenon. Damage to the chloroplast envelope indicates the severity of the adverse effect of salt stress. It is suggested that the swelling of thylakoids is induced by lipid peroxidation caused by ROS such as H_2O_2 and $^{\bullet}$OH derived from H_2O_2 but not by $O_2^{\bullet-}$ [49]. [51] suggested that lipids released upon thylakoid damage may be responsible for the increase in plastoglobuli which was described by [52] as the localizers of toxicants in the chloroplasts. Therefore, the increased number of plastoglobuli detected in this study could be an adaptive response to the increased salinity. These responses of chloroplast under salt stress

are in view with the observations of [53] in potato, [54] in sweet potato, [55] in *Cistus albidus* and [56]) in *Kandeliacandel*. Reduced net photosynthesis due to destruction of photosynthetic apparatus [55] and/or the use of soluble sugars to contribute to the osmotic adjustment may be responsible for the reduced number of starch grains observed in salt-stressed untreated plants.

Ultrastructural damage, prevented by SA pretreatment suggests that SA may be responsible to inhibit generation of ROS such as H_2O_2 and $^{\bullet}OH$ derived from H_2O_2 assuring protection from lipid peroxidation. Less number of plastoglobuli observed in SA-pretreated plants may be attributable to SA-induced inhibition of lipid peroxidation. [57] suggested that ion toxicity or ionic imbalance might induce the swelling of thylakoids, while osmotic effects might cause the destruction of the chloroplast envelope. This phenomenon along with the observation (keeping the chloroplast envelope intact while the thylakoid structure was damaged) on the plants pretreated with 1.0 mmoldm-3 SA, leads us to conclude that SA may be more capable of preventing the damage caused by osmotic stress than the damage caused by ion toxicity or ionic imbalance in chloroplast.

