Evaluating the Use of Rhizobacterin on Cowpea Plants Grown under Salt Stress

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Abstract: The effect of biofertilizer rhizobacterin on growth, yield and metabolites of cowpea Vigna sinensis grown at 0, 25, 50 and 75 mM NaCl was investigated. Growth and yield were progressively declined by increasing NaCl concentrations. Treatment with rhizobacterin mitigated the harmful effect of NaCl and the greatest growth and yield were obtained from control plants fertilized by rhizobacterin. Rhizobacterin improved salt tolerance in cowpea by enhancing the accumulation of nontoxic metabolites such as total soluble sugars, proline and glycine betaine as well as N, P and K as protective adaptation.

Keywords: Cowpea; Salt Stress; Rhizobacterin

INTRODUCTION

Salinity is one of the most important abiotic factors limiting plant growth and productivity. This is especially acute in arid and semi-arid regions where cowpea Vigna unguiculata is a widely cultivated species\(^\text{[33]}\). Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. Parida and Das\(^\text{[26]}\) stated that the ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites, specific proteins and certain free radical scavenging enzymes that control ion and water flux and support scavenging of oxygen radicals. Several physiological and biochemical processes are affected by salinity, particularly nitrate assimilation, which largely influences plant growth\(^\text{[14]}\). Wilson et al.\(^\text{[14, 35]}\) indicated that salt stress produced a strong non-linear reduction effect on cowpea biomass accumulation and that various cowpea cultivars may be differentially affected by increasing salinity. They concluded that salt tolerance is mainly concerned with leaf area and dry weight. Additionally, they found that increasing stomatal closure with increasing salinity might limit net photosynthetic rate per unit leaf mass \((P_{\text{n}})\) or net photosynthetic rate per unit leaf area \((P_{\text{a}})\). Cavalcanti et al.\(^\text{[6]}\) stated that the salt treatment \((200\text{mM NaCl})\) caused an almost complete cessation in the relative growth rate of both leaves and roots.

Raptan et al.\(^\text{[23]}\) and Yupsanis et al.\(^\text{[17]}\) found that salinity decreased fresh and dry weights of mungbean shoot. Rabie\(^\text{[27]}\) found that salinity decreased the growth of mungbean. Ghoulam et al.\(^\text{[15]}\) stated that salinity affected all parameters, high NaCl concentrations caused a great reduction in growth parameters such as fresh and dry weight of leaves and a decrease in the K\(^+\) concentrations but proline content was increased.

Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic ionic stress caused by high salinity. These mechanisms include osmotic adjustment by accumulation of compatible solutes such as proline, glycine betaine\(^\text{[7]}\) and lowering the toxic concentration of ions in the cytoplasm by restriction of Na\(^+\) influx or its sequestration into the vacuole\(^\text{[6]}\). Mahajan and Tuteja\(^\text{[21]}\) stated that high salt depositions in the soil generate a low water potential zone in the soil making it increasing difficult for the plant to acquire both water as well as nutrients. Therefore, salt stress essentially results in a water deficit condition in the plant and takes the form of a physiological drought. Also salt stress caused disruption of ionic equilibrium, influx of Na\(^+\), dissipates the membrane potential and facilitates the uptake of Cl\(^-\) down the chemical gradient. Na\(^+\) is toxic to cell metabolism and has deleterious effect on the functioning of some of the enzymes\(^\text{[22]}\). High concentration of Na\(^+\) causes osmotic imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion. High Na\(^+\) levels also lead to reduction in photosynthesis and production of reactive oxygen species\(^\text{[10]}\). Salinity can induce oxidative damage to the plant\(^\text{[10]}\) and it affected in plant metabolism by the enhanced production of reactive oxygen species (ROS), such as superoxide \((O_{2}^{\cdot-})\), hydrogen peroxide \((H_{2}O_{2})\), singlet oxygen and hydroxyl radical\(^\text{[11]}\). The accumulation of harmful ROS depends on an imbalance between the rates of production and elimination through several biochemical reactions\(^\text{[8]}\). These ROS are extremely cytotoxic. As a
consequence, a series of cellular degenerative processes are triggered, including peroxidation of membrane lipids and programmed cell death\(^{[12]}\). In order to avoid the damage caused by ROS compounds, plants have evolved molecular defence systems that both limit the formation of ROS and promote its removal\(^{[4]}\).

The present study aims to investigate the effect of addition rhizobacterin, which may improve the salt tolerance of cowpea *Vigna sinensis* plants grown under different levels of NaCl.

