



The Mechanism Behind The Promotive Effect of Foliar Application of 5-Aminolevulinic Acid (ALA) in Tomato Plants Under Salt Stress

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Abstract

The effect of foliar ALA application on the internal ALA concentration in tomato plants grown in soil containing high levels of NaCl was investigated. Six week old plants were treated with 100, 50, and 25 mmol/L NaCl on a weekly basis and they were simultaneously treated with 5-ALA at a concentration of 5% by foliar spray. The effect of foliar ALA application on plant growth, chlorophyll contents and internal ALA concentration was studied. The internal ALA shoot concentrations ranged between 27.50 ± 2.12 and $34.35 \pm 1.48 \mu\text{g g}^{-1}$ dry weight with no significant difference ($p < 0.05$) recorded between plants treated with NaCl alone and those treated with both NaCl and ALA. The concentrations of chlorophyll a and b were elevated only in tomato plants treated with NaCl and ALA, whereas their levels decreased in plants treated with NaCl only. An adverse significant effect ($p < 0.05$) of salinity stress was recorded on plants length, number of leaves, shoot and root fresh and dry weight. However, no significant difference ($p < 0.05$) was observed in plants treated with NaCl alone with those treated with NaCl plus ALA in comparison to the control. The results of the present study suggested that foliar ALA treatment had no effect on the Na and Cl uptake, the internal ALA concentration, and had no role in adverting the effects of salinity on plant growth. The present study has proven that foliar ALA is directly used by the plant for the synthesis of chlorophyll in order to increase the photosynthetic rate and thus to help tomato plants to survive the salinity stress.

Key words: ALA, Chlorophyll, *Lycopersicon esculentum*, Salinity Stress, Tomato.

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Introduction

5- aminolevulinic acid (ALA) is a natural amino acid that plays a role in the improvement of many physiological and biochemical characteristics of plants such as the improvement of plants tolerance to stress including cold and hot temperatures, salinity, and water scarcity (Hotta *et al.*, 1998; Watanabe *et al.*, 2000; Balestrasse *et al.*, 2010; Al-Qureshi and Awad, 2011).

ALA formation is the first step in the biosynthesis of chlorophyll. Two molecules of ALA are condensed to form porphobilinogen (PBG), a unique single pyrrole structure. Subsequently, four molecules of PBG then condense to form a large ring (Uroporphyrinogen III) which is oxidized to form protoporphyrin IX. This pathway will further branches depending on the type of metal added to the porphyrin centre. If magnesium is inserted, then additional steps are needed to convert the molecule into chlorophyll (Taiz and Zeiger, 2002).

Several studies have demonstrated the promotive effect of ALA on the photosynthetic rate and chlorophyll contents in a number of plants. The chlorophyll content increased significantly by 21- 30.1% while CO₂ concentration decreased after ALA treatment in Kudzu plant species (Xu *et al.*, 2010). Several studies on date palms (Al-Khateeb *et al.*, 2006), horseradish, rice seedlings (Hotta *et al.*, 1997), and lettuce (Xu *et al.*, 2012) have also demonstrated similar results. Foliar application of ALA has also enhanced the chlorophyll content; photosynthetic rate in Pakchoi leaves (Memon *et al.*, 2009). These results coincided with the study of Tanaka *et al.* (1993) which suggested that exogenous treatment with ALA may result in higher level of chlorophyll a which is a prerequisite for chlorophyll b formation (Wang *et al.*, 2005; Awad, 2008).

Many plants live under salt stress condition in different regions of the world. Salt stress can adversely affect various plant processes such as reduction in the seed germination, yield, and the photosynthetic rate (Wang *et al.*, 2001; Perveen *et al.*, 2010). The positive effect of the liquid fertilizer PENTAKEEP which contains ALA on plant photosynthesis, growth, and stress resistance has been documented (Watanabe *et al.*, 2000; Nishihara *et al.*, 2003; Zsembeli *et al.*, 2008). The foliar application of ALA plays an important role in reducing the salt-induced effects in many plants such as the Pakchoi (*Brassica campestris*) (Memon *et al.*, 2009), spinach (*Spinacia oleracea*) (Nishihara *et al.*, 2003), cotton seedlings (Watanabe *et al.*, 2000), and sunflower (*Helianthus annus L*) (Akram *et al.*, 2012).