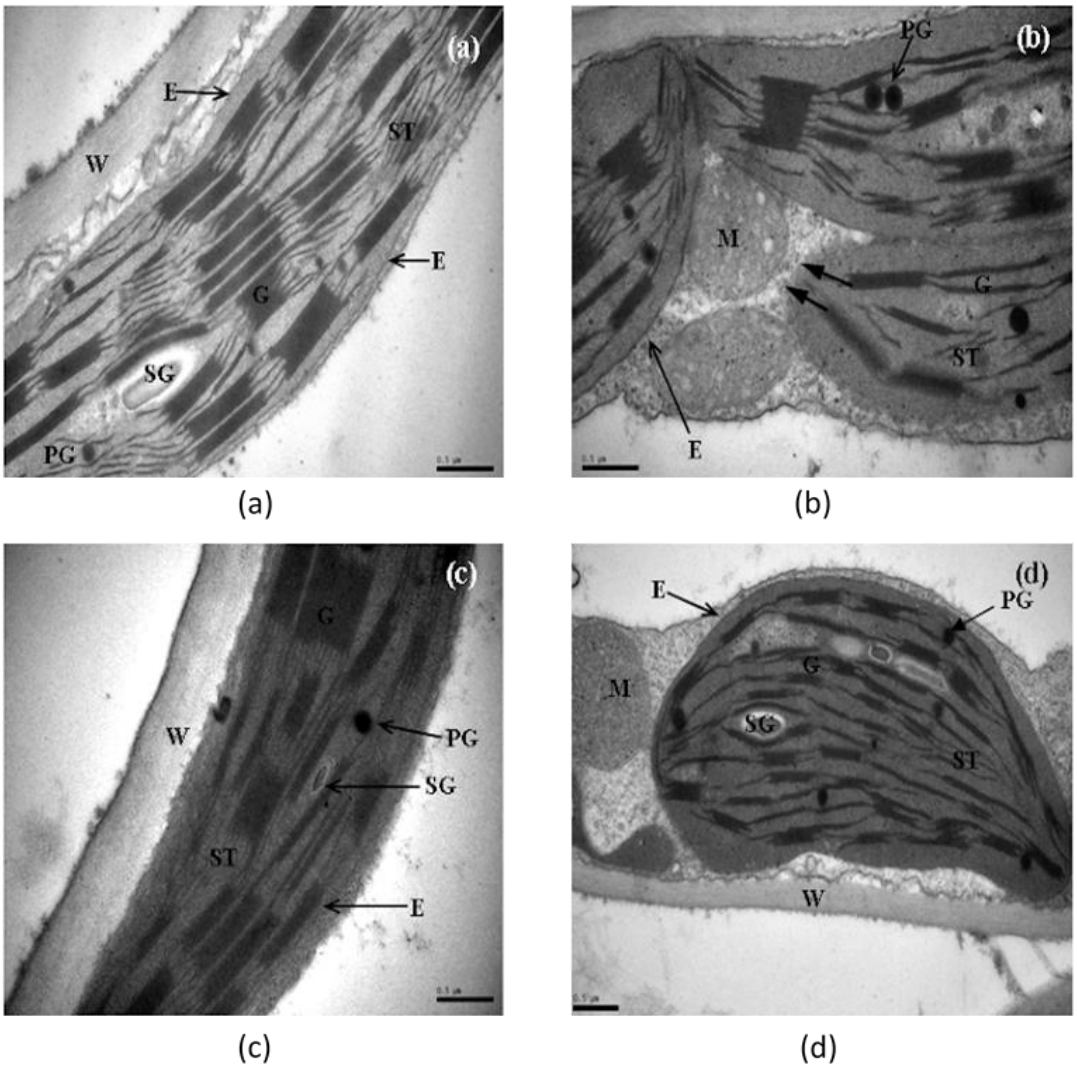

Fig. 3. Transmission electron micrographs (TEM) of leaf mesophyll cells in gerbera plants under control (a), untreated salt-stressed (b), 0.5 mM SA-pretreated salt-stressed (c) and 1.0 mM SA-pretreated salt-stressed (d) conditions. E- chloroplast envelope, G- grana, ST- stromal thylakoids, M- mitochondria, PG- plastoglobuli, SG- starch grains, W- cell wall. Scale bars represent 0.5 µm

According to [58], the effect of salinity on leaf chlorophyll may originate from damage of chloroplast structure (or from an increased chlorophylase activity). However, higher salt concentrations appear to be required to bring about loss of chlorophyll than to cause chloroplast ultrastructural damage [59]. Furthermore, [52] stated that, net photosynthesis is primarily inhibited at chloroplast level but not due to stomatal closure. In the present study also, the magnitude of percentage variation in *Pn* and the pattern of variation in chlorophyll content hint that there should be other major aspect/s responsible for the variation in net assimilation rate. Therefore, in view of all these reports and the results of the present study, it can be deduced that the response of photosynthesis observed under each treatment may be due to a combination effect of stomatal limitation and non-stomatal limitations such as photosynthetic pigments and chloroplast ultrastructure variations by respective treatment.