**MATERIALS AND METHODS**

Cowpea *Vigna sinensis* and commercial product rhizobacterin were obtained from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Cowpea seeds, which were previously sterilized, were planted in pots of 35 cm diameter and 40cm depth; each pot contained 7 kg soil. The soil characteristics were as follows: sandy loam in texture, sand 84.2%; silt 12.9%; clay 2.9%; pH 7.8; EC 0.5dSm\(^{-1}\) and organic matter 1.2%. Eight seeds per pot and five replicates were used for each treatment. Irrigation was applied every two days basis to achieve soil water field capacity level. Cowpea seeds were subjected to four different salinity levels 0, 25, 50 and 75 mM NaCl. Once every 15 days pots were rinsed with water to avoid accumulation salt. Five plants after seeding thinning were let to grow in each pot. Treatments were as follows:

Control and Salt Treatment:
- a- control (0 NaCl)
- b- 25 mM NaCl
- c- 50 mM NaCl
- d- 75 mM NaCl

**Salt Treatment and Rhizobacterin Biofertilizer:** A second set was subjected to 0, 25, 50 and 75 mM NaCl and 10g/pot rhizobacterin biofertilizer.

Growth measurements, fresh and dry weights, were determined at 30, 60 and 90 days old. For standardizing data, the results could be expressed as the relative reduction of fresh, dry weights and yield in comparison to the control using the following formula:

\[
\text{Relative reduction(%) = } \left[1 - \frac{\text{salinized}}{\text{control}}\right] \times 100
\]

Yield components (numbers of pods plant\(^{-1}\), yield plant\(^{-1}\) and weight of 100seeds) were determined at the end of the experiment (150 of sowing date).

**Shoot analysis:** Photosynthetic pigments were determined according to Metzner et al.\(^{[22]}\). Total soluble carbohydrates were carried out according to Dubios et al.\(^{[3]}\). Proline content was determined as described by Bates et al.\(^{[8]}\). Glycine betaine content was estimated as described by Grieve and Grattan\(^{[14]}\). N, P and K were determined according to A.O.A.C.\(^{[11]}\). The crude protein content was calculated by multiplying the total nitrogen by 6.25. Protein electrophoresis, SDS polyacrylamide gel electrophoresis was performed in 10% acrylamide slab gels according to laemmli\(^{[19]}\). For gel analysis, gels were photographed, scanned and analyzed using Gel Doc 2000 Bis Rad system.

The data were statistically analyzed using the one-way analysis of variance as described by Snedecor and Cochran\(^{[22]}\). The means were compared by LSD using SPSS version 10.

**RESULTS AND DISCUSSION**

**Growth Parameters:** Shoot Fresh and dry weights at all stages of development were reduced progressively with increasing NaCl concentrations while reversibly, use of rhizobacterin had stimulated plant growth at three stages of development studied (Fig.1). Control plants treated with rhizobacterin recorded 143.8% and 144.2% fresh and dry weights, respectively, compared to the control at 90 days old. Biofertilized plants treated with 75mM NaCl recorded 124.4% and 147.4% fresh and dry weights, respectively, compared to the nonfertilized plants at 90 days old. Treatment with 75mM NaCl caused reduction in growth while slight decreases in fresh and dry weights were obtained with 25 mM NaCl treated plants. Reduction percentage in fresh and dry weights of salinized and biofertilized plants are represented in Fig. 3. The results obtained indicate that rhizobacterin has ameliorated the deleterious effects of salinity. Silveira et al.\(^{[21]}\) found that addition of high level of NaCl (100 mol m\(^{-3}\)) induced a decrease in nitrate uptake and assimilation parallel to a reduction in the shoot growth of cowpea plants while 50 mol m\(^{-3}\) NaCl resulted in a slight reduction in the shoot dry mass compared to the control. Ghoulam et al.\(^{[13]}\) stated that salinity affected all of the considered parameters, high NaCl concentrations (100 and 200 mM NaCl) caused a great reduction in growth parameters, fresh and dry weights of sugar beet leaves. Wilson et al.\(^{[15]}\) stated that salinity treatment results in a progressive decline in growth among the four cowpea cultivars. Jebara et al.\(^{[14]}\) mentioned that inoculation with salt tolerant rhizobia could lead to a best growth of the host chickpea plant under salinity conditions.

**Yield and Yield Components:** Number of pods/plant, yield/plant and weight of 100seeds attained the highest values for control plants and were declined progressively by increasing NaCl concentration (Fig. 2). Rhizobacterin fertilized plants gave significantly higher yield and yield components than non-fertilized plants. As shown from Fig. 3 the least reduction percentage in
cowpea yield (7%) was attained at 25mM NaCl applying rhizobacterin. These results agree with Ahmad et al.\textsuperscript{[3]} who stated that pod fresh weight, seed yield and weight of 100 seeds of Vigna radiata L. showed a reduction as the salinity levels increased. Mhadhbi et al.\textsuperscript{[23]} concluded that rhizobial strain seemed to allow a best tolerance to chickpea under salt stress. Yield potential of chickpea depends on the
Fig. 3: Reduction percentage in fresh and dry weights and yield of cowpea plants grown under different concentrations of NaCl.

Fig. 4: Metabolic products of cowpea plants grown under different concentrations of NaCl.

Photosynthetic Pigments: Salinity stress (25, 50 and 75mM NaCl) resulted in significantly progressive decline the photosynthetic pigments (chlorophyll a, b, a+b and carotenoids) at 30, 60 and 90 days old (Table 1). Biofertilized plants gave the highest amount of photosynthetic pigments than non-fertilized plants. Rhizobacterin mitigated NaCl induced effect on pigments reduction. The results are parallel to those of Ahmad et al.\textsuperscript{[1]} and Mohammed\textsuperscript{[24]} who found that salinity reduced the total chlorophyll content of mungbean leaves, and may result from stomatal closure due to osmotic stress or salt induced damage of photosynthetic apparatus.