ALA helps in regulating the physiological processes which are associated with plant growth under salt stress (Zhao *et al.*, 2014) such as improving the reactive oxygen species by scavenging (Nishihara *et al.*, 2003; Akram *et al.*, 2012), regulating the NA⁺ uptake (Watanabe *et al.*, 2000), improving seed germination (Hotta *et al.*, 1997a, b), improving the photosynthesis (Wang *et al.*, 2008), enhancing photosynthetic capacity (Zhao *et al.*, 2014), and controlling aquaporin expression (Zhao *et al.*, 2015).



Tomato is a glycophyte plant that has been classified as a moderate salt-tolerant crop (Maas, 1986) using an exclusion strategy that restrict the excessive transport of Na and Cl to the roots as well as from roots to shoots of plants grown in saline substrates (Bergmann, 1992; Marschner, 1995). Tomato is an important crop facing a serious constraint when grown in areas with optimal climate for tomato cultivation however suffering from salinization of soil which is considered as a serious problem facing many countries specially in arid and semi-arid areas (Yurtseven *et al.*, 2005; Abdel Latef, 2010).

Most studies available today have investigated the importance of ALA external application in reducing the salt-induced effects in many plants. However, the present study is a first attempt to examine the effect of salinity on ALA internal contents in tomato plants treated with foliar ALA and therefore its correlation to salt tolerance. To our knowledge, no research has investigated the interactive effects of external ALA application with internal ALA concentration under saline conditions in any plant including tomato.

Materials and Methods

Plant material and growth conditions

Tomato seeds (*Lycopersicon esculentum* L. cv. Vanessa, F1) were sowed in Bio-Mix Peat Potting Substratum (Jiffy Products International BV, Holland) in plastic tray. Bio-Mix Peat Potting Substratum properties were (organic matter \geq 85%, Ca \geq 5%, pH 3.5-6.5, Na \leq 250mg/L). Four weeks old germinated seeds were transferred into pots filled with Bio-Mix Peat Potting Substratum and were kept in a greenhouse under natural light conditions at an average temperature of 20-35°C during the experiment span of time at the University of Bahrain, College of Science, Sakhir campus. The experiment was conducted during the period from 25 October 2016 till 8 January 2017.

Six week old plants were treated with 100, 50, and 25 mmol/L NaCl on a weekly basis and they were simultaneously treated with PENTAKEEP® *super* EC Fertilizer containing 5-ALA provided by Cosmo Seiwa Agriculture CO., LTD, Japan at a concentration of 5% by foliar spray. The upper and lower leaf surface were sprayed until wetted by hand-held sprayer due to the fact that the absorption of lower leaf surface is more rapid and effective (Hull *et al.*, 1975). The concentrations of NaCl were selected based on various salinity-induced studies in different plants (Watanabe *et al.*, 2000; Tari *et al.*, 2002; Nishihara *et al.*, 2003; Naeem *et al.*, 2012; Zhang *et al.*, 2013). The same salt treatment was repeated on another set of plants without the use of the ALA foliar application. In the control treatment, distilled water was used instead of salt solution. Each treatment was replicated four times and plants were harvested after 10 weeks of salt and ALA treatments.

Plants were washed with distilled water, blotted to dry, divided into shoots and roots, and weighed. Plants height, and number of leaves were measured and counted. Shoots and roots were then placed in an oven at 60°C to dry until reaching a constant weight and their dry weight was then recorded. Shoot and root samples were mechanically grinded to fine power, kept in closed glass bottles, and stored in a dry dark and cool place until analysis.



Chlorophyll determination

Two grams of leaf samples were used for each replicate/treatment to determine the concentrations of chlorophyll a and b. Chlorophyll contents were extracted using 10 mL acetone (90%). The extracts were then centrifuged for 10 minutes at 4000x g, and chlorophyll a and b were measured by spectrophotometer (Perkin Elmer Lambda XLS), and calculated using the appropriate equations following the UNESCO protocol (Vohra, 1966).

ALA extraction and analysis

ALA extraction was carried out following the procedure of Kolosov and Rebeiz (2005) with some modifications as described by Freije and Alkhuzai (2015). One gram of the powdered plant leaves with a total of four replicate from each specimen/treatment were used for the ALA analysis. Fluorometric measurement of ALA was performed on the extracted samples according to the method of Lee *et al.* (2004) with some modifications as described by Freije and Alkhuzai (2015).