4. CONCLUSION

Traits, such as gas exchange characteristics, photosynthetic pigment contents and chloroplast ultrastructure which are responsible for the photosynthetic capacity were adversely affected by $NaCl_{(aq)}$ salt stress in gerbera at reproductive stage. Exogenous foliar application of SA alleviated the salt-induced photosynthetic inhibition by enhancing gas exchange, photosynthetic pigment content and maintaining chloroplast ultrastructure. Correlations among most of the vital attributes were significant. Of the two concentrations tested, 0.5 $mmoldm^{-3}$ SA seemed to be more effective. These findings hint that crop plants under salt stress may, in future, be benefited by exogenous application of SA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. del Martínez-Ballesta MC, Silva C, López-Berenguer C, Cabañero FJ, Carvajal M. Plant aquaporins: new perspectives on water and nutrient uptake in saline environment. Plant Biol. 2006;8(5):535–46.
2. Khan MIR, Fatma M, Per TS, Anjum NA, Khan NA. Salicylic acid-induced abiotic stress Tolerance and underlying mechanisms in plants. Front. Plant Sci. 2015;6:462.
   DOI: 10.3389/fpls.2015.00462
3. Mahajan S, Tuteja N. Cold, salinity and drought stresses: An overview. Arch BiochemBiophys. 2005;444(2):139–158.
   DOI: 10.1016/j.abb.2005.10.018
4. Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 2003;218(1):1–14.
5. Yamaguchi T, Blumwald E, Yamaguchi T, Blumwald E. Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci. 2006;10:615-20.
   DOI: 10.1016/j.tplants.2005.10.002
6. Parida AK, Das AB, Salt tolerance and salinity effects on plants: A review. Ecotoxicol Environ Saf. 2005;60(3):324–49.
   DOI: 10.1016/j.ecoenv.2004.06.010
7. Paranychianakis NV, Chartzoulakis KS. Irrigation of Mediterranean crops with saline water: From physiology to management practices. Agric Ecosyst Environ. 2005;106(2):171–87.
   DOI: https://doi.org/10.1016/j.agee.2004.10.006
8. Janda T, Horváth E, Szalai G,Paldi E. Role of salicylic acid in the induction of abiotic stress tolerance in Salicylic acid: A plant hormone, Springer. 2007;91–150.
9. Hernández JA, Olmos E, Corpas FJ, Sevilla F, del Río LA. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. 1995;105(2):151–67.
   DOI: https://doi.org/10.1016/0168-9452(94)04047-8
10. Hernandez JA, Campillo A, Jimenez A, Alarcon JJ, Sevilla F. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol. 1999;141(2):241–51.
11. Agastian P, Kingsley SJ, Vivekanandan M. Effect of Salinity on Photosynthesis and Biochemical Characteristics in Mulberry Genotypes. Photosynthetica. 2000;38(2): 287–90.
    DOI: 10.1023/A:1007266932623
12. Husen A, Iqbal M, Aref IM. IAA-induced alteration in growth and photosynthesis of pea (*Pisumsativum* L.) plants grown under salt stress. J. Environ Biol. 2016;37:421–9.
13. Mimouni H, Wasti S, Manaa A, Gharbi E, Chalh A, Vandoorne B, Lutts S, Ahmed HB. Does salicylic acid (SA) improve

tolerance to salt stress in plants? A study of SA effects on tomato plant growth, water dynamics, photosynthesis, and biochemical parameters. OMICS J Integr Biol. 2016;20:180–90.

