EFFICIENCY OF NATIVE HETEROCYSTOUS CYANOBACTERIAL ISOLATES FOR SUPPLYING NITROGEN AND IMPROVING RICE PLANT GROWTH

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ABSTRACT

The use of chemical fertilizers in rice fields has many environmental hazards because of soil water logging. Nitrogenous fertilizers that are very soluble, undergoes higher transformations in such conditions and are subjected to the losses and reduction in their efficiency. They thus contaminate surface and groundwater. In such situations heterocystous cyanobacteria possessing biological nitrogen fixation and plant growth promoting abilities, can be useful in supplying nitrogen to the plant. Some isolates of cyanobacteria native to the northern Iran, were effective N$_2$ fixers as well as producing plant growth stimulants under laboratory conditions. In this study, the inoculation effects of two heterocystous cyanobacterial isolates (B0 = control, B1= Anabaena sp. GGuCy-33 and B2= Anabaena sp. GGuCy-17 ) in rice plant (Oryza sativa L. cv. Hashemi) with three levels of nitrogen fertilizer (0, 75 and 150 mg N/kg soil as urea) on growth and uptake of N, P and K were studied in greenhouse conditions. The results showed that B2 isolate increased shoot dry weight and plant height, but B1 isolate was effective in increasing dry weight of root. Nitrogen uptake of root in the presence of B2 was increased by 18.5 percent compared to the non-bacterial control. Also the B2 isolate increased shoot P uptake by 15.7% and K uptake by 33.4% in shoot compared to the control. B1 was also able to increase root nitrogen by 53.3%, root P by 29.4% and root K by 28.7% compared to the control. In the presence of B2, plant dry weight reached a highest level by using 75 mg N/kg, however a significant reduction in plant growth was recorded at 150 mg N/kg level.

Key Words : Cyanobacter, Heterocyst, Nitrogen, Phosphorus, Rice

INTRODUCTION

Nitrogen is a major limiting nutrient in production of rice plant in Iran paddy soils. Despite economically aspects, application of chemical nitrogen fertilizers in paddy soils has environmental risks due to rapid movement of soluble nitrogen compounds in surface waters as well as its leaching to the ground water or volatilization to the atmosphere. Cyanobacteria (Blue-Green Bacteria) are one of the major components of the nitrogen fixing organisms in rice fields. The agricultural importance of cyanobacteria in rice cultivation is directly related to their ability to fix nitrogen and other positive effects for plants and soil. These photosynthetic prokaryotes could enhance soil organic matter after their death and decomposition and improve soil aeration by producing O$_2$ which results in detoxification of sulfides, Mn$^{2+}$ and Fe$^{2+}$ under water logging conditions. Nitrogen fixation by cyanobacteria is catalyzed in specialized cells named heterocysts. Therefore, only heterocystous cyanobacteria are capable for N$_2$ fixation. Biological N$_2$-fixation by cyanobacteria has been mostly estimated from acetylene reducing activity measurements. Published data vary from a few to 80 kg N ha$^{-1}$ which corresponds to increase crop yield by 10-15%. Nostoc and Anabaena are the most abundant heterocystous genus in rice fields with efficient N$_2$ fixation. The most fast-growing strains (doubling time of 5–12 h) belong to the genus Anabaena, have short filaments. Excess mineral nitrogen is known to inhibit the biological nitrogen fixation in cyanobacteria. Adverse effects of NO$_3^-$ and NH$_4^+$ on nitrogenase activity in N$_2$ fixers has mostly been reported. Nitrogen broadcasting, widely practiced by farmers, not only inhibits N$_2$
fixation but also causes N losses by ammonia volatilization. In addition to the nitrogen supply by the bacteria, they can also exert other beneficial effects on plant growth by several ways such as phosphate solubilization, releasing potassium from K bearing minerals, production of siderophores, phytohormons (such as auxins and gibberellins), vitamins and amino acids. Moreover, they can increase soil water holding capacity through their jelly structure by producing exopolysaccharides.

This study was aimed to evaluate the efficiency of two native cyanobacterial strains belonging to the *Anabaena* genus, isolated from paddy soils of Guilan Province, Iran, in supplying nitrogen, phosphorus and potassium and growth promotion of rice plant under different levels of compound nitrogen.

