Effects of Inoculation Method of Plant Growth-Promoting Rhizobacteria and Silicon Rate on Rice Grain Yield

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Abstract

A field experiment was conducted in Lahijan, north of Iran, to determine the effects of inoculation method of plant growth-promoting rhizobacteria (Azospirillum + Azotobacter) and silicon rate on rice grain yield. The experimental design was a factorial based on randomized complete block with three replications. Factors were silicon rates (0, 150, 300, 450, and 600 kg ha\(^{-1}\)), and bio-fertilizer application methods (un-inoculated, seed treatment, and seedling root dip). A liquid bio-fertilizer containing Azospirillum spp. and Azotobacter spp. bacteria was applied according to manufacture instruction. Analysis of variance showed that plant height, grain yield, tiller number per m\(^2\), grain number per panicle, 1000-grain weight, biological yield, and grain N uptake were significantly influenced by silicon rate and biofertilizer application method, but the interaction effects of two factors was not significant for all of the traits. Grain yield, averaged across silicon rates, was significantly increased by 27% and 16% when biofertilizer was applied as seed and seedling root dip treatment, respectively, compared to non inoculated treatment. Grain N uptake was higher for the seed inoculated plants than non inoculated plants averaged over silicon rates. Averaged across inoculation methods, rice grain yield was significantly increased as Si application rate increased from 0 to 450 Kg ha\(^{-1}\), but did not significantly increase at higher Si rate. The trend in rice biological yield was similar to that of rice grain yield. With increasing Si rate from 0 to 450 kg ha\(^{-1}\), grain N uptake increased from 47.4 to 94.8 kg ha\(^{-1}\), but declined slightly at higher Si rate. This experiment illustrated that silicon fertilization and biofertilizer application, especially seed treatment method, can increase rice grain yield.

Keywords: Azospirillum, Azotobacter, rice, seed and seedling root dip treatments, silicon rate

Introduction

Rice (Oryza sativa L.), a staple food crop for over half the world's people, is cultivated widely in the tropical to temperate climate regions. In Iran, the total area under rice cultivation is more than 600 thousand hectares and more than 80 percent of this area is distributed in the two northern provinces, i.e. Mazandaran and Guilan. Nearly 85% of total rice production is produced in these provinces. In these regions, transplanting is the most common method of crop establishment for rice cultivation, and rice seedlings are transplanted during May and harvested from mid-August to mid-September depending on the cultivar, climate conditions, and availability of labour. Although total rice production in Iran has been increased by 32% in recent years, the country imports around 400 to 500 thousand tonnes of its rice consumption. Therefore, it is essential to enhance rice production in the country.

Crop fertilization is one of the most important cultural practices for increasing plant production. At the same time, it is also proved that continuous and excessive use of chemical fertilizers has led to serious problems such as air, water, and soil pollutions, loss of organic humus, reduction in soil porosity, and depletion of soil nutrients, eventually
resulting in reduction of crop yield (Brown et al., 2012). Nitrogen is an essential plant nutrient that plays a major role for achieving the maximum grain yield in rice and other crops. Rice absorbs the ammonium (NH4+) as its primary source of nitrogen (Yoshida, 1981). Inside the plant, N is converted to amino acids, the building blocks for proteins. These amino acids are utilized in producing enzymes, structural and stored proteins. Also, N is a major component of chlorophyll, a green pigment which is vital for photosynthesis, and is therefore important in plant growth and final production. Most of the nitrogen fertilizer applied to rice crops is lost from the soil through different mechanisms, including ammonia volatilization, denitrification, leaching, and run off. These losses reduce N use efficiency in paddy fields (Choudhury and Khanif, 2001, 2004) and may cause environmental issues such as polluting the atmosphere, aquatic systems, and groundwater (Reeves et al. 2002). These issues cannot be alleviated completely, but they can be reduced to a considerable extent by various techniques. Nitrogen fertilizer rate in rice fields can be minimized by applying plant growth promoting microorganisms like *Azotobacter* and *Azospirillum*. These microorganisms can reduce N fertilizer rate by growth promotion through the production of auxins, cytokinins, gibberellins, and ethylene (Dobbelaere et al., 2003). The use of plan-growth promoting microorganisms can increase plants’ capacity to utilize fertilizer N efficiently. Hence, use of these organisms can reduce N losses (Choudhury and Kennedy, 2004). Moreover, biofertilizers are less expensive and are more environmentally-friendly than chemical fertilizers. Azospirillum inoculation can increase ammonium uptake by rice (Murty and Ladha, 1988). Thus, if plant growth promoting microorganisms such as *Azotobacter* and *Azospirillum* are used in rice cultivation, the environmental pollution issues from N losses can be reduced.

