Developing a Salt Tolerant Cowpea Using Alpha Tocopherol

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Abstract: A pot experiment was carried out at the greenhouse of the National Research Center, Dokki, Cairo, Egypt during 2005 summer season to study the role of alpha tocopherol (vitamin E) on vegetative growth, dry matter photosynthetic pigments and chlorophyll stability index in cowpea plants. Continuous depression in plant height, fresh weight of stem, leaves and whole plant was observed by increasing the salt concentration up to EC 9.37 dS/m salt level. Similar results were detected in dry matter of stem, leaves and whole plant. Foliar application of tocopherols at the rate of 200 ppm improved growth characters of cowpea plants this improvement was only significant in plant height and stem dry weight. Chlorophyll a concentration slightly increased at high salt concentration. Chlorophyll b increased significantly 3.13 dS/m. Also irrigation by water with 6.25 dS/m salt level raised the concentration of chlorophyll (b) compared with that of the control plants. Concerning the total carotenoids, a continuous increase was detected by the increase in the concentration of salts in water of irrigation and tended to decrease with the higher salts level but still slightly more than the control, however the differences were not enough to reach the level of significance. The total chlorophyll (chlorophyll a + chlorophyll b) showed similar response as that of carotenoids. Chlorophyll a responded significantly to tocopherol application by decreasing although chlorophyll b and total carotenoids concentrations insignificantly increased. The results of total chlorophyll (in the control plants which was sprayed by water and that sprayed by vitamin E) seemed to be similar in their results.

Keywords: Cowpea, Salinity, Tocopherols, Growth, Chlorophyll-Carotenoids, Chlorophyll stability index.

INTRODUCTION

Salinity is a wide spread environmental stress for crop plants it is common in arid, and coastal regions[4]. Production of salt tolerant plants is one of the ways to utilize the waste saline water and lands[4]. There are a range of plants that are capable of growing under conditions of saline soil and water. At the lower levels of salinity (<15 dS m⁻¹) both legumes and grasses with moderate salt tolerance are capable of providing 5-10 t of edible dry matter (DM) year⁻¹, particularly when the availability of water is high. At high salt concentrations (>25 dS m⁻¹), production levels drop and the plant options decrease significantly[15].

Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields and among, the environmental stresses, salinity stress is one of the most adverse factor of plant growth and productivity[19,5,2]. Transgenic expression of Vitamins biosynthetic genes has been investigated for over-production of these dietary supplements in microorganisms and plants. In plants, successful efforts have been reported with vitamins A, C, E and B-9, however information is lacking for other vitamins. Vitamin B-6 is an essential cofactor for numerous enzymatic reactions, and has also been showed to be a potent antioxidant involved in protecting phytopathogenic Cercospora fungi from their own toxin, cercosporin[10]

In plants, Tocopherols are believed to protect chloroplast membranes from photo-oxidation and help to provide an optimal environment for the photosynthetic machinery. Most of proposed tocopherols functions are related to their antioxidant properties, the most prominent of which is protection of polyunsaturated fatty acids from lipids peroxidation by quenching and scavenging various reactive oxygen radicals. (ROS) including singlet oxygen, superoxide radicals and alkyl peroxyradicals. Also, in plants, tocopherol levels vary in different tissues and fluctuate during development and in response to abiotic stresses.
Tocopherols and its effect on growth and metabolism of plants and its role in amelioration of plants against stresses were studied by many authors: Gossett, et al.,[9], Lucas et al.,[13], Jahnke and White[11], and Bosch[6].

Thus, antioxidant defenses have evolved to protect cells from the potential hazardous effects: of reactive oxygen species (ROS). In plants, unfavorable environmental conditions such as salt stress lead to increased production and accumulation of ROS[16, 1].

Therefore, this work was designed to evaluate the influence of tocopherols on growth, photosynthetic pigments and chlorophyll (SI) of cowpea plants grown under saline conditions.

MATERIALS AND METHODS

A pot experiment was carried out in a greenhouse at the National Research Center, Dokki, Cairo, Egypt during the summer season of (2005) to study the role of alpha tocopherols (vitamin E) on vegetative growth, dry matter, photosynthetic pigments and chlorophyll stability index in Cowpea plants under saline conditions.

