Effect of Poly-β-Hydroxybutyrate (PHB) and Glycogen Producing Endophytic bacteria on yield, growth and nutrient contents in rice cultivated in saline soil

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ABSTRACT

The saline soil is important problem in Egypt because of a wide areas are Influenced by salts that obstruct attempts to increase the cultivated land. The main problem in the saline soil is how to prepare suitable conditions for plants cultivation and it’s known that microorganisms could make a suitable root zone for seeds to diminish saline effect on the plant. The Poly-β-Hydroxybutyrate (PHB) and Glycogen produced by endophytic bacteria and Rhizobia as carbon storage polymers can support the survival and reproduction of these bacteria in adverse conditions (soil salinization) and improve tolerance to osmotic stress, hence these bacteria could increase the soil fertility, improve growth and the yield of crops to be a significant alternative to chemical fertilizers in agriculture. In this study strains of endophytic bacteria Enterobacter aerogenens, ET.101(T), Enterobacter gergoviae, ET.111(S), Enterobacter aerogenens (L), ET.102 and Rhizobium leguminosarum bv. Viceae viz Icarda 441 (R) were examined for elevation plant resistance to soil salinity. Laboratory and field experiments were conducted and the Laboratory experiment showed that the amounts produced from Poly-β-hydroxybutyrate (PHB) and glycogen, were superior in strain (T) followed by strain (S), (R) and (L) respectively where the percentage of PHB in these cells was between 19.66 and 39.09 % of dry cell weight while the content of glycogen in these tested strains were ranged from 0.093 - 0.211 g/l. Strain (T) has progressed in enhanced stress resistance capability. In plant traits, results indicated that the high amount of PHB and glycogen producing by strain (T) improved the growth, grain yield and 1000 grain weight of rice, saving 50% N fertilizer. These treatments also significantly effected in pH, organic matter, and N, P, K content of the soil.

Key words: Saline soil, Poly-beta-hydroxybutyrate(PHB), Glycogen, endophytic bacteria, Rhizobia.

Introduction

The majority of salt-affected soils in Egypt are located in the Northern 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 (FAO, 2003). Using of biofertilizers can improve soil chemical and biological characteristics and leads to using low doses of chemical fertilizers, therefore agricultural production will be free from contaminants (Salimpour et al., 2010). Bio- fertilizers play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone productivity, and designed to improve soil fertility. Endophytes are defined as microbes that colonize living, internal tissues of plants without causing any negative effects (Bacon and White, 2000). Many reports strongly suggest that these endophytes have an excellent potential to be used as plant growth promoters with legumes and non-legumes (Bai et al., 2002). (Tantawy, 2009) proved that Endophytic strains of Enterobacter aerogenens, and Enterobacter gergoviae, that isolated from Rice tissue had the ability to tolerate the high soil salinity and was excellent as PGPB producers. Rhizobia considered as endophytic bacteria in the nodules (Chi et al., 2005 and Yanni and Dazzo 2010). (Nair et al., 1993) defined the PHB as a carbon storage polymer. In the same way, Braunegg et al., (1998) reported that PHB is accumulated as intracellular granules by many prokaryotic organisms as they enter the stationary phase of growth, to be used later as an internal reserve of carbon and energy.

Glycogen is a major energy storage compound in many bacteria, including Enterobacter (Steiner, and Preiss, 1977). It is a polysaccharide consisting of glucose units. In most bacteria, glycogen accumulates in the stationary growth phase and under conditions of limited growth in the presence of an excess of carbon and energy (Preiss and Romeo, 1994). Therefore, glycogen is generally assumed to be a storage compound serving as a carbon and energy reserve (Preiss, 1996).
Accumulation of cellular glycogen and PHB is initiated only under growth-limiting conditions such as saline soil. When the external carbon source is exhausted, glycogen and PHB are metabolized by the cells, sustaining their longevity and thus act as true reserve materials (Zevenhuizen, 1981). Ceyhan and Ozdemir, (2011) isolated the Enterobacter aerogenens strain 12Bi, and they reported that it was excellent PHB granules producer, as its main carbon storage compound and it was found that PHB production beginning from 16.66 (%).