**MATERIAL AND METHODS**

**Bacterial Strains**

Two heterocystous cyanobacterial strains, *Anabaena sp.* GGuCy-33 and *Anabaena sp.* GGuCy-17 were obtained from Biotechnology Institute of Tabarestan, Sari, Iran. They were propagated in BG11 medium to reach OD<sub>650</sub> = 0.6 and then 100 ml of bacterial suspension was mixed with 130 g sterile perlite to get bacterial inoculum.

Some plant growth promoting (PGP) traits of bacterial strains are summarized in Table 1.

**Table 1 : Some PGP traits of two bacterial strains**

<table>
<thead>
<tr>
<th>Measured traits</th>
<th><em>Anabaena sp.</em> GGuCy-17(B2)</th>
<th><em>Anabaena sp.</em> GGuCy-33(B1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate solubilization (μg mg&lt;sup&gt;-1&lt;/sup&gt; DW)</td>
<td>614.24</td>
<td>40.52</td>
</tr>
<tr>
<td>Siderophore production (μmol mL&lt;sup&gt;-1&lt;/sup&gt; day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.95</td>
<td>3.07</td>
</tr>
<tr>
<td>Auxin (IAA) production (μg μg&lt;sup&gt;-1&lt;/sup&gt; chl.a day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Nitrogenase activity (nmol C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22.63</td>
<td>21.11</td>
</tr>
</tbody>
</table>

**Plant culture and bacterial inoculation**

Seeds of rice plant (*Oryza sativa* L. cv. Hashemi) were surface sterilized with 1% sodium hypochlorite and washed three times with sterile water. They then germinated within moist filter paper at 25-27 °C. Five germinated seeds were sown in each pot containing 2 kg of autoclaved sandy loam soil. Each pot was inoculated with either *Anabaena sp.* GGuCy-33 (B1) or *Anabaena sp.* GGuCy-17 (B2) or left uninoculated as control (B0). Each pot received 13 g of bacterial inoculum as a thin layer under the seeds. Three levels of nitrogen fertilizer (0, 75 and 150 mg N kg<sup>-1</sup> soil as urea), 15 mg P kg<sup>-1</sup> as KH<sub>2</sub>PO<sub>4</sub> and 50 mg K kg<sup>-1</sup> as both K<sub>2</sub>SO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub> were applied to the pots. The pots were water logged and the height of water at soil surface was one cm. The seedlings were thinned to three per pot after two weeks. A factorial complete randomized block design with three replications was carried out to statistical analysis. At harvest, plant growth characteristics including shoot and root dry weights, leaf chlorophyll index (Hansatech CL-01), nitrogen, phosphorus and potassium concentrations in shoot and root were measured.

**RESULTS AND DISCUSSION**

**Shoot and root biomass**

Shoot dry weight (SDW) was significantly increased by increasing level of nitrogen to 75 mg kg<sup>-1</sup> but a marked decrease was observed towards the 150 mg kg<sup>-1</sup> although it was still higher than control (zero addition of N). At 75 mg kg<sup>-1</sup> the B2 strain significantly increased SDW by 30% compared to the nonbacterial control but at 150 mg kg<sup>-1</sup> both strain were efficient and the increase was 15 and 15% with B1 and B2, respectively (Fig.1). Root dry weight (RDW) showed same trend as SDW by increasing nitrogen level. Bacterial strain B1, was more efficient than B2 in this case and significantly (p<0.05) enhanced RDW by 70% and 38% at both 0 and 75 mg kg<sup>-1</sup> nitrogen.
levels, respectively but they showed no significant difference with nonbacterial control at 150 mg.kg$^{-1}$ (Fig.2). Considering shoot and root biomass decline at the highest level of nitrogen, it seems that there is an inhibitory effect of nitrogen fertilizer on both bacteria and plant at this level. Root growth enhancement caused by B1 strain can be explained by its higher auxin production (Table 1).

**Fig. 1**: Effects of bacterial inoculation and nitrogen levels on shoot dry weight.

**Fig. 2**: Effects of bacterial inoculation and nitrogen levels on root dry weight.

**Chlorophyll index**

Leaf chlorophyll index (LCI) was positively affected by both nitrogen fertilizer and bacterial inoculation. LCI was enhanced by increasing level of nitrogen although there was no significant difference between 75 and 150 mg.kg$^{-1}$. Both bacterial isolates also caused a marked increase in LCI compared to the nonbacterial control but there was no difference between B1 and B2 in this respect (Fig.3). Nitrogen is considered as a main nutrient in chlorophyll synthesis and its supplying by chemical fertilizer or cyanobacterial inoculation had led to LCI increase.

