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Effect of Cyanobacteria Isolates on Rice Seeds Germination in Saline Soil

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Abstract:

Cyanobacteria are prokaryotic photosynthetic communities which are used in biofertilization of many plants especially rice plant. Cyanobacteria play a vital role to increase the plant's ability for salinity tolerance. Salinity is a worldwide problem which affects the growth and productivity of crops. In this work three cyanobacteria strains (*Nostoc calcicola*, *Anabaena variabilis*, and *Nostoc linkia*) were isolated from saline soil at Kafr El-Sheikh Governorate; North Egypt. The propagated cyanobacteria strains were used to withstand salinity of the soil and increase rice plant growth (Giza 178). The length of roots and shoot seedlings was measured for seven and forty days of cultivation, respectively. The results of this investigation showed that the inoculation with *Nostoc calcicola*, *Anabaena variabilis*, and *Nostoc linkia* increased root length by 27.0, 4.0, 3.0 % and 39, 20, 19 % in EC5 and 10 (ds/m), respectively. Similarly, they increased shoot length by 121, 70, 55 %, 116, 88, 82 % in EC5 and 10 (ds/m), respectively. In EC15and more concentrations, control rice plants could not grow while those to which cyanobacteria were inoculated could withstand only EC15 but not other elevated concentrations. These results encourage using *Nostoc calcicola,Anabaena variabilis*, and *Nostoc linkia* as biofertilizer for rice plant in the saline soil for increasing growth and decrease soil electrical conductivity.

Keywords: Cyanobacteria, Rice, Germination, Salinity, Growth

Introduction:

Salinity has a negative effect on agricultural productivity, including plant growth and minimizing the use of this land. Salinity affects about 6% of the world's total land and 20% of the world's irrigated areas (1). It is estimated that the total agricultural land in Egypt is about 8.4 million feddans (3.5 million ha) which represents about 3.5 percent of the total area. FAO (2) stated that about one million ha in the irrigated areas suffer from salinization problems, water logging, and sodicity. Most of the salt-affected soils in Egypt are located in the North central part of the Nile Delta and on its Eastern and Western sides. About 900,000 ha suffer from salinization problems in cultivated, irrigated areas, 6 % of the Northern Delta region is salt-affected, 20 % of the Southern Delta and Middle Egyptian region and 25 % of the Upper Egypt region (3).

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The cyanobacteria showed high rates of nitrogen fixation at 10–20% NaCl (4). Cyanobacteria play an important role in the biofertilization of rice plants through excretions of growth hormones (5, 6). The present work aimed to increase the efficiency of rice plants in saline soil and decrease the electric conductivity of soil by inoculation with nitrogen-fixing cyanobacteria as biofertilizers to avoid chemical hazardous (fertilizer) input to the soil.

Materials and Methods:

Isolation and Identification of cyanobacterial strains

Cyanobacterial strains were isolated from saline soil in Seidy-Salem, El Hamoul, El-Ryad and Baltim at Kafr El-Sheikh Governorate. Isolation and culturing techniques were carried out in BG-11 culture medium (7). Three strains, *Nostoc calcicola*, *Anabaena variabilis* and *Nostoc linkia* were identified according to Venkataraman (8) and used for inoculation process.

Effect of cyanobacterial filtrate on rice seedlings

Rice seeds (Giza 178), were obtained from Rice Breeding Section, Agricultural Research

Center, Kafr El-Sheikh, Egypt. Seeds were used to study the effect of the filterate of *Nostoc calcicola*, *Anabaena variabilis* and *Nostoc linkia* on the growth of the rice seedlings.

Rice seeds were carefully washed with distilled water followed by sterilization with ethanol for 3- 4 sec, sodium hybochloride (4%) for 3 min then rewashed with distilled water. Petri-dishes (9 cm in diameter) were used in three replicates, which were supplied with a filter paper of the same diameter then oven-sterilized at 160 ^{o}C for 23 h. The filter papers were saturated with different NaCl concentrations (0, 5, 10, 15, 20 and 25 dS/m). Ten rice seeds were arranged in each petri-dish to which 5 ml filtrate of cyanobacterial isolates were inoculated and then incubated at 30 ^{o}C for 3 days in the dark.