Silicon (Si) is the second most abundant element in the soil (Epstein, 1999) and is considered as a quasi-essential element for higher plants (Marschner, 1995). In Si accumulator’s crops, such as rice, wheat and sugarcane, it is found at much higher concentration compared to macronutrient elements (e.g. nitrogen, phosphorous and potassium). Rice plants take up Si from soil solution as salisilic acid (Epstein, 1999). Si plays important roles in plants including: (1) promoting plant resistance to pest and pathogen (Savvas et al., 2009) and assisting plants to withstand abiotic stresses, such as heavy metal stress (Doncheva, 2009), drought (Chen et al., 2011), salinity (Ashraf et al., 2010), (2) Si increases rice grain yield as well as yield components (Zhang et al., 2008; Kamenidou et al., 2009). The magnitude of the response depends on the time and rate of Si application. For example, Anderson et al. (1987) found that a single application of Si before rice planting increased rice grain yield about 50%. Snyder et al. (1986) reported that rice straw should contain approximately 30 g kg⁻¹ of Si for optimum grain production (dry weight basis), (3) playing an important role in hull formation, which in turn seems to influence rice grain quality.

Therefore, this experiment was conducted to determine the effects of silicon rate and inoculation method of plant growth-promoting rhizobacteria (*Azospirillum* + *Azotobacter*) on rice grain yield and yield components.

### Materials and Methods

A field experiment was conducted in Lahijan, north of Iran, in 2012-2013 rice growing season. Some soil properties were presented in Table 1. The experimental design was a factorial arrangement based on randomized complete block with three replications. Factors were silicon rates (0, 150, 300, 450, and 600 kg ha⁻¹), and biofertilizer application methods (uninoculated, seed treatment, and seedling root dip). A liquid biofertilizer containing *Azospirillum* spp. and *Azotobacter* spp. bacteria was applied according to manufacture instruction. The colony forming unit (cfu) of *Azotobacter* and *Azospirillum* were 10⁸ cells per gram of powder. The inoculated and uninoculated rice seeds were sown in a nursery bed on 7 April 2013. The seedlings of uninoculated rice seeds were used for root dipping treatment. Consistent with the lowland paddy field practices in north of Iran, the three-leaf stage seedlings were transplanted in experiment plots (3m x 4m) at a hill spacing of 20 cm x 20 cm with three to four seedlings per hill on 30 April 2013.
Table 1 Some soil properties (0-30 cm) of experimental field prior to transplanting

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>49.4</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>19.2</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>31.4</td>
</tr>
<tr>
<td>Texture</td>
<td>Loam clay sand</td>
</tr>
<tr>
<td>EC (ds m⁻¹)</td>
<td>1.09</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>3.25</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.248</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>19</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>122</td>
</tr>
</tbody>
</table>

N (25 kg ha⁻¹ as urea), phosphorus (100 kg ha⁻¹ as triple superphosphate) and potassium (60 kg ha⁻¹ as KCl) were applied and incorporated before transplanting. N as urea was also applied at 20 days after transplanting (25 kg ha⁻¹), just before hand weeding. After transplanting, 5-cm water depth was maintained in the experimental plots. In this experiment, no herbicide was used and weeds were removed by hand at 20 and 36 days after transplanting. Fifteen days before harvest, the plots were drained to facilitate harvesting.

Results and Discussion

Analysis of variance showed that effects of silicon rate and biofertilizer application method were significant for plant height (H), grain yield (Y), tiller number per m² (TN), grain number per panicle (GN), 1000-grain weight (ThGW), biological yield (BY), and grain N uptake (GNU), but the interaction between Si rate and biofertilizer application method was not significant for all of the traits (Table 2). Moreover, the main effects of silicon rate and biofertilizer application method and the interaction between them were significant neither for harvest index, nor for grain N concentration.

Averaged across silicon rates, the tallest rice plants were observed in plots which biofertilizer was applied as seed treatment, while the shortest ones was observed when biofertilizer was not applied (Table 3).

Regardless of silicon rate, grain yield was significantly increased by 27% and 16% when biofertilizer was applied as seed and seedling root dip treatment, respectively, compared to non-inoculated treatment (Table 3). This result is in accordance with that of Bagyaraj and Indira (1994), who found that grain yield of cereals like rice, sugarcane, and wheat increased up to 11% through *Azotobacter* and *Azospirillum* inoculation. Mukhopadhyay et al. (2013) reported that rice grain yield was significantly increased when bio-fertilizer was applied.