**Fig. 3**: Effects of bacterial inoculation and nitrogen levels on leaf chlorophyll index
Plant nitrogen
Both bacterial isolates significantly \((p<0.05)\) enhanced shoot nitrogen at all nitrogen levels compared to the non-bacterial control. The highest shoot nitrogen was recorded at 75 mg.kg\(^{-1}\) in the presence of B2 isolate but a decreasing trend in shoot N uptake was seen toward the 150 mg.kg\(^{-1}\) \((\text{Fig.4})\). This may be as result of inhibiting effect of higher nitrogen on plant growth and N\(_2\) fixation by bacteria. In contrast to the shoot, nitrogen uptake by root was pronounced in the presence of B1 at all N levels, although a significant decrease was observed by increasing N level from 75 to 150 mg.kg\(^{-1}\) \((\text{Fig.5})\). Since nutrient uptake is affected by both plant biomass and its nutrient concentration, therefore the relatively higher dry mass of root in B1 treatment \((\text{Fig.2})\) can be responsible for higher N uptake in this treatment. This can be explained by relatively higher auxin production by B1 bacterium \((\text{Table 1})\).

Plant phosphorus
The highest phosphorus uptake was recorded by B2 isolate at nitrogen level of 75 mg.kg\(^{-1}\) but there was no significant difference between bacterial and non bacterial treatments at 0 and 150 mg.kg\(^{-1}\), although P uptake at 150 was more than zero addition of nitrogen \((\text{Fig.6})\). The higher P uptake by plant in the presence of B2 can be attributed to the higher P solubilization activity of this isolate \((\text{Table 1})\). Root P uptake was not affected by nitrogen levels but at nitrogen levels of 0 and 75 mg.kg\(^{-1}\), the B1 isolate caused a marked increase in P uptake compared to the B2 and non bacterial treatments \((\text{Fig.7})\). This can be explained by higher auxin (IAA) production of B1 which resulted in higher biomass of root \((\text{Fig.2})\). Regardless of bacterial inoculation, P uptake by shoot was inhibited by the highest level of nitrogen (150 mg.kg\(^{-1}\)). Such inhibition effect was also seen in plant biomass and nitrogen uptake as described above.
Fig. 6: Effects of bacterial inoculation and nitrogen levels on phosphorus uptake by shoot.

Fig. 7: Effects of bacterial inoculation and nitrogen levels on phosphorus uptake by root.

**Plant potassium**
Regardless of bacterial inoculation, both nitrogen levels (75 and 150 mg.kg$^{-1}$) had stimulatory effects on K uptake by shoot, perhaps by increasing plant biomass. Both bacterial isolates were efficient in K uptake by shoot at nitrogen level of 75 mg.kg$^{-1}$. The inhibitory effect of the highest N level on shoot K uptake was pronounced in both bacterial treatments (Fig. 8). It seems that the higher nitrogen level exerts adverse effects both on plant growth and bacterial activities. Potassium uptake by root showed same trend as shoot as result of N fertilizer addition. The B1 isolate was more efficient in improvement of K uptake at zero level of nitrogen. (Fig. 9)

Fig. 8: Effects of bacterial inoculation and nitrogen levels on potassium uptake by shoot
CONCLUSION

Anabaena species as heterocystous cyanobacteria are efficient N₂ fixers in aquatic ecosystems and rice fields as well¹⁻⁸. In addition to molecular nitrogen fixation, they exert other beneficial effects on the ecosystems, such as enhancing organic matter, increasing available phosphorus¹³, producing phytohormons¹⁵ and siderophores¹⁴. Both isolate used in this experiment showed the above mentioned PGPR traits and could improve the rice growth and nutrient uptake. In the presence of B2 isolate, plant dry weight reached the highest level by using 75 mg.kg⁻¹ of nitrogen, however a significant reduction in plant growth was recorded at 150 mg.kg⁻¹. Considering importance of shoot biomass in rice production, both bacterial isolates (B1 and B2) were efficient in improvement of its growth and supplying N, P and K at nitrogen level of 75 mg.kg⁻¹. Nitrogen fixing ability and other plant growth promoting effects of both cyanobacterial isolates make them efficient candidates for their implication in rice fields, although further field experiments are necessary for this recommendation. Moreover, the higher efficacy of these isolates at moderate level of nitrogen (75 mg.kg⁻¹) results in reduced application of chemical N fertilizers in paddy soils, hence, declining hazardous impact of N fertilizers on environment.

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REFERENCES