After germination, petri-dishes were transferred to light for 7 days and the length of seedling roots was measured.

Pots experiment

This experiment was used to study the effect of cyanobacterial inoculum on the growth of rice plant and salinity concentration and to evaluate the most salt tolerant cyanobacterial isolate. Rice seeds were soaked in water for 24 hours, and then were placed in the dark, at 30^{o} C for 48 hours. Plastic pots (of 7 cm internal diameter and 9 cm height), were filled each with 200 g of soil with artificial salinization. The pots divided into salinization levels (5, 10, 15, 20 and 25 dS/m) to test the salinity tolerance of cyanobacterial isolates (3 replicates for each were done).

Artificial salinization of soil with neutral salt

Before rice transplantation, representative air-dried soil samples were salinized to the final concentration as follows (5, 10, 15, 20, and 25 (dS/m). Appropriate amounts of the required neutral dominant salts of NaCl and $CaCl_2$ (studied in the utilized soil) were well mixed and homogenized to modify the original (Electrical Conductivity) EC value to the desired (EC). Artificial salinization of soil was prepared at a constant sodium adsorption ratio (SAR) using manual of salinity research methods (9).

Seedling growth in the pots

Three seedlings were transferred to pots after preparation stage. One week after transferring the seedlings, 10 ml of cyanobacterial inoculum were added to the soil. After four weeks, plant height, root length, EC of soil were measured**.**

Statistical Analysis

The obtained, collected data were subjected to the statistical analysis, using the analysis of variance (ANOVA). The LSD range tests were used to compare between the means (10).

Results and Discussion:

Effect of the cyanobacterial filtrate on root length of rice seedlings

The experiment was conducted to investigate the effect of the cultural filtrates of *N. calcicola*, *A. variabilis*, and *N. linkia* grown in BG_{11} medium containing different concentrations of NaCl (5, 10, 15, 20 and 25 dS/m) in the laboratory for seven days on rice seed germination. Results illustrated in Fig. 1 showed that there is a highly significant decrease in root length with increasing salinity levels. The highest mean value is 3.667 cm recorded at EC 5 dS/m. On the other hand, the lowest mean value was 1.875 cm recorded at EC 25 dS/m. Application of filtrates of *N. calcicola*, *A. variabilis*, and *N. linkia* to rice seedlings stimulated their growth. *N. calcicola* showed always high stimulation of rice seedling growth than *A. variabilis*, and *N. linkia* filtrates in all salinity concentrations used.

Similar results were obtained by Rodríguez et al. (11) who found that NaCl (5g/L) reduced shoot length by 54%, root length of *Oryza sativa* by 62%, shoot and root dry weight by 37% and 59%, respectively. They also concluded that the extracellular products of the Cyanobacterium *Scytonema hofmanni* reverted partially or completely many of the NaCl-induced effects on growth and even the biochemical alterations of rice seedlings. Our results are in agreement with those obtained by Monu et al. (12) who concluded that exopolysaccharides (EPS) produced by cyanobacteria alleviated the salt effect on germination of three crops wheat, maize, and rice.

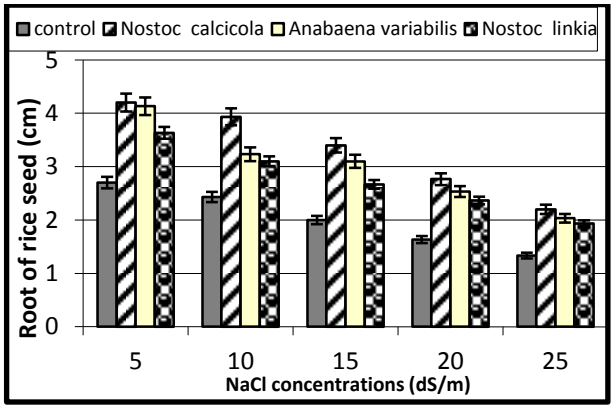

Figure 1. Effect of cyanobacterial filtrates on the root length (cm) of germinated rice seeds (Giza 178) under different levels of salinity in laboratory conditions after 7 days of planting.

