STUDIES ON THE IMPROVEMENT OF RHIZOBIUM- GREEN GRAM (VIGNA RADIATA (L.) WILCZEK) SYMBIOSIS IN LOW NUTRIENT, ACID STRESS SOILS

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ABSTRACT

A field study was conducted during Kharif seasons of 2003 and 2004 in low nutrient acid stress soil. Seven strains of Rhizobium were compared for their symbiotic performance with the host legume, green gram. The rhizobial strain, VRM3 obtained from the host grown under acid soil performed better than the other strains. This acid tolerant strain demonstrated a comparative advantage of over acid sensitive strains in their ability to nodulate and thereby promoting the growth and yield of green gram. Combined inoculation of this strain with phosphobacteria and plant growth promoting rhizobacteria (PGPR) resulted in improvement of crop yield over single inoculation under acid soil conditions.

INTRODUCTION

Biological nitrogen fixation represents the major source of nitrogen in tropical agricultural soils including those in acid regions. The major N₂ fixing systems are the Rhizobium - legume symbiotic systems, which can play a significant role in improving the fertility and productivity of low nitrogen soils.

Soil acidity is a major problem facing agricultural production in many areas of India and limits legume productivity. Acid soils are characterized by limited availability of nitrogen, phosphorus, potassium, calcium, magnesium, molybdenum, boron and many other micro- and macro- nutrients. Acidic soils with pH less than 5.0 usually have high concentrations of aluminium and manganese. Excess of aluminium and iron in acid soils leads to soil encrustation and hardening problems. Acidic soils constraints nodulation and N₂ fixation in many Rhizobium - legume symbiosis, limiting Rhizobium survival and persistence in soils and reducing nodulation (Ibekwe et al., 1997).

However, Rhizobium exhibit varied responses to acidity. Some Rhizobium form effective symbiosis with their host under acidic stress conditions (Clarke et al., 1993). Hence selection of acid tolerant Rhizobium to inoculate legume hosts under acidic conditions will ensure the establishment of symbiosis and also successful performance. The objective of the present investigation was to select an acid tolerant strain of Rhizobium for green gram and to find out its combined inoculation effect with phosphobacteria and PGPR.

MATERIAL AND METHODS

Field experiments were conducted in the soils of National Pulses Research Centre, Vamban, Tamil Nadu during Kharif seasons of 2003 and 2004. Soil characteristics of the experimental field were pH 4.8, EC-0.1 to 0.2 dsm⁻¹, available N - 14.23 kg.ha⁻¹, P₂O₅ - 39.7 kg.ha⁻¹ and K₂O - 229.4 kg.ha⁻¹ and organic carbon - 0.43%. The study was conducted with green gram var. VBN 3, which is a recommended crop variety for such soil conditions.

The treatments in the field study included the native standard rhizobial strains obtained from different Regional Pulses Research Centres of India. The local rhizobial strain VM 3 was a selection based on nitrogen fixing efficiency and its tolerance to pH of <4.5 under in vitro conditions.
RESULTS AND DISCUSSION

Symbiotic performance of rhizobial isolates in acidic soil: In the studies on the performance of different rhizobial strains, inoculation with each rhizobial strain as well as application of recommended dose of nitrogen fertilizer had improved the nodule number, nodule dry weight, plant growth and grain yield of green gram grown in acidic soil (Table 1). However, strains performed differently in their efficiency in nodule formation and promoting yield. The strains CRM 6 and VRM 3 formed higher number of nodules and with more dry weight than the other strains used in the study. Other strains which grow and nodulate under identical soil conditions performed poorly in soil acidity. Strains of a given species varied widely in their pH tolerance. Van Rossum et al. (1994) compared 12 strains of Bradyrhizobium for their symbiotic performance with groundnut in acidic soil and found that some strains were totally ineffective under acidic stress (pH 5.0 – 6.5) while others performed well under these conditions. The performance of the Rhizobium trifolium – Trifolium pretense symbiosis under acidic conditions is best when the rhizobial strains were isolated from the host legume grown in acidic soil (Lindstrom et al.,1985). In the present study, the better symbiotic performance of the strain, VRM 3 was obvious as the inoculation with this strain resulted in the formation of more number of nodules with