(Preiss, 1984) studied the widespread existence of glycogen in enteric genera, including Citrobacter, Enterobacter, Klebsiella, Serratia and Shigella, suggested that, under conditions of nutritional and other stresses associated with environmental survival, the accumulation of energy-storage compounds such as glycogen would be important.

Lodwig, et al. (2005) mentioned that Rhizobium leguminosarum synthesizes polyhydroxybutyrate and glycogen as its main carbon storage compounds. It has been hypothesized that PHB accumulated by the bacteria prior to infection serves to fuel infection and/or bacteriodifferentiation.

Rice (Oryza sativa L.) is a cereal foodstuff which forms an important part of the diet of more than three billion people around the world (Cramer, 1967) and for its importance in Egypt, this study assessed the ability of three strains of endophytes and strain of Rhizobium leguminosarum bv. Viciae 441 to produce PHB, Glycogen in culture media and use them to enhance production of rice (Oryza sativa L.) and improve soil properties.

Materials And Methods
Bacterial strains and growth conditions

Strains of endophytic bacteria Enterobacter aerogenens, ET.101 isolated previously from Rice root (T), Enterobacter gergoviae, ET.111 isolated from rice shoot (S), Enterobacter aerogenens, ET.102 isolated from Rice leaves (L) (Tantawy, 2009) were used in the present study, beside the strain of R. leguminosarum bv. Viciae viz Icarda 441, that kindly provided from the Biofertilizers Production Unit, Agricultural Microbiol. Dept., Soils, Water and Environ. Res. Inst., ARC, Giza, Egypt. The strains grown and maintained on yeast extract mannitol agar media (YEM) Vincent (1970).

Laboratory experiments

Cultures of four bacterial strains under study were grown in 100 ml Erlenmeyer flasks containing 25 ml of yeast extract mannitol (YEM) medium. The growth, PHB and glycogen were determined after 72 h of incubation at 30 ± 1°C.

Screening for plant growth-promoting traits

Determination of growth

Cell dry weight was studied for biomass production. Cell dry weight was determined by filtering the culture medium through pre-weighed Whatman filter paper No. 44. Filter papers contained biomass were placed in an oven at 80°C for 18 hrs until a constant weight was measured (Corvini et al., 2000).

Determination of PHB

Determination of the amount of PHB was performed chemically according to Law and Slepecky, (1961) and Kuniko et al., (1989).

Glycogen detection.

Glycogen was extracted from cells and determined by anthrone, according to the procedure of Chun and Yin (1998).

Nitrogenase enzyme activity

Nitrogenase activity was determined in the rhizosphere of the plant sample uprooted at 75 days of planting by Acetylene reduction assay and determined by GLC (Leth Bridge et al., 1982).

Salt tolerance

The tolerance of strains to salt was studied in cultures grown on YEM. Sodium chloride was added before autoclaving at concentrations of 0, 1.5, 3, 5, 6 and 8% the pH was adjusted to 6.8 (El-shelkh and wood, 1989). Stock culture (0.1 ml) of the selected strains was inoculated to the above prepared media. Colonies were counted after incubation at 30°C for 48h. Viable rhizobial cells were estimated by plate counts on YEM agar medium plus 10 ml/L of Congo red solution.

Field experiment

A field experiment was set up on Rice plant (Oryza sativa) sppin El- Ezdehar village Sahl El-Hossinia region (El-Sharkia governorate, Egypt) this area irrigated with El- Salam (Nile water mixed with agriculture drainage water of rate 1:1).

Soil analysis

The main soil properties of the experimental field were determined as shown in Table (1) as described by Black (1965). Soil pH and Total soluble salt (EC) was measured as in Jackson (1967). Available N was measured according to
the modified Kjeldahel method (Black, 1965). Available P was determined colorimetrically according to Olsen’s method (Jackson, 1967). Available K was determined using the Flame-Photometer, (Soltan Pour and Schwab, 1977).