Effect of cyanobacterial filtrate on shoot length of rice seedlings

Influence of salt-tolerant cyanobacteria strains on rice plants under different salinity levels is illustrated in Fig. 2. Salinity caused a highly significant decrease in shoot length. However, inoculation treatments with Cyanobacterial filtrate gave a highly significant increase in shoot length.

The shoot length of rice seeds was reduced with rising salinity concentrations (5, 10, 15, 20, 25 dS/m), however, inoculation with cyanobacterial filterate showed a highly significant increase in root length rice seeds compared with control. The highest mean value of shoot length was 3.441 cm which recorded at EC 5 dS/m, while, the lowest mean value of shoot length was 2.075 cm which recorded at EC 25 dS/m.

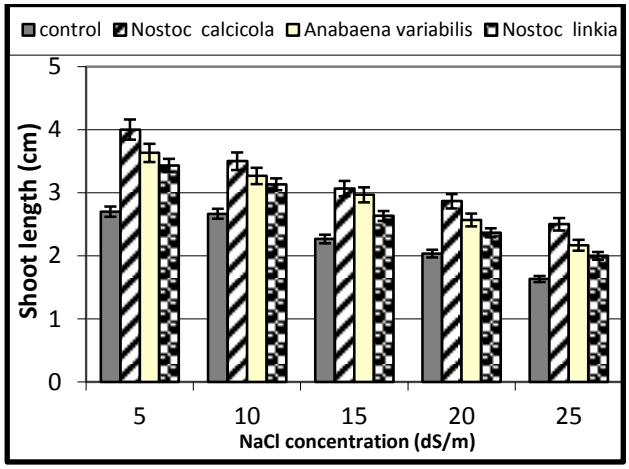

Figure 2. Impact of cyanobacteria isolates on the shoot length of rice germinated seeds treated with different concentrations of NaCl under laboratory conditions after 7 days of planting

Pots experiment

This experiment aimed to study the role of inoculation with tested cyanobacteria strains *N. calcicola*, *A. variabilis*, and *N. linkia* on the root, shoot length (cm) and E.C of soil at different levels of salinity in the seedling stage after 40 days of rice seedling cultivation.

Data illustrated in Fig. 3 indicated that the growth of rice plants was inhibited completely at 15 and 20 dS/m. Root length (cm) of rice plant showed a highly significant reduction in with elevating or increasing salinity concentrations (5, 10, 15, 20 and 25 dS/m). On the contrary, inoculation showed a highly significant increase in root length of rice plant compared with un-inoculated control length (3.933, 1.200, 0.000, 0.000 and 0.000 cm, respectively). In addition, inoculation with *Nostoc calcicola* gave significant root length of rice plant (6.600, 5.133, 3.133, 0.000 and 0.000 cm), followed in descending order by inoculation with *Anabaena variabilis* (4.333, 3.267, 3.200, 0.000 and 0.000 cm) and by inoculation with *Nostoc linkia* (4.200, 3.133, 2.800, 0.000 and 0.000 cm).

Similarly, the increase in salinity concentration induced marked reduction in shoot length comparable to those in root length as illustrated in Fig.2. Complete reduction of shoot length (as in roots) occurred at elevated NaCl conc. (15, 20, 25 dS/m).

The gradual increase in shoot length by the inoculated cyanobacteria was inversally proportional to salinity concentrations.

Results in this work are in agreement with Saadatnia and Riahi (13) who observed that the results of pot experiment showed an increase of 53% in plant height; 66% in root length compared with untreated plants with cyanobacteria.

From the above-mentioned results, it could be concluded that root and shoot length of rice plant were decreased with increasing salinity levels. These results might be attributed to the inhibition in the number of metabolites synthesized by plant grown under saline conditions. In this context Strogonov (14) revealed that the inhibition of growth in saline soil must be explained not on the basis of hunger, of plants, but also by the inhibitory effect of salt on growth due to inhibition of carbohydrates and nitrogenous substances are not fully utilized in the formation of new cells and tissues