Regardless of silicon rate, panicle number per m² was significantly lower for non biofertilizer application treatment in comparison to seed treatment and seedling root dip treatment, which did not differ (Table 3). The highest (134.9 grain per panicle) and the lowest (90.2 grain per panicle) grain number per panicle was observed in seed inoculated and non-inoculated plants, respectively. The highest 1000-grain weight (31.6 g) was recorded when bio-fertilizer was applied as seedling root dip, while the lowest one (27.6 g) was recorded in seed inoculated plants. Moreover, 1000-grain weight was significantly higher in uninodulated plants than seed inoculated plants (Table 3), which may be due to lower grain number per panicle. Mukhopadhyay et al. (2013) found that panicle number and grain number per panicle of rice were significantly increased when bio-fertilizer was applied. Contrary to this result, Mukhopadhyay et al. (2013) reported that 1000-grain weight significantly increased as bio-fertilized was applied.

The seed and seedling root dip inoculated plants were accumulated more biomass compared to non inoculated plants when averaged across silicon rates (Table 3). There was no significant difference for harvest index between inoculated and non-inoculated plants. In contrast, Mukhopadhyay et al. (2013) reported that maximum harvest index value was observed in plots receiving bio-fertilizer.

There were no significant differences for grain N concentration among inoculation methods. The seed inoculated plants exhibited higher grain N uptake than non-inoculated plants as a result of higher grain N uptake for the former (Table 3). This result is in accordance with some previous findings (Cong et al., 2009; Choudhury and Bhuiyan, 1994).
necrosis and

Roots and, in turn, is

cle (GN), cytokinins (Keyeo et al., 2011). Many soil bacteria produce auxin, which may be involved in plant growth promotion (Prigent-Combaret et al. 2008). Okon and Gonzales (1994) concluded that crop colonization on roots affects both the morphology and physiology of host plants. Proliferation of lateral roots and root hairs increased after inoculation with Azospirillum, which, in turn, is accompanied by changes in root physiology, such as increased mineral and water uptake, increased root respiration, delay in leaf senescence and increased dry weight (Dobbelaere and Okon 2007). Moreover, plant growth-promoting rhizobacteria produce auxin-like compounds, gibberellins and cytokinins (Keyeo et al., 2011). Many soil bacteria including strains of A. lipoferum encode a deaminase (acdS gene), degrade the direct precursor of ethylene, i.e. 1-aminocyclopropane-1-carboxylic acid (ACC) (Glick, 2005). This prevents the adverse effects of ethylene on root elongation. In the meantime, some plant growth-promoting bacteria produce N-acyl homoserine lactone which may be involved in plant growth promotion (Pirigent-Combaret et al. 2008). Okon and Labandera-Gonzales (1994) concluded that crop yields were significantly increased, from 5 to 30%, after inoculation with Azospirillum, especially under low chemical N fertilizer. However, they believed that the growth-promoting effect was probably linked to phytohormone production rather than nitrogen fixation. In contrast, Rodrigues et al. (2008) reported significant nitrogen fixation after inoculation of rice with certain strains of A. amazonense. This result is in accordance with the

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>df</th>
<th>H</th>
<th>Y</th>
<th>TN</th>
<th>GN</th>
<th>ThGW</th>
<th>BY</th>
<th>HI</th>
<th>GNC (%)</th>
<th>GNC (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (R)</td>
<td>2</td>
<td>270</td>
<td>828255</td>
<td>8218</td>
<td>63</td>
<td>2</td>
<td>845478</td>
<td>0.012</td>
<td>0.03</td>
<td>269</td>
</tr>
<tr>
<td>Silicon rate (S)</td>
<td>4</td>
<td>980</td>
<td>5795503</td>
<td>10363</td>
<td>113</td>
<td>8</td>
<td>28943296</td>
<td>0.010</td>
<td>0.1</td>
<td>2702</td>
</tr>
<tr>
<td>bio-fertilizer</td>
<td>2</td>
<td>1723</td>
<td>2944218</td>
<td>26854</td>
<td>12350</td>
<td>66</td>
<td>12416381</td>
<td>0.0006</td>
<td>0.5</td>
<td>2430</td>
</tr>
<tr>
<td>SxB</td>
<td>8</td>
<td>32</td>
<td>86618</td>
<td>1913</td>
<td>59</td>
<td>1</td>
<td>2189112</td>
<td>0.004</td>
<td>0.02</td>
<td>60</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>4</td>
<td>11</td>
<td>16</td>
<td>7</td>
<td>4</td>
<td>14</td>
<td>14</td>
<td>9</td>
<td>22</td>
</tr>
</tbody>
</table>

* and ** represent significance at 0.05 and 0.01 probability levels, respectively. ns represents non-significance.