Table 1. Some physical and chemical properties of soil before planting

<table>
<thead>
<tr>
<th>Coarse sand (%)</th>
<th>Fine sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>Texture</th>
<th>O.M (%)</th>
<th>CaCO$_3$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.56</td>
<td>43.92</td>
<td>31.98</td>
<td>20.54</td>
<td>Clay</td>
<td>0.24</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH (1:2.5)</th>
<th>EC (ds/m)</th>
<th>Cations (meq/l)</th>
<th>Anions (meq/l)</th>
<th>pH (1:2.5)</th>
<th>EC (ds/m)</th>
<th>Cations (meq/l)</th>
<th>Anions (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.45</td>
<td>16.64</td>
<td>17.19</td>
<td>27.6</td>
<td>121</td>
<td>0.62</td>
<td>12.83</td>
<td>105</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Macronutrients (mg/kg)</th>
<th>Micronutrients (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N 27.18</td>
<td>P 3.64</td>
</tr>
<tr>
<td>K 183</td>
<td>Fe 1.19</td>
</tr>
<tr>
<td>Mn 6.91</td>
<td>Zn 0.81</td>
</tr>
<tr>
<td>Cu 0.66</td>
<td></td>
</tr>
</tbody>
</table>

**Soil tillage**

First, the soil surface leveled used by laser technique. Second, the deep sub-soiling plough, and establishment of field drains at a distance of 10m between each of two drains and at 90 cm at drain beginning. The plot units are subjected to continuously and alternatively leaching processes before rice planting. The experiment was designed in split-plot design with three replicates, where the bio-fertilizers are allocated in the main plots as follow:

- Control without biofertilizers.
- Enterobacter aerogenens ET.101.
- Enterobacter gergoviae, ET.111.
- Enterobacter aerogenens, ET.102
- R. leguminosarum bv. Viceae viz Icarda (441).

Nitrogen fertilizers were randomly distributed in sub-plots as follows:

- 100% of recommended dose (100 kg N/fed).
- 75% of recommended dose (75 kg N/fed).

Mineral nitrogen added in the form of urea (46% N) at rates of 100 and 50 kg N/fed as a recommended and half recommended dose, respectively, added at equal 3 doses after 21, 42 & 60 days of rice planting. Calcium super-phosphate (15% P$_2$O$_5$) and was added at a rate of 30 kg P$_2$O$_5$/fed during soil preparation, while potassium sulphate (48 % K$_2$O) was added at a rate of 100 kg K$_2$O/fed two equal split doses during soil tillage and after 42 days of planting.

**Bacterial preparations**

Bacterial strains were grown and maintenance individually on (YEM) (Vincent, 1970) and a broth culture contain $10^9$ cell ml$^{-1}$ of either strain. Equal portion of each strain were mixed with gamma irradiated peat and vermiculite neutralized with 5% CaCO$_3$ (2:1 w/v) and the moisture content of final product was adjust 50%.

**Grains inoculation**

Rice grains (oriza sativa L.) culivar (Giza 178) was obtained from the Field Crop, Res. Inst., ARC. Giza, Egypt. Rice grains were coated with carrier (peat and vermiculite)-based inoculants of the endophytic bacteria and Rhizobia individually using Arabic gum as adhesive agent to form a bio-film of bacteria around seeds before planting, this process performed on the day of sowing and dried in shadow before planting. Inoculated plots received a liquid bacterial culture after 30 and 60 days of planting. After planting the recommended agriculture practices were carried out along the season.

**Determination of plant growth parameters**

Both Rice crop quality and quantity were assessed as follows: Rice was harvested and grain was separated to Straw and grains and recorded as yield in ton / fed. Samples of ten plants were collected from each plot and grain was separated. Grain and straw, were oven dried at 70°C, weighed to obtain their dry matter per plant. The selected samples of plant were ground and then 0.5 g of each sample was digested using the methods described by Page et al. (1982). N, P and K contents in seeds and straw were determined according to Cottenie et al. (1982), and the Crude protein in rice grains by (AOAC, 2005).