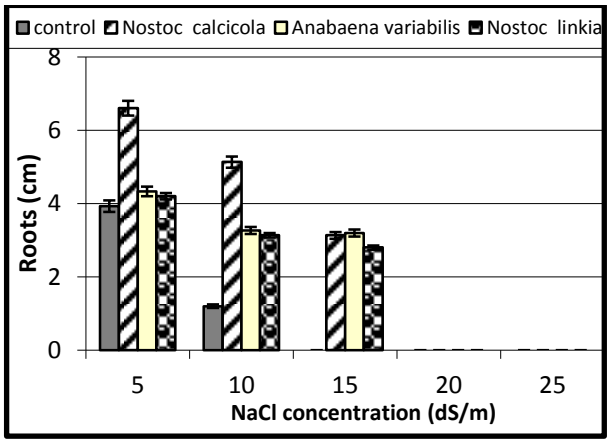

Figure 3. Effect of cyanobacterial isolates on the root length (cm) of rice seedlings (Giza 178) under different levels of salinity in laboratory conditions after 40 days of cultivation.

It was obvious from the obtained results recorded in Fig.3 and 4, that the root and shoot length (cm) were significantly positively affected by inoculation with all tested cyanobacteria strains. The increase in these parameters due to cyanobacteria inoculation could be attributed to the role of free oxygen as well as fixing N which improve the vegetative growth of rice plant. These results are in harmony with those obtained by many

investigators (15-17), who concluded that rice seedlings treated with *Nostoc* extracts cause an increase in root, epicotyl and hypocotyl growth, number of roots and plant fresh and dry weights.

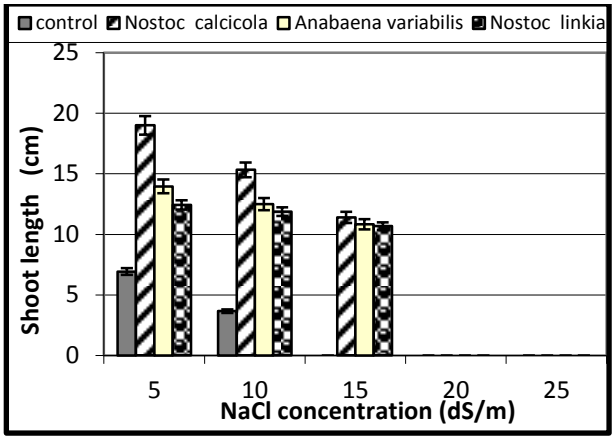

Figure 4. Effect of cyanobacterial isolates on shoots length (cm) of rice plant under different levels of salinity after 40 days of cultivation.

Effect of cyanobacterial isolates on electrical conductivity (EC) of soils after 40 days of rice seedlings

Results presented in and Fig. 5 revealed that there were a highly significant decrease in EC with increasing salinity concentrations (5, 10, 15, 20 and 25 $dS.m^{-1}$), compared with un-inoculated soil in control (4.857, 9.843, 14.820, 19.837 and 24.903 cm) while in case of inoculation with *Nostoc linkia* the EC were 4.467, 8.833, 12.867, 17.663 and 23.760 cm., followed in descending order by inoculation with *Anabaena variabilis* (4.350, 8.590, 12.830, 17.280 and 23.407 cm) followed by inoculation with *Nostoc calcicola* (4.167, 8.193, 12.830, 17.280 and 23.407 cm). Results showed that *Nostoc calcicola* was the most cyanobacterial species which decrease EC after the inoculation.

These results coincide with those of other authors who worked on the effect cyanobacterial isolates on EC. Wilson (18) stated that soil salinity decrease by the application of the algae. Hashem (19) showed that cyanobacteria can also have a positive influence on the natural environment by reducing soil salinity.

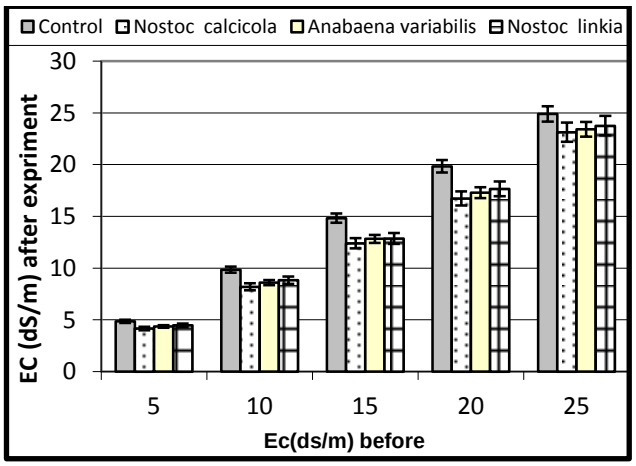

Figure 5. Effect of cyanobacterial isolates on EC of soils after 40 days of rice seedlings cultivation.