The experiment included 4 saline water treatments (EC: 3.13, 6.25 and 9.37 dS/m, and tap water as a control) and 2 tocopherol treatments (0 and 200 ppm) and tap water sprayed as a control) with 5 replicates. Ten metallic pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 Kg. of air dried clay loam soil. The inner surface of the pots were coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel (particles about 2-3 cm in diameter), so the movement of water from the base upward.

Seeds of (Vigna sinns L) were sown in the first day of December. Plants were thinned twice, the first after 8 days from sowing and the second 12 days later to leave five uniform plants /pot. Calcium super phosphate (15.5 % P₂O₅) and potassium sulfate (48.5 % k2O) in the rate of 3.0 and 1.50 g/pot were added twice, before sowing and two weeks later. Irrigation with diluted Mediterranean Sea water of different concentrations, were started 20 days after sowing (two irrigations by salt water followed by tap water, alternatively). This alternative approach is needed to control salinity by leaching of soluble salts from the root zone soil by giving an additional amount of fresh water of irrigation as a leaching fraction. Vitamin E treatments were sprayed twice 20 and 30 days after sowing.

Two plants from each treatment were collected, cleaned, and dried in electric oven at 70°C and ground in stainless steel mill.

Chemical Analysis:

**Photosynthetic pigments:** Photosynthetic pigments were determined by the spectro photometric method recommended by Von Wettstein[23].

**Biochemical analysis:** The biochemical characters were estimated at all phenophases of the crop growth, the most critical stage i.e. flowering stage. The chlorophyll a, chlorophyll b and total carotenoids contents were estimated in a fully expanded leaf and expressed as mg/g on fresh weight basis. The chlorophyll a/b ratio was also worked out. The chlorophyll stability index (CSI) was calculated by combining chlorophyll a/b content of cowpea crop before and after being subjected to salinity stress using a formula noted by Kumari, *et al.*

\[ \text{CSI} \% = \left( \frac{\text{chlorophyll before the stress} - \text{chlorophyll under stress}}{\text{chlorophyll before the stress}} \right) \times 100. \]

**Statistical analysis:** Data collected for vegetative and fruiting growth were analyzed according to the methods described by Snedecor and Cochran[21].

RESULTS AND DISCUSSIONS

Data in Table (1) showed that cowpea plant was seriously affected during growth period when using saline water for irrigation. There was a steady decrease in growth characters (plant height, fresh weight of stem and leaves) when increasing salinity up to 9.37 dS/m. Similarly, obtained results by dry matter of stem, leaves and whole plant. The percentage of decrease at 9.37 dS/m reached 75.65, 76.75, 71.69, 74.66, 88.35, 77.91 and 84.66% for plant height, fresh weight of (stem, leaves, whole plant) and dry weight of (stem, leaves and whole plant) compared to unstressed plants.

Although low salinity level of 3.13 dS/m did not show a significant decrease in above mentioned growth parameters Table (1).

Presented data in Table (2) indicated that spraying plants with α-tocopherol at the rate of 200 ppm improved growth characters of cowpea plants, although improvement was only significant for plant height and stem dry weight.

Bosch[6] reported that a plethora of plant reactions exist to circumvent the potentially harmful effects caused by light, drought, salinity extreme temperatures, pathogen infections and other stress. α-Tocopherol is the major vitamin E compound found in leaf chloroplasts, where it is located in the chloroplast envelope, thylakoid membranes and plastoglobuli. This antioxidant deactivates photosynthesis-derived reactive oxygen species (mainly 'O₂ and OH), and prevents the
tocopherol was significantly effective in increasing plant height reaching 130.0 cm, although, at higher salinity levels of water of 6.25 and 9.37 dS/m, antioxidant application showed a positive reduction in plant height reaching 30.5 cm. On the contrary, the fresh weight of cowpea leaves at 3.13 dS/m in addition to antioxidant application showed a significant increase compared to the control. The same trend was observed for the fresh weight of the whole plant. For the whole plant dry weight a slight increase was observed only under high salinity levels (6.25 and 9.37 dS/m) in addition to antioxidant application compared to their controls 3.09 and 1.19 g, respectively. The interaction between salinity and alpha tocopherol treatment was highly significant for plant height and significant for stem dry weight only. Demiral and Turkan[3], indicated that improved tolerance to salt stress in root system of rice tissues may be

Table 1: Growth parameters of cowpea plants as affected by salinity.