Statistical analysis

The obtained data were statistical analyzed using the Minitab 16. One way analysis of variance was used to test the difference in the means of height, leaves, shoot wet , shoot dry weight, shoot ALA , chlorophyll a and b, root wet, root dry weight among the different treatments of water, water plus salt, and water plus salt plus ALA. Independent samples to test was also performed to test if there were significant differences between the presence and absence of ALA. Results were presented in 4 replicates of mean values and standard deviations (\pm SD). A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The effect of different salt treatment alone and in combination with foliar ALA application on plant growth in terms of height, number of leaves, shoot and root fresh and dry weight is presented in Table 1 . Dunette test was used to find out the significant level of different salt treatments alone and in combination with ALA in comparison to control plants (Table 2). A significant inhibitory effect ($p < 0.05$) was observed in plants subjected to salinity stress and salinity plus ALA treatments as compared to their respective control. This decrease was higher in plants grown under 50mmol/L NaCl condition compared to control groups; while the growth of all plants treated with 100mmol/L NaCl was terminated after 10 days of salt treatment. Moreover, no significant difference was recorded in any growth parameters measured between plants treated with NaCl alone and those treated with both NaCl and ALA.



Table 1: Effects of salinity and ALA on shoot length, number of leaves, and biomass of *Lycopersicon esculentum* L. plants. Figures in the same column having the same superscript are not significantly different at $p < 0.05$.

Treatment	Length (cm)	No. of leaves	Shoot of fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control (H ₂ O)	26.30±2.12 ^a	9.00±0.00 ^a	19.97±0.21 ^a	2.16±0.11 ^a	3.33±0.82 ^a	0.29±0.04 ^a
50 mmol/L NaCl	12.35±0.49 ^b	5.50±0.71 ^b	4.45±0.43 ^b	0.75±0.07 ^b	0.83±0.11 ^b	0.03±0.01 ^b
25 mmol/L NaCl	17.15±1.48 ^c	8.50±0.71 ^c	12.10±0.49 ^c	1.29±0.01 ^c	1.11±0.21 ^c	0.07±0.05 ^c
Control (H ₂ O) + ALA	27.15±0.64 ^a	9.50±0.71 ^a	20.66±4.91 ^a	2.23±0.61 ^a	3.51±0.36 ^a	0.34±0.04 ^a
50 mmol/L NaCl + ALA	13.10±0.14 ^b	5.50±0.71 ^b	4.31±0.52 ^b	0.82±0.19 ^b	0.89±0.06 ^b	0.03±0.01 ^b
25 mmol/L NaCl + ALA	18.00±0.14 ^c	8.50±0.71 ^c	11.24±0.11 ^c	1.35±0.21 ^c	0.91±0.10 ^c	0.10±0.01 ^c

Table 2: Correlation and interaction analysis between ALA and salt treatment and shoot length, number of leaves, biomass, photosynthetic pigments and ALA concentrations in *Lycopersicon esculentum* L.

Correlation parameter	Significant level (p)	Correlation/ interaction
Length and salt treatment	0.000	Correlation
Length and ALA treatment	0.250	No correlation
Length, ALA and salt treatment interactions	0.997	No interaction
No. of leaves and salt treatment	0.000	Correlation
No. of leaves and ALA treatment	0.670	No correlation
No. of leaves, ALA and salt treatment interactions	0.824	No interaction
Shoot wet weight and salt treatments	0.000	Correlation
Shoot wet weight and ALA treatment	0.935	No correlation
Shoot wet weight, ALA and salt treatment interactions	0.867	No interaction
Shoot dry weight and salt treatments	0.001	Correlation
Shoot dry weight and ALA treatment	0.234	No correlation
Shoot dry weight, ALA and salt treatment interactions	0.485	No interaction
Shoot ALA level and salt treatment	0.002	Correlation