Caiola et al. (20) and Sharma et al., (21) reported that exopolysaccharides (EPS) have a significant role in providing protection to the cell as a boundary layer, contributing to soil aggregation due to its gluing properties and binding heavy metals due to the presence of several active functional groups onto it. Looking into the characteristics of EPS it was thought worthwhile to examine its possible application for binding sodium ions from the saline medium, thereby alleviating salt stress for germinating seeds (22-24). Cyanobacterial EPS has a rich array of ligands that can chelate metal ions (25). Singh and Dhar (26) reported a recommendation of possible usage of cyanobacteria for desalination and the reclamation of saline soils in India. Aziz and Hashem (27) stated that fertility of saline soils might be restored by cyanobacterial inoculum, which increased the organic matter content of the post-harvest soils from 1.82 to 2.02%. Recently, Chittapun (28) concluded that cyanobacteria promoted rice seedling growth and yield. Sheng et al. (29) mentioned that the total EPS content increased with NaCl concentration, suggesting a protective response by the bacterium and therefore, the high level of NaCl enhanced the flocculation in photosynthetic bacteria.

Conclusion:

Cyanobacterial strains (*Nostoc calcicla*, *Anabaena variabilis*, and *Nostoc linkia*) were isolated from different saline regions at Kafr El-Sheikh Governorate. Rice plants were inoculated by the three isolates. Shoot and root length (cm) showed a highly significant decrease with rising salinity concentrations while, inoculation with isolates showed a highly significant increase in shoot and root length compared with un-inoculated soils.

A significant positive effect of inoculation with the tested cyanobacterial strains on Electrical

Conductivity (EC) was detected. Inoculation with cyanobacterial isolates *(Nostoc calcicola*, *Anabaena variabilis*, and *Nostoc linkia*), cause decreased EC of soils compared with (un-inoculated control). These results recommended using these isolates as biofertilizer in the saline soil to increase the efficiency of rice plant and decrease the electric conductivity of the soil.

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تأثير العزلات السيانوبكترية على نمو بذور الأرز فى التربة الملحية

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الخلاصة:

السيانوبكتريا هى كائنات أولية النواة تقوم بالبناء الضوئى وتستخدم فى التسميد الحيوى لعدة نباتات منها محصول الأرز. كما ان السيانوبكتريا تلعب دورا هاما فى تحمل الملوحة. الملوحة هى مشكلة عالمية تؤثر على نمو وانتاجية النباتات. فى هذا البحث تم عزل 3 سلالات من السيانوبكتريا هى أنابينا كالسيكولا- أنابينا فاريابلس و نوستوك لنكيا من اراضى ملحية من محافظة كفر الشيخ شمال مصر. وقد استخدمت السلالات المعزولة فى جعل التربة تتحمل الملوحة وزيادة انتاجية الأرز صنف جيزة 178. تم قياس طول الجزر والمجموع الخضرى لبادرات الأرز فى الفترة من 7 الى 40 يوم. وقد أظهرت النتائج أن حقن التربة المزروعة بنبات الأرز بالنوستوك لنكيا، أنابينا فاريابلس و أنابينا كالسيكولا قد زاد من طول الجذر بنسبة 4,27 و 3% على التوالى. وبالمثل تم زيادة طول المجموع الخضرى بنسبة 121، 70و55% على التوالى. فى تركيز الملوحة EC15 واعلى من هذا لم تستطيع النباتات الغير معاملة بالسيانوبكتريا النمو بينما النباتات المعاملة بالسيانوبكتريا قد تحملت الملوحة EC15 وليس أعلى من ذلك. هذه النتائج تشجع على استخدام الثلاث سيانوبكتريا فى التسميد الحيوى لنبات الأرز فى التربة الملحية لزيادة النمو والانتاجية وتقليل الملوحة.

الكلمات المفتاحية: الأرز، الملوحة، السيانوبكتريا، النمو، الانبات.