**Statistical analysis**

The obtained data were statistically analyzed using the general linear models procedure of SAS (1999). When significant effects were found (p<0.05), means were separated using Duncan’s multiple range test.

**Results And Discussion**

**PHB and Glycogen production**

Table (2) showed that In spite of the results of cell dry weight content was high in the strain L followed by S, T and R but the results of yield of PHB and Glycogen content in Enterobacter and Rhizobia strains were in the different
trend where strain (T) was preceding followed by strain (S) then strain (R) in the third rank and the strain (L) came at last rank and these results referred that the strains L, R and S were produced high amount of PHB and glycogen from low dry weight content of cells. The production of PHB was ranged from 0.236-0.430 g/l, and the percentage of PHB in these cells was between 19.66 and 39.09% of dry cell weight. Also, it is clear from Table (2) that the content of glycogen in tested strains of endophytic bacteria and rhizobia ranged from 0.093-0.211 g/l with pioneer by strain (T) which ranking in terms of the production of glycogen same as order such as PHB produced. These results are in harmony with those obtained by Ceyhan and Ozdemir (2011) when reported that E. aerogenes produce high amount of PHB. In the same direction, Caiola et al. (2004) proved that the endophytic bacteria formed PHB and glycogen inside plant and reported that the Azospirillum Brasilense penetrated the epidermis root hairs and outer cortex cells and within the root it formed cysts-like cells very rich in poly-B-hydroxybutrate granules and glycogen. On the other hand Tombolini and Nuti (1989) indicated that the content of PHB in rhizobia ranges from 30 to 55% of dry cell weight and added that rhizobia synthesizes Poly-b-hydroxybutyrate under specific conditions. Mercan (2002) indicated that the content of PHB in Rhizobium strains was 0.01-0.5 g/l, and the percentage of PHB in these cells was between 1.38 and 40.0% of dry cell weight. Borah et al. (2002) proved that β-hydroxybutyrate is accumulated during the growth phase. Hence, PHB accumulation appeared to be growth associated.

### Table 2. Cell dry weight, PHB, Yield of PHB, Glycogen content by strains of endophytic bacteria and rhizobia sp.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Cell dry weight (g L⁻¹)</th>
<th>PHB (g L⁻¹)</th>
<th>Yield of PHB (%)</th>
<th>Glycogen (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenens (T)</td>
<td>1.10⁹</td>
<td>0.430⁶</td>
<td>39.09⁶</td>
<td>0.211⁶</td>
</tr>
<tr>
<td>Enterobacter gergoviae (S)</td>
<td>1.15⁹</td>
<td>0.422⁶</td>
<td>36.69⁶</td>
<td>0.152⁶</td>
</tr>
<tr>
<td>Enterobacter aerogenens (L)</td>
<td>1.20⁹</td>
<td>0.236⁶</td>
<td>19.66⁶</td>
<td>0.093⁶</td>
</tr>
<tr>
<td>R. leguminosarum (R)</td>
<td>0.859⁹</td>
<td>0.308⁸</td>
<td>35.5⁸</td>
<td>0.151⁴</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan’s test.

Bacteria able to synthesize PHB can be divided into two groups (Lee, 1996). The first group, accumulate PHB during the stationary phase requires an excess of the carbon sources. The second group accumulate PHB during the log phase (Bormann et al., 1998). Regarding of glycogen (Preiss, 1984) emphasized that glycogen is existing in enteric genera, including Enterobacter under conditions of stresses associated with infection and environmental survival. (Zevenhuizen, 1981) said that under growth-limiting conditions, free-living rhizobial cells produce glycogen simultaneously with PHB. This suggests that glycogen metabolism may fulfil a similar role to PHB (Dunn et al., 2002).