Shoot ALA level and ALA treatment	0.831	No correlation
Shoot ALA level, ALA and salt treatment interactions	0.484	No interaction
Chlorophyll a content and salt treatment	0.016	Correlation
Chlorophyll a content and ALA treatment	0.000	Correlation
Chlorophyll a content, ALA and salt treatment interactions	0.000	Interaction
Chlorophyll b content and salt treatment	0.878	No correlation
Chlorophyll b content and ALA treatment	0.010	Correlation
Chlorophyll b content, ALA and salt treatment interactions	0.024	Interaction
Chlorophyll c content and salt treatment	0.000	Correlation
Chlorophyll c content and ALA treatment	0.000	Correlation
Chlorophyll b content, ALA and salt treatment interactions	0.000	Interaction
Root wet weight and salt treatments	0.000	Correlation
Root wet weight and ALA treatment	0.359	No correlation
Root wet weight, ALA and salt treatment interactions	0.455	No interaction
Root dry weight and salt treatments	0.000	Correlation
Root dry weight and ALA treatment	0.208	No correlation
Root dry weight, ALA and salt treatment interactions	0.600	No interaction

The effect of different salt treatment alone and in combination with foliar ALA application on the photosynthetic pigments Chlorophyll a and b is shown in Table 3. All photosynthetic pigments investigated were significantly decreased ($p < 0.05$) with increasing NaCl levels in comparison to the control plants. However, a significant increase ($p < 0.05$) in all photosynthetic pigments studied was recorded in plants treated with ALA in combination with NaCl; whereas no significant difference in the photosynthetic pigments content was recorded in plants treated with ALA alone compared with control plants as shown in Table 2.

Table 3: Effects of salinity and ALA on photosynthetic pigments and ALA concentrations in *Lycopersicon esculentum* L. leaves.

Treatment	Chlorophyll a ($\mu\text{g/L}$)	Chlorophyll b ($\mu\text{g/L}$)	Shoot ALA $\mu\text{g g}^{-1}$ dry weight
Control (H_2O)	14.80 \pm 1.03	5.51 \pm 0.98	28.75 \pm 1.77
50 mmol/L NaCl	9.09 \pm 0.71	3.28 \pm 0.49	33.15 \pm 0.92
25 mmol/L NaCl	9.62 \pm 0.01	3.52 \pm 0.15	33.10 \pm 0.99
	14.33 \pm 0.07	5.73 \pm 0.80	27.50 \pm 2.12



Control (H ₂ O) + ALA			
50 mmol/L NaCl + ALA	16.44±0.37	6.82±1.53	33.70±0.71
25 mmol/L NaCl + ALA	18.81±0.08	6.14±0.01	34.35±1.48

ALA concentrations in plant shoots treated with NaCl alone and in combination with ALA showed no significant difference in comparison to control plants (Tables 2 and 3). Similarly, ALA foliar treatment had no significant effect on the internal ALA concentration in all types of treatment nor on the photosynthetic pigments in the control plants.

The adverse effect of salinity on different types of plants including tomato has been fully elucidated in several studies (Naeem *et al.*, 2012; Zhang *et al.*, 2013; Zhao *et al.*, 2014, 2015). The result of the present study is consistent with the previous studies; it has shown a significant decrease in tomato plants length, number of leaves, shoot and root wet and dry weight when treated with different concentrations of NaCl.

On the other hand, the promotive effect of ALA foliar application on the photosynthetic rate and chlorophyll contents have been elucidated in plants under different types of stress such water stress (Luo *et al.*, 2014), cold stress (Balestrasse *et al.*, 2010) hot arid climate (Al-Qurashi *et al.*, 2011), dry conditions (Al-Thabet, 2006), as well as salt stress (Xu *et al.*, 2011; Zhao *et al.*, 2014, 2015).

Conclusion

The current study has focused on the internal concentration of ALA and chlorophyll content in tomato plants grown under salt stress in comparison to no stress. The result of the current study has revealed a promotive effect of ALA foliar application only in plants grown under salt stress condition. A significant increase in the chlorophyll a and b contents was only seen in tomato plants treated with 25 and 50 mmol/L NaCl as well as ALA. However, the ALA foliar application had no significant effect on the photosynthetic pigments in the control plants. Furthermore, internal ALA contents were not affected by the foliar application of ALA in all plant samples including salt stressed.

The results of the present study support the findings of the previous study of Freije and Alkhuzai (2015) which has shown that the internal ALA concentration is specific in plants living at their natural habitats and is mainly used to maintain plants' specific chlorophyll content and photosynthetic parameters; whereas the external ALA application is directly converted to chlorophyll by plants and thus enhance plants adaptation to environmental stresses including salt stress.

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