<table>
<thead>
<tr>
<th>Bio-fertilizer</th>
<th>Trait(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
</tr>
<tr>
<td>No application</td>
<td>118 c</td>
</tr>
<tr>
<td>Seed treatment</td>
<td>139 a</td>
</tr>
<tr>
<td>Seedling root dip</td>
<td>133 b</td>
</tr>
</tbody>
</table>

\(^2\) Means within a column followed by the same letter are not significantly different at the 5% level according to Fischer's Protected LSD test.
findings of Mukhopadhyay et al. (2013) and Isawa et al. (2010) reporting grain yield, panicle number, grain number per panicle for rice were significantly increased when bio-fertilizer was applied.

Regardless of inoculation method, rice plant height increased linearly from 119 to 143 cm, as silicon rate increased from 0 to 600 kg ha\(^{-1}\) (Figure 1). Elawad et al. (1982) reported that plant height was quadratically related to the rate of Si application.

A positive quadratic equation expressed the relationship between Si application rate and rice grain yield (Figure 2). Averaged across inoculation methods, rice grain yield was significantly increased from 2314 to 4251 kg ha\(^{-1}\) as Si application rate increased from 0 to 450 Kg ha\(^{-1}\), but did not significantly increase at higher Si rate. The trend in rice biological yield was similar to that of rice grain yield (Figure 3). This result is in accordance with that of Zhang et al. (2008), who found that the shoot biomass of rice was increased by 125\%–171\% after Si application. Aziz et al. (2001) found that shoot dry matter of cotton plants increased three fold with Si application as compared to control. Winslow (1992) reported that rice grain yield nearly doubled due to Si application. Deposition of Si enhances the strength and rigidity of cell walls and thus increases the resistance of plants to lodging and several important diseases of rice, including blast, brown spot, sheath blight, leaf scald and grain discoloration (da Cunha and do Nascimento, 2009; Savvas et al., 2009, Vaculík et al., 2009), and abiotic stresses (Doncheva, 2009, Ashraf et al., 2010, Chen et al., 2011). Increases in rice grain yield after Si fertilization was attributed to increased resistance against diseases (Deren et al., 1994). Moreover, in dense stand of cereals, light penetration into plant community may limit photosynthesis. The modifying effect of Si on leaf erectness can increase light interception and penetration in dense plant population and, hence, increase photosynthesis (Savant et al., 1999). The maintenance of photosynthetic activity due to Si fertilization could increase dry matter production (Agurie et al., 1992). At the same time, Si fertilization enhances water use efficiency probably due to the prevention of excessive transpiration.
Tiller number was significantly increased from 166 to 225 tillers per m² as Si rate increased from 0 to 300 kg ha⁻¹, but remained unchanged at higher Si rates (Figure 4). A quadratic equation \( Y = 0.41X^2 - 0.14X + 99.07, R^2 = 0.96 \) provided a good description of the relationship between filled grain number per panicle and Si rate. Filled grain number per panicle increased from 99 to 106 as Si increased from 0 to 450 kg ha⁻¹, but increased slightly at higher Si application rate (Figure 5). 1000-grain weight increased linearly \( y = 0.52X + 27.88, R^2 = 0.78 \) with increasing Si rate (Figure 6).

There were no significant differences for grain N concentration among Si rates. With increasing Si rate from 0 to 450 kg ha⁻¹, grain N uptake increased from 47.4 to 94.8 kg ha⁻¹, but declined slightly at higher Si rate (Figure 7). This was mainly due to greater grain yield at higher silicon rates. Contrary to this result, Wallace (1989) reported that application of N tends to decrease silicon uptake in rice.

**Conclusions**

This experiment illustrated that silicon fertilization and biofertilizer application have positive effect on rice growth and grain yield. Grain yield, regardless of silicon rate, was significantly increased by 27% and 16% when biofertilizer was applied as seed and seedling root dip treatment, respectively, compared to non inoculated treatment. Rice grain yield was significantly increased from 2314 to 4251 kg ha⁻¹ as Si application rate increased from 0 to 450 kg ha⁻¹, but did not significantly increase at higher Si rate.

**References**


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