Total Soluble Carbohydrates: Total soluble carbohydrates increased in salinized plants compared with control (Fig.4). Biofertilized plants showed higher total soluble carbohydrate content than the nonfertilized
Table 1: Photosynthetic pigments of cowpea plants grown under different concentrations of NaCl

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Chlorophyll a+b</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM NaCl</td>
<td>32.9</td>
<td>48.9</td>
<td>60.6</td>
<td>30.0</td>
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<tr>
<td></td>
<td>+ Rhizobacterin</td>
<td>39.6</td>
<td>57.5</td>
<td>68.5</td>
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<tr>
<td>25 mM NaCl</td>
<td>32.1</td>
<td>43.5</td>
<td>45.9</td>
<td>27.4</td>
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<td></td>
<td>+ Rhizobacterin</td>
<td>36.2</td>
<td>47.7</td>
<td>57.9</td>
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<td>50 mM NaCl</td>
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<td>45.5</td>
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<td></td>
<td>+ Rhizobacterin</td>
<td>35.0</td>
<td>46.1</td>
<td>48.1</td>
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<td>75 mM NaCl</td>
<td>30.4</td>
<td>38.6</td>
<td>43.9</td>
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<tr>
<td></td>
<td>+ Rhizobacterin</td>
<td>36.2</td>
<td>42.9</td>
<td>46.7</td>
</tr>
</tbody>
</table>

LSD at 5% → 1.6 2.3 3.1 1.2 1.8 2.6 3.4 3.8 4.2 1.5 1.3 1.0

Figure 5: NPK of cowpea plants grown under different concentrations of NaCl.

Plants. Mahajan and Tuteja[21] reviewed that high salinity causes both hyperionic and hyperosmotic stress, can lead to demise and a major consequence of NaCl stress is the loss of intra-cellular water. To prevent this water loss from the cell and protect the cellular proteins plants accumulate many metabolites such as sugars, mainly fructose and sucrose, these solutes do not inhibit the normal metabolic reactions. Hajar et al.[17] also showed that carbohydrate accumulation in Nigella Sativa increased the ability for water absorption under salinity stress. **Proline and Glycine Betaine (GB):** Proline and GB contents of cowpea leaves increased with increasing NaCl levels and increased further more in biofertilized plants (Fig. 4). These results agree with those obtained by Silveira et al.[31] and Girija et al.[13] who stated that the salt treatment caused an increase in the concentration of proline and GB in cowpea and peanut plants. Proline and GB accumulation is a general response to salinity stress. Silveira et al.[30] showed that in case of cowpea, proline is accumulated largely in leaves only under a drastic salt stress. Girija et al.[13]
Table 2: Presence (1) absence (0) of protein banding pattern of cowpea plants grown under different concentrations of NaCl

<table>
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<tr>
<th>M.W.</th>
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<th>25</th>
<th>50</th>
<th>75</th>
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<td>1</td>
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<tr>
<td>Total</td>
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<td>14</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>13</td>
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</tbody>
</table>

Fig. 6: SDS-PAGE protein banding of cowpea plants grown under different concentrations of NaCl.

and Mahajan and Tuteja[21] proposed that proline and GB accumulation may contribute to osmotic adjustment at the cellular level, may acts as an enzyme protectant and stabilizing the structure of macro-molecules. Proline also acts as a major reservoir of energy and nitrogen for utilization upon exposure to salinity.

Crude Protein Content: Crude protein content was higher in rhizobacterin salinized plants than nonfertilized plants (Fig. 4)

Mineral Content: Leaf N and P contents rose steadily with rise of NaCl concentration (Fig.5). It is noticed that rhizobacterin application resulted in a further rise of leaf N and P over the non-biofertilized plants. Increasing NaCl concentration has decreased leaf K content. The present findings agree with that obtained by Abu-Ghalia and El-Khallal[20]. While Na⁺ is deleterious for plant growth, K⁺ is a macronutrient required in quite large quantities for maintaining the osmotic balance, as activator for many enzymes as well as for its role in regulating opening and closing of stomata.

SDS-PAGE Protein: Fig. 6 demonstrates the SDS protein profiles of all treatments while Table 2 reveals their computer analysis and represents the occurrence of bands as (1) and absence as (0). A maximum number of 14 bands were detected at approximately molecular weights ranging between 15.13KDa to 175.75 KDa. The minimum number of bands was 12 and recorded in (lane 4) of plants under severe salinity stress by absence of bands No. 1 and 5 of 175.75 and 99.77 KDa. Plants treated with rhizobacterin revealed absence of band No 1 of 175.75 KDa under all salinity treatments. Sayed[29] reported that the results of water non-soluble protein fractions of alfalfa under different stresses did not show clear-cut markers tolerance of stresses.

REFERENCES