Table 1. Symbiotic performance of isolates of Rhizobium with green gram in acidic soil*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nodules (No./plant)</th>
<th>Nodule dry wt. (mg/plant)</th>
<th>Plant height (cm)</th>
<th>Plant biomass (g/pl)</th>
<th>Grain yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 13-98</td>
<td>3.43</td>
<td>1.89</td>
<td>33.04</td>
<td>3.87</td>
<td>449.3</td>
</tr>
<tr>
<td>M 20-98</td>
<td>5.55</td>
<td>2.42</td>
<td>39.74</td>
<td>3.99</td>
<td>530.3</td>
</tr>
<tr>
<td>DM 100</td>
<td>9.86</td>
<td>4.03</td>
<td>40.66</td>
<td>3.78</td>
<td>475.8</td>
</tr>
<tr>
<td>M-1-00</td>
<td>9.10</td>
<td>4.11</td>
<td>31.41</td>
<td>3.55</td>
<td>475.9</td>
</tr>
<tr>
<td>PMR 2001</td>
<td>7.96</td>
<td>4.16</td>
<td>49.10</td>
<td>3.76</td>
<td>551.2</td>
</tr>
<tr>
<td>VRM 3</td>
<td>13.56</td>
<td>9.83</td>
<td>51.74</td>
<td>4.42</td>
<td>598.4</td>
</tr>
<tr>
<td>CRM 6</td>
<td>11.38</td>
<td>9.95</td>
<td>45.09</td>
<td>3.32</td>
<td>556.3</td>
</tr>
<tr>
<td>20 kg N. ha⁻¹</td>
<td>4.89</td>
<td>0.86</td>
<td>38.11</td>
<td>3.48</td>
<td>421.8</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>5.32</td>
<td>1.03</td>
<td>25.08</td>
<td>2.89</td>
<td>368.0</td>
</tr>
</tbody>
</table>

*CD (P=0.05) 3.21 1.62 1.53 0.44 23.4

*pooled analysis data of 2003 and 2004.
higher dry weight. The performance of the other strains which were obtained from normal soils was poor under soil acidic conditions. Taylor et al. (1991) had reported that the colonization of soil and soybean roots by Bradyrhizobium japonicum may be adversely affected by acidity, an effect which will result in reduced nodulation.

Inoculation with rhizobial strains significantly increased the plant height and dry matter over uninoculated control. The effect of inoculation on dry matter production is equivalent to application of 20 kg. ha\(^{-1}\) fertilizer N. Application of rhizobial strains and fertilizer N increased the grain yield of green gram. Strains behaved differently in acid soil in accentuating the yield of crop. The magnitude of response varied depending on the nature of the strain. The strain VRM showed tolerance to soil acidity as indicated by improved dry matter and grain yield. The growth, nodulation and yield of Vicia faba was improved after inoculation with strains of R. leguminosarum bv. viciae in acid soils (Carter et al., 1994).

The performance of the strain VRM was comparable to the application of 20 kg N.ha\(^{-1}\). The strain which supported the host in nodulation, plant height and accumulation of plant biomass, improved the grain yield indicating a positive correlation between nodulation status of the host and the grain yield.