14. Khan MIR, Asgher M, Khan NA. Alleviation of salt-induced photosynthesisand growth inhibition by salicylic acid involves glycinebetaineand ethylene in mungbean (*Vignaradiata* L.). Plant PhysiolBiochem. 2014;80:67–74.
15. Senaratna T, Touchell D, Bunn E, Dixon K. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul. 2000;30(2):157–61.
DOI: 10.1023/A:1006386800974
16. Don KKG, Xia YP, Zhu Z, Le C, Wijeratne AW. Some deleterious effects of long-term salt stress on growth, nutrition, and physiology of gerbera (*Gerbera jamesonii* L.) and potential indicators of its salt tolerance. J Plant Nutr. 2010;33(13):2010–27.
17. Kapila GDK, Xia Yi, Ping BBMVS, Zhu Zhujun. Ultrastructural changes induced by long-term nacl salt stress in leaf and root cells of gerbera. Indian J Hortic. 2009; 66(4):551–53.
18. Jini D, Joseph B. Physiological Mechanism of Salicylic Acid for Alleviation of Salt Stress in Rice. Rice Science. 2017; 24(2):97-108.
19. Klessig DF, Malamy J. The salicylic acid signal in plants. Plant Mol Biol. 1994;26(5):1439–58.
DOI: 10.1007/BF00016484
20. Yildirim E, Turan M, Guvenc I. Effect of foliar salicylic acid applications on growth, chlorophyll, and mineral content of cucumber grown under salt stress. J Plant Nutr. 2008;31(3):593–612.
DOI: 10.1080/01904160801895118
21. He YL, Liu Q. Thermotolerance related to antioxidation induced by salicylic acid and heat acclimation in tall fescue seedlings. J Plant PhysiolMol Biol. 2002; 28:89-95.
22. Khan W, Prithiviraj B, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. J Plant Physiol. 2003;160(5):485–92.
DOI: 10.1078/0176-1617-00865
23. Barkosky RR, Einhellig FA. Effects of salicylic acid on plant-water relationships. J Chem Ecol. 1993;19(2):237–47.
DOI: 10.1007/BF00993692
24. Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zeamays* L.) grown under salinity. J Plant Physiol. 2007;164(6):728–36.
DOI: 10.1016/j.jplph.2005.12.009
25. Bhatia R, Singh KP, Jhang T, Sharma T. Assessment of clonal fidelity of micropropagated gerbera plants by ISSR markers. Sci. Hortic. 2009;119:208–11.
DOI: 10.1016/j.scienta.2008.07.024
26. Sonneveld C, Straver N. Nutrient solutions for vegetables and flowers grown in water or substrates. Voedingspolossingen Glas. 1994;8(1)33.
27. Williams M, Senaratna T, Dixon K, Sivasithamparam K. Benzoic acid induces tolerance to biotic stress caused by Phytophthoracinnamomi in Banksia attenuata. Plant Growth Regul. 2003;41:89–91.
28. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 1987;148:350–82.
29. Meng R, Saade S, Kurtek S, Berger B, Brien C, Pillen K, et al. Growth curve registration for evaluating salinity tolerance in barley. Plant Methods. 2017;13:18.
Available:https://doi.org/10.1186/s13007-017-0165-7
30. Schober P, Boer C, Schwarte LA. Correlation Coefficients: Appropriate Use and Interpretation, Anesthesia & Analgesia. 2018;126(5)1763-68.
DOI: 10.1213/ANE.0000000000002864
31. NikaljeGC, Variyar PS, Joshi MV, Nikam TD, Suprasanna P. Temporaland spatialchangesin ion homeostasis,antioxidantdefenseand accumulation of flavonoidsand glycolipidin ahalophyte *Sesuvium portulacastrum* (L.) L. PLo SONE. 2018;13(4):e0193394.
Available:https://doi.org/10.1371/journal.pone.0193394
32. Mäkelä P, Kontturi M, Pehu E, Somersalo S. Photosynthetic response of drought-and salt-stressed tomato and turnip rape plants to foliar-applied glycinebetaine. Physiol Plant. 1999;105(1):45–50.
33. Heuer B. Photosynthetic carbon metabolism of crops under salt stress.In: Pessarakli M, editor.Handbook of photosynthesis. New York; C.R.C. Press. 1997;887–96.