**Effect of salt stress on endophytic strains and rhizobia.**

In the current study, the endophytic stains and rhizobia sp. showed different responses to salt stress. They showed gradually decreased in growth of bacterial cells with increasing salt concentration until 5%. At 6% NaCl the reduction was acute, and least number of colonies was observed at 8%. The tolerance was greater in case of strain (T) as compared to strains (S), (L) and (R), this suggest a definite role for PHB and glycogen in cell protection in saline condition (Fig. 1). These results were in the same line with results obtained by Tantawy (2009) when showed that the same isolates of Enterobacter that used in the current work and declared their halo-tolerant in maintenance in the salt media and alleviate the salt suppression.
Also, Thrall et al. (2008) found that increasing salt concentrations may have a detrimental effect on rhizobial populations as a result of direct toxicity as well as through osmotic stress. Arora et al., 2006 suggested that PHB has a definite role in protection of the bacterial cells from osmotic stress, where minimum PHB content was accumulated at low or zero salinity, maximum was observed by the salt- tolerant strains at higher salt concentrations. In addition to PHB is synthesized by many species of bacteria has been shown to improve survival during starvation (Kadoury et al., 2002), as well as improve tolerance to osmotic stress (Kadoury et al., 2003).

Nitrogenase activity

Inoculation of rice grain with R. leguminosarum bv. Viciae strain 441 and strain (S) significantly enhanced nitrogenase 2.01 and 1.69 µmole C₂H₄/g dry nod/h in case of strains R and S plus 50% mineral nitrogen fig (2). Strains T and L gave lower result. Wang et al. (2007) suggest that both PHB and glycogen can result in increased infection and are influential in the nodulation and nitrogen fixation capabilities of S. meliloti. Caiola et al. (2004) suggested that the endophytes can fix nitrogen in intracellular spaces of plant roots therefore the nitrogen-fixing does not appear in the root system of the plant.

Concerning with Rhizobia Bergersem and Turner (1993) showed that Rhizobia free living cells bacteroids can be used to support nitrogen fixation in vitro. Also, Wang et al. (2007) assured that the ability to synthesize PHB is important for nitrogen fixation in Rhizobia. On the other hand, store energy in the lipid poly-hydroxybutyrate (PHB), which may enhance rhizobial survival when they are carbon limited (in salinity), either in nodules or in the soil (Ratcliff et al., 2008) and they added Bacteria form the carbon storage compounds polyhydroxybutyrate (PHB) and glycogen under growth-limiting conditions for the increased symbiotic efficiency. It most likely is due to increased nodulation rather than increased nitrogenase activity.

Plant yield parameters

The values for grain yield presented in Table (3) revealed that all the tested strains had the capability to increase the grain yield and straw of rice significantly in comparison with uninoculated control. Overall, the increase in grain yield as result of inoculation with all the strains under study ranged from 0.410 to 0.759 (ton/ fed) over uninoculated control. Whereas strains T and S plus 50% N were the highest producers of rice straw yield (1.76 and 1.28 ton/ fed respectively). On the other hand, Strains L and R although improved the straw Yield 0.91 and 0.90 ton/ fed, respectively, over uninoculated control, the improvement was statistically not significant compared with un-inoculated control.

Positive significant effect on 1000 grains weight in comparison with un-inoculated control was recorded. Maximum weight of 1000 grains was achieved with strain T, S, L and R respectively Table (3). The yield and plant growth enhancement effects of bacteria used in this study on rice could be explained with N₂- fixing, PHB and glycogen capacity of bacteria. Zeiny (2007) proved that the better plant yields obtained with strains based inoculants may be attributed to its efficiency in supplying the growing plants with biologically fixed nitrogen and induce exudates of some hormonal substances like auxins which could stimulate nutrients absorption. In accordance with the findings of Tal and Okon (1985) the ability of endophyte strains and rhizobia to tolerate salinity stress and promote plant growth also may be affected by PHB accumulation, which resulted in stress endurance. Wang et al. (2007) proved that the ability to synthesize PHB is important for N₂ fixation in Medicago truncatula nodules and younger Medicago sativa nodules and the blocking of glycogen synthesis resulted in lower levels of N₂ fixation on M. truncatula and on M. sativa and proved that mutants defective in PHB and glycogen resulted in significantly lower nodule dry weight, shoot dry weight and ARA. On M. sativa compared with wild-type.
The ability of plant host to grow and survive in saline conditions with helped by endophytic bacteria is improved, this facts have been emphasized by Tantawy and Shaban (2010) when they are inoculated the Rice plant with salt tolerant strains of endophytic bacteria and the results showed their role in elevation plant resistance in saline soil by producing exopolysaccharids (EPS) and indole acetic acid (IAA) as plant growth promoting substances.