<table>
<thead>
<tr>
<th>Salinity dS/m</th>
<th>Plant height</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem Leaves</td>
<td>Whole plant</td>
<td>Stem Leaves</td>
</tr>
<tr>
<td>0.38</td>
<td>115.0</td>
<td>19.53</td>
<td>13.79</td>
</tr>
<tr>
<td>3.13</td>
<td>116.8</td>
<td>19.30</td>
<td>10.47</td>
</tr>
<tr>
<td>6.25</td>
<td>55.0</td>
<td>10.58</td>
<td>5.72</td>
</tr>
<tr>
<td>9.37</td>
<td>28.0</td>
<td>4.54</td>
<td>3.89</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>13.5</td>
<td>6.51</td>
<td>5.03</td>
</tr>
</tbody>
</table>

Table 2: Growth of cowpea as affected by alpha-tocopherol (Vit E).

<table>
<thead>
<tr>
<th>Tocopherols (Vit E) ppm</th>
<th>Plant height</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem Leaves</td>
<td>Whole plant</td>
<td>Stem Leaves</td>
</tr>
<tr>
<td>0</td>
<td>78.0</td>
<td>12.51</td>
<td>8.40</td>
</tr>
<tr>
<td>200</td>
<td>87.38</td>
<td>14.45</td>
<td>8.51</td>
</tr>
<tr>
<td>L.S.D. at 5%</td>
<td>4.05</td>
<td>N.S</td>
<td>N.S</td>
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</tbody>
</table>

Table 3: Growth of cowpea plants as affected by alpha tocopherol and salinity

<table>
<thead>
<tr>
<th>Salinity dS/m</th>
<th>Tocopherols vit E ppm</th>
<th>Plant height (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stem Leaves Whole plant</td>
<td>Stem Leaves Whole plant</td>
<td></td>
</tr>
<tr>
<td>0.38</td>
<td>0</td>
<td>108</td>
<td>19.53</td>
<td>13.21</td>
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<tr>
<td></td>
<td>200</td>
<td>122</td>
<td>19.52</td>
<td>14.26</td>
</tr>
<tr>
<td>3.13</td>
<td>0</td>
<td>103.5</td>
<td>18.8</td>
<td>12.63</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>130</td>
<td>19.79</td>
<td>8.31</td>
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<tr>
<td>6.25</td>
<td>0</td>
<td>93</td>
<td>7.73</td>
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<td></td>
<td>200</td>
<td>13.42</td>
<td>6.98</td>
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<tr>
<td>9.37</td>
<td>0</td>
<td>35.5</td>
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<td></td>
<td>200</td>
<td>30.5</td>
<td>5.08</td>
<td>4.49</td>
</tr>
<tr>
<td>L.S.D at 5%</td>
<td>8.11</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
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</tbody>
</table>

The propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. α-Tocopherol levels change differentially in response to environmental constraints, depending on the magnitude of the stress and species-sensitivity to stress. Lucas et al.[13] noticed that alpha-tocopherol concentrations were higher in Acala cotton cultivars than the salt sensitive cultivars at both 0 or 150 mM NaCl level.

The effect of salinity levels and/or tocopherols application on some growth parameters of cowpea plants was presented in (Table 3). It was obvious that under normal conditions using tap water in addition to antioxidant application increased plant height reaching 122.0 cm compared to untreated plants. At salinity level of 3.13 dS/m, application of α-tocopherol was significantly effective in increasing
attributed to increased capacity of antioxidant system. Amor, et al.,[2] concluded that the limitation of plant growth at 200 mM NaCl was concomitant with lesser efficiency of protective enzymes such as superoxide dismutase, catalase and peroxidase but malondialdehyde levels in both roots and shoots remained close to the control.

Chlorophyll content can be considered as one of the indices to screen plant growth. In Table (4) the effect of α-tocopherol on photosynthetic pigments was introduced to prove that Chlorophyll a responded negatively but chlorophyll b and total carotenoids concentrations increased although their increases were not significant. The two values of total chlorophyll (in the control plants which was sprayed by water and that sprayed by vitamin E seemed to be equal. Furthermore, chlorophyll a/b ratio quality was depressed, however, total chlorophyll : total carotenoids ratio drastically was depressed with the vitamin E treatment. Yamani, et al.,[23] in his experiment, incubated leaf segments in 200 mM NaCl for 24 h in light after pre-incubation with antioxidants for 12 h in light. Pretreatment with ascorbate and benzoate, which scavenged \( \text{H}_2\text{O}_2 \) and \( \text{OH}^- \), respectively, effectively suppressed the reduction of chlorophyll content and the destruction of chloroplasts by NaCl in light.