34. Lazar T, Taiz L, Zeiger E. Plant physiology. 3rd ed. Ann Bot. 2003;91(6):750–51. DOI: 10.1093/aob/mcg079
35. Athar H, Ashraf M. Photosynthesis under drought stress. In: Pessarakli M, editor. Handbook of Photosynthesis. 2nd (Ed.). New York; C.R.C. Press. 2005;795–810.
36. Dubey RS. Photosynthesis in plants under stressful conditions. Handbook of photosynthesis. Marcel Dekker. 1996;859–75.
37. Pinheiroa HA, Silva JA, Endres L, Ferreira VM, Câmarac CA, Cabral FF et al. Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinuscommunis* L) seedlings subjected to salt stress conditions. Ind Crops Prod. 2008;27(3):385–92.
38. Stevens J, Senaratna T, Sivasithamparam K. Salicylic Acid Induces Salinity Tolerance in Tomato (*Lycopersiconesculentum* cv. Roma): Associated Changes in Gas Exchange, Water Relations and Membrane Stabilisation. Plant Growth Regul. 2006;49(1):77–83. DOI: 10.1007/s10725-006-0019-1
39. Li T, Hu Y, Du X, Tang H, Shen C, Wu J. Salicylic acid alleviates the adverse effects of salt stress in *Torreyagrandis cv.* merrilliiseedlings by activating photosynthesis and enhancing antioxidant systems. PLoS ONE. 2014;9(10):9:e109492. DOI: 10.1371/journal.pone.0109492
40. Szepesi A, Csiszár J, Bajkán S, Gémes K, Horváth F, Erdei L. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt-and osmotic stress. ActaBiol Szeged. 2005;49(1-2):123–25.
41. Rai VK, Sharma SS, Sharma S. Reversal of ABA-Induced Stomatal Closure by Phenolic Compounds. J Exp Bot.1986;37(1):129–34. DOI: 10.1093/jxb/37.1.129
42. Downton WJS, Grant WJR, Robinson SP. Photosynthetic and stomatal responses of spinach leaves to salt stress. Plant Physiol. 1985;78(1);85–88.
43. Santos CV. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci. Hortic (Amsterdam). 2004;103(1):93–99.
44. Tari I, Csiszár J, Szalai G, Horváth F, Pécsváradi A, Kiss G, et al. Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. Acta Biol Szeged. 2002;46:55-56.
45. El-Tayeb MA. Response of barley grains to the interactive e.ect of salinity and salicylic acid. Plant Growth Regul. 2005;45(3):215–24. DOI: 10.1007/s10725-005-4928-1
46. Khodary SEA. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int J Agric Biol. 2004;6(1):5–8.
47. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7(9):405–10. DOI: 10.1016/s1360-1385(02)02312-9
48. Arfan M, Athar HR, Ashraf M. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress?. J Plant Physiol. 2007;164(6):685–694. DOI: 10.1016/j.jplph.2006.05.010
49. Yamane K, Kawasaki M, Taniguchi M, Miyake H. Pretreatment with Antioxidants Decreases the Effects of Salt Stress on Chloroplast Ultrastructure in Rice Leaf Segments (*Oryza sativa* L.). Plant Prod. Sci. 2004;7:292–300. DOI: 10.1626/pps.7.292
50. Foyer CH, Descourvieres P, Kunert KJ. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ. 1994;17(5):507–23.
51. Barhoumi Z, Djebali W, Chaibi W, Abdelly C, Smaoui A. Salt impact on photosynthesis and leaf ultrastructure of *Aeluropuslittoralis*. J Plant Res. 2007;120(4):529–37.
52. Torgasheva EG. Ultrastructural changes in the chloroplasts of leaf mesophyll in *Morus alba* (moraceae) under conditions of aluminiumplant. Bot Zhurnal. 1984;69:921-925.
53. Gao HJ, Yang HY, Bai JP, Liang XY, Lou Y, Zhang JL, et al. Ultrastructural and physiological responses of potato (*Solanum tuberosum* L.) plantlets to gradient saline stress. Front Plant Sci. 2015;13(5):787. DOI: 10.3389/fpls.2014.00787 PMID: 25628634; PMCID: PMC4292236.
54. Mitsuya S, Takeoka Y, Miyake H. Effects of sodium chloride on foliar ultrastructure of sweet potato (*Ipomoea batatas* Lam.) plantlets grown under light and dark conditions in vitro, J Plant Physiol. 2000;157(6):661–67.

DOI: 10.1016/S0176-1617(00)80009-7

55. Sánchez-Blanco MJ, Rodríguez P, Olmos E, Morales MA, Torrecillas A. Differences in the effects of simulated sea aerosol on water relations, salt content, and leaf ultrastructure of rock-rose plants. J Environ Qual. 2004;33(4):1369–75.
DOI: 10.2134/jeq2004.1369

56. Qiu D, Lin P, Su J. Relationship of leaf ultrastructure of mangrove *Kandeliacandel* (L.) Druce to salt tolerance. J For Sci. 2005;51:476-80.
DOI: 10.17221/4581-JFS

57. Yamane K, Kawasaki M, Taniguchi M, Miyake H. Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. J Plant Physiol. 2003;160(5):573–75.
DOI: 10.1078/0176-1617-00948

58. Günes A, Inal A, Alpaslan M. Effect of salinity on stomatal resistance, proline, and mineral composition of pepper. J Plant Nutr. 1996;19(2):389–96.
DOI: 10.1080/01904169609365129

59. Flowers TJ, Duque E, Hajibagheri MA, McGonigle TP, Yeo AR. The effect of salinity on leaf ultrastructure and net photosynthesis of two varieties of rice: Further evidence for a cellular component of salt-resistance. New Phytol. 1985; 100(1):37–43.

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