Concerning inoculation with rhizobia, Shamseldin and Werner (2005) reported that the ability of host plant to grow and survive in saline conditions with helped by endo

Table 3. Effect endophytic bacteria and rhizobia sp. on growth characters of rice at harvest

<table>
<thead>
<tr>
<th>Treat</th>
<th>Dry weight (g plant⁻¹)</th>
<th>Weight (1000 grains)</th>
<th>Weight crop (ton/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Straw</td>
<td>Grains</td>
<td>Straw</td>
</tr>
<tr>
<td>N mineral 100%</td>
<td>14</td>
<td>9.49</td>
<td>11.00</td>
</tr>
<tr>
<td>Strain,T + 50% N</td>
<td>21</td>
<td>13.17</td>
<td>28.00</td>
</tr>
<tr>
<td>Strain,T + 100% N</td>
<td>18</td>
<td>11.89</td>
<td>26.00</td>
</tr>
<tr>
<td>Strain,S + 50% N</td>
<td>19</td>
<td>9.48</td>
<td>24.00c</td>
</tr>
<tr>
<td>Strain,S + 100% N</td>
<td>16</td>
<td>6.79</td>
<td>20.66d</td>
</tr>
<tr>
<td>Strain,L + 50% N</td>
<td>17</td>
<td>7.48</td>
<td>20.00f</td>
</tr>
<tr>
<td>Strain,L + 100% N</td>
<td>15</td>
<td>5.86</td>
<td>18.00g</td>
</tr>
<tr>
<td>Rhizoba + 50% N</td>
<td>12</td>
<td>6.75</td>
<td>17.00e</td>
</tr>
<tr>
<td>Rhizoba +100% N</td>
<td>10</td>
<td>5.96</td>
<td>14.00h</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan’s test.

Nutrient element (NPK) contents of rice

Tables 4 and 5 depict the statistical behavior of total N P K uptake by rice plant. The inoculation by endophytic strains and rhizobia increased N, P and K contents of grain and straw in rice in compared to full dose of N fertilizer (control). The improvement of NPK uptake were scored insignificant differences in case of N uptake, with preceding by stain T that recorded 1.42 and 2.65 % in grain and straw respectively in case of N uptake, (0.36 and 0.280 %) in case of P uptake while recorded (2.18 and 2.21 %) in the K uptake by grain and straw respectively.

Table 4. Concentration of nutrients contents in grains at harvest

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mineral 100%</td>
<td>1.38a</td>
<td>0.26a</td>
<td>1.75a</td>
<td>7.93a</td>
</tr>
<tr>
<td>Strain,T + 50% N</td>
<td>1.42a</td>
<td>0.36a</td>
<td>2.18a</td>
<td>8.16a</td>
</tr>
<tr>
<td>Strain,T + 100% N</td>
<td>1.39a</td>
<td>0.32a</td>
<td>2.18a</td>
<td>7.99a</td>
</tr>
<tr>
<td>Strain,S + 50% N</td>
<td>1.40a</td>
<td>0.34ab</td>
<td>1.02b</td>
<td>8.05b</td>
</tr>
<tr>
<td>Strain,S + 100% N</td>
<td>1.34b</td>
<td>0.29b</td>
<td>2.05b</td>
<td>7.70b</td>
</tr>
<tr>
<td>Strain,L + 50% N</td>
<td>1.37c</td>
<td>0.32b</td>
<td>2.09b</td>
<td>7.87b</td>
</tr>
<tr>
<td>Strain,L + 100% N</td>
<td>1.35cd</td>
<td>0.26d</td>
<td>1.89bcd</td>
<td>7.76c</td>
</tr>
<tr>
<td>Rhizoba + 50% N</td>
<td>1.37c</td>
<td>0.29c</td>
<td>1.99bc</td>
<td>7.87c</td>
</tr>
<tr>
<td>Rhizoba +100% N</td>
<td>1.32c</td>
<td>0.24c</td>
<td>1.85cd</td>
<td>7.59c</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan’s test.