In Table (5) under normal conditions (using tap water) it was clear that Chlorophyll a has twice the concentration of chlorophyll b and five times that of carotenoids. Vitamin E application increased the contents of chlorophyll b and carotenoids. Under saline conditions of (3.13 dS/m) photosynthetic pigments showed a slight increase in chlorophyll a, chlorophyll b and carotenoids while vitamin E application increased chlorophyll b content only. Non significant variations was observed among chlorophyll a, carotenoids, total chlorophyll, chlorophyll a: chlorophyll b and total chlorophyll / carotenoids, only chlorophyll b showed a significant increase when increasing salinity up to 9.37 dS/m compared to the control. Antioxidant application increased chlorophyll b content under different salinity levels. On the other hand, application of vitamin E and interaction between salinity and vitamin E was significant for chlorophyll a only.

For carotenoids contents, increasing salinity increased carotenoids content under different salinity levels compared to the control, the highest content was attained under 6.25 dS/m salinity level in addition to vitamin E application (Table 5).

Sairam, et al.,[20] indicated that NaCl decreased relative water content, chlorophyll content, membrane stability index and ascorbic acid. Madan, et al.,[14] reported that salinity stress marginally decreased the rate of photosynthesis and chlorophyll content in the salt tolerant salinity however in the sensitive one showed greater reduction. Yamane, et al.,[24] suggested that salt induced injury in chloroplasts is dependent on light, and that \( \text{H}_2\text{O}_2 \) and \( \text{OH}^- \) are responsible for the deleterious effects of salt stress in chlorophyll content and chloroplast ultra structure.
A decrease in Chlorophyll a/b ratio due to salinity was observed in Table (5). Also, Singh and Jam reported the same trend in chickpea. The Vitamin E application showed lower chlorophyll a/chlorophyll b ratio than the control. This disruption of the fine structure of the chloroplast, instability of the pigment protein complex and enhanced chlorophylase activity are attributed to decrease in chlorophyll content under saline conditions. The stability of chlorophyll b content for salt stress plants can be ranged as an index of tolerance, which might produce higher photosynthetic rate and eventually show higher yield. Moreover, the total chlorophyll carotenoids ratio was calculated (Table 5) and it was obvious that the ratio decreased with increasing salinity level up to 9.37 dSm⁻¹ but antioxidant (Vitamin E) application increased that ratio compared to that of the control.

Concerning the chlorophyll stability index (CSI), it was an important index for screening of plant cultivar for abiotic stresses. In the current study the chlorophyll stability index (Figure 1) increased at 9.37 dSm⁻¹ (high salinity level) when treated with vitamin E reached 0.4 and at 6.25 dSm⁻¹ the CSI reached 0.28, although application of Vitamin E increased CSI to reach 0.49 % indicating the plant tolerance for salt stress, results were supported by Raga Babo, et al.,

**Conclusion:** Tocopherols are a group of compounds that can play different roles in plant metabolism and can play important roles in amelioration of biotic and abiotic stresses, but in order to understanding their functions a necessary need an intensive research on the optimal rate, method and time of application and its wide different responses. Apse and Blumwald mentioned that recent progress has been made in the identification and characterization of the mechanisms that allow plants to tolerate high salt concentrations. The understanding of metabolic fluxes and the main constraints for the production of compatible solutes (i.e. feedback inhibition and the limitation of substrate supply) open up the possibility of genetically engineering entire pathways that could lead to the production of osmoprotectants. This, together with the identification of the different sodium transporters (in particular vacuolar and plasma membrane Na/H antiporters) that could provide the needed ion homeostasis during salt stress opens the possibility of breeding crop plants with improved salt tolerance.

**REFERENCES**

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![Fig. 1: Effect of salinity and alpha tocopherol (Vit. E) application on chlorophyll stability index (CSI) %](image_url)