**Table 2. Effect of combined inoculation of Rhizobium, phosphobacteria and PGPR on green gram**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nodules (No./plant)</th>
<th>Nodule dry wt. (mg/plant)</th>
<th>Plant height (cm)</th>
<th>Plant biomass (g/pl)</th>
<th>Grain yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inoculation</td>
<td>6.29</td>
<td>1.86</td>
<td>36.53</td>
<td>1.07</td>
<td>512.8</td>
</tr>
<tr>
<td>Rhizobium (VRM)</td>
<td>13.13</td>
<td>11.6</td>
<td>40.26</td>
<td>1.74</td>
<td>579.5</td>
</tr>
<tr>
<td>Phosphobacteria (PSB)</td>
<td>7.80</td>
<td>2.43</td>
<td>43.70</td>
<td>1.42</td>
<td>532.5</td>
</tr>
<tr>
<td>PGPR</td>
<td>6.76</td>
<td>2.16</td>
<td>43.55</td>
<td>1.45</td>
<td>514.1</td>
</tr>
<tr>
<td>Rhizobium + PSB</td>
<td>16.20</td>
<td>12.10</td>
<td>50.85</td>
<td>2.03</td>
<td>602.2</td>
</tr>
<tr>
<td>Rhizobium + PGPR</td>
<td>14.87</td>
<td>12.58</td>
<td>50.92</td>
<td>1.83</td>
<td>523.6</td>
</tr>
<tr>
<td>PGPR + PSB</td>
<td>8.41</td>
<td>2.05</td>
<td>45.88</td>
<td>1.77</td>
<td>528.1</td>
</tr>
<tr>
<td>Rhizobium + PSB + PGPR</td>
<td>19.53</td>
<td>13.86</td>
<td>50.92</td>
<td>3.16</td>
<td>651.3</td>
</tr>
</tbody>
</table>

CD (P=0.05) 1.17 1.08 3.11 0.86 36.3


**Synergistic performance of Rhizobium, phosphobacteria and PGPR:** In the present study coinoculation of Rhizobium, with phosphobacteria and PGPR had enhanced the nodulation, plant biomass and grain yield of green gram than single inoculation with each of the biofertilizer. Also the inoculation of Rhizobium with phosphobacteria or PGPR had enhanced the nodulation and the grain yield of green gram than Rhizobium alone (Table 2). Sowmiya (2001) reported that inoculation of Rhizobium with PGPR (Bacillus sp.) and phosphobacteria (Bacillus megatherium var phosphaticum) enhanced the growth, nodulation and yield of black gram.

The application of Rhizobium with phosphobacteria improved the yield of green gram in the present investigation. Phosphorus appears essential for both nodulation and nitrogen fixation. Acid soils have little available phosphorous. Phosphates are rendered unavailable due to formation of insoluble iron and aluminium phosphates. Rhizobial P deficiency is a real possibility especially under acidic conditions. The benefit of phosphate solubilizing bacteria in releasing the single phosphates from bound phosphates and thereby improving the growth, nodulation and yield is well documented in crops like black gram (Prabakaran et al., 1996).
The inoculation of PGPR with Rhizobium was found to increase the yield of green gram. Production of growth hormones, siderophores are said to be the mechanisms by which PGPR help the crop plants. Co-inoculation of soyabean with PGPR with Serratia liquefaciens and Bradyrhizobium japonicum resulted in increased grain yield and grain protein yield (Dashti et al., 1997) of soyabean. The combined inoculation of bioinoculants is not always successful. Kamble et al. (2000) reported that the competitive behaviour of Azospirillum, Pseudomonas fluorescens and phosphobacteria in the rhizosphere of paddy played an important role. Hence selection of strains for combined inoculation becomes necessary.

Strains of Rhizobium appear to vary in their symbiotic efficiency under acidic conditions. Selection of acid tolerant rhizobia to inoculate legume hosts under acidic conditions will ensure the establishment of the symbiosis and also successful performance. Improvement of growth, nodulation and yield of mung bean in low fertility acid soils could be achieved by combined inoculation of Rhizobium with phosphobacteria and plant growth promoting rhizobacteria.

REFERENCES

Prabakaran, J., et al. (1996). 37th Annual Conference of the Association of Microbiologists of India, IIT, Chennai, Dec.4-6, p.120.