In endophytes nutrient uptake, Johnston-Monje and Raizada (2011) decided the ability of endophytic bacteria to enhance plant growth and nutrient uptake and attributed that to secretion of auxin within the plant into plant tissue directly. Also, Zarrin et al. (2008) suggested that legume yield and N accumulation are directly related to the magnitude and efficiency of symbiotic N2-fixation occurring in root nodules. Biswas et al. (2000) were used six rhizobial diazotrophs including Rhizobium leguminosarum in inoculation of Rice plant and they decided that increased in rice grain, straw yields and NPK uptake in plant and referred that to nitrogen fixation and PGBS like IAA.
Available (NPK) in soil after harvest

Available NPK in soil remained after rice harvesting as shown in Table (6) increased insignificantly over the control in all treatments. Inoculation with any of endophytic bacteria strain T and strain L or Rhizobia compound with 100% N scored the highest values by corresponding values 54, 51 and 50 mg/ kg soil, respectively. While, the Rhizobia with both 50 and 100% N was higher in case of available- P and these treatments joined with endophytic bacteria strain L + 100% N to be superior in case of available-K. These trends stands in well agreement with Yanni et al. (1997), when they Emphasized that the symbiotic relationship of Rhizobia and legumes plants excreted the nitrogen element in soil as a results of nitrogen fixation and for over 7 centuries, production of rice (Oryza sativa L.) in Egypt has benefited from rotation with Egyptian berseem clover (Trifolium alexandrinum). The nitrogen supplied by this rotation replaces 25- 33% of the recommended rate of fertilizer-N application for rice production. They added that the benefit of rhizobial inoculation to the rice increased availability fixed N consequence Rhizobia have the potential to promote rice growth and productivity under field conditions.

Concerning the phosphorus element, Peix et al. (2001) recorded that the inoculation of soil with rhizobia not only based on the effectiveness to their nitrogen fixation potential, since these microorganisms can increase the growth of plants by phosphate solubilization mechanisms.

Presently, information is rarely available on the types of endophytic bacteria and their ability for availability the elements, but Tantawy and Shaban (2010) reported that the inoculation with endophytic bacteria maximized the quantity of NPK that remained in soil after harvest.

Table 6. Concentration of soil available NPK (mg/ kg\(^{-1}\) soil) after harvest

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mineral 100%</td>
<td>40</td>
<td>3.82(^a)</td>
<td>194(^a)</td>
</tr>
<tr>
<td>Strain,T + 50% N</td>
<td>47</td>
<td>3.93(^b)</td>
<td>198(^b)</td>
</tr>
<tr>
<td>Strain,T + 100% N</td>
<td>54</td>
<td>3.96(^c)</td>
<td>195(^c)</td>
</tr>
<tr>
<td>Strain,S + 50% N</td>
<td>46</td>
<td>3.95(^d)</td>
<td>197(^d)</td>
</tr>
<tr>
<td>Strain,S + 100% N</td>
<td>48</td>
<td>3.98(^e)</td>
<td>199(^e)</td>
</tr>
<tr>
<td>Strain,L + 50% N</td>
<td>47</td>
<td>3.88(^f)</td>
<td>198(^f)</td>
</tr>
<tr>
<td>Strain,L + 100% N</td>
<td>51</td>
<td>3.93(^g)</td>
<td>202(^g)</td>
</tr>
<tr>
<td>Rhizoba + 50% N</td>
<td>47</td>
<td>4.02(^h)</td>
<td>207(^h)</td>
</tr>
<tr>
<td>Rhizoba +100% N</td>
<td>50</td>
<td>4.06(^i)</td>
<td>209(^i)</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan’s test

Soil PH and EC as affected by endophytic bacteria.

Results obtained in Table (7) show the effect of endophytic bacteria and rhizobia inoculation on pH and EC of the soil after rice harvesting. All strains with rice decreased both pH and EC as compared to the full nitrogen dose (the control). However, strain R with 100% nitrogen gave the least pH degree (8.22) and the least EC (7.5 dSm\(^{-1}\)) although, the soil EC started with 16.64 (dSm\(^{-1}\)) when assessed that there were decreasing in cations of Na\(^+\) and Ca\(^++\) and anions of HCO\(_3\) and SO\(_4\)\(^2-\) as effective of endophytic bacterial inoculants and these results means that the bio-fertilizer alleviate soil salinity. These results confirmed more over by Vishal et al. (2013) who proved that number of organic acids are produced by the bacterial endophytes like indol acetic acid, gibberelic acid, and abscisic acid etc. support plant growth by solubilization of minerals and by root growth promotion and lowering the EC and pH in the rhizosphere and these organic acids provided a substantial modification of soil physical and chemical properties.

Table 7. Some chemical properties of the soil after harvesting rice as affected by endophytes and rhizobia

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH (1:2.5)</th>
<th>EC (dSm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mineral 100%</td>
<td>8.40(^a)</td>
<td>12.67(^a)</td>
</tr>
<tr>
<td>Strain,T + 50% N</td>
<td>8.37(^b)</td>
<td>10.34(^b)</td>
</tr>
<tr>
<td>Strain,T + 100% N</td>
<td>8.34(^c)</td>
<td>9.72(^c)</td>
</tr>
<tr>
<td>Strain,S + 50% N</td>
<td>8.30(^d)</td>
<td>10.45(^d)</td>
</tr>
<tr>
<td>Strain,S + 100% N</td>
<td>8.27(^e)</td>
<td>11.25(^e)</td>
</tr>
<tr>
<td>Strain,L + 50% N</td>
<td>8.29(^f)</td>
<td>10.30(^f)</td>
</tr>
<tr>
<td>Strain,L + 100% N</td>
<td>8.24(^g)</td>
<td>8.30(^g)</td>
</tr>
<tr>
<td>Rhizoba + 50% N</td>
<td>8.26(^h)</td>
<td>9.90(^h)</td>
</tr>
<tr>
<td>Rhizoba +100% N</td>
<td>8.22(^i)</td>
<td>7.50(^i)</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan’s test

Moreover Shaban and Attia (2009) reported that Application of biofertilizers combined with different mineral nitrogen fertilizer levels led to a decrease in soil salinity because bio-fertilizer could improve the soil properties as a results of influence of bio-fertilizer on the total porosity, and improving soil aggregation and possible moving soil soluble
salt with irrigation water which promote plant growth and had an effect to reduce the detrimental effect of the salinity stress. Tran et al. (2004) stressing that biofertilizer exerts beneficial effect on soil physical, chemical and biological characteristics and increase of organic matter. Omar et al. (1993), reported that the biofertilizers that inoculated in plants produce several phytohormones such as indole acetic acid and cytokinins which promote plant growth and had an effect to reduce the EC and the salinity stress. Shaban and Omar (2006) showed that the increasing in dehydrogenase activity leads to the hydrogen moles production which react in root zone to form hydrocarbon acid and decrease the soil pH.

**Conclusion**

The present study demonstrated a significant positive effect of endophytic bacteria on plant cultivated in salt stress soil, strains T and S improved growth, N, P and K contents of rice plants in grains and straw compared to inoculation with endophytic strain L or Rhizobium. These data suggest that endophytic strains T and S can be used in further investigations as a potential agent of new biofertilizer for improved rice production. Also, these data have important implications for understanding the role of PHB and glycogen that produced by the tested bacterial strains in soil salinity adverse conditions to support growth and resistant of rice plant in the interaction of plant and microorganisms.

**References**


