SYMBIOTIC DIVERSITY AMONG ACID-TOLERANT BRADYRHIZOBIAL ISOLATES WITH COWPEA

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ABSTRACT

A total of eight acid tolerant strains of bradyrhizobia isolates from indigenous cowpea plants grown in acid soil in Varanasi, Uttar Pradesh, India, were examined for their ability to survive in soil and yeast extract mannitol broth at low pH levels. All these isolates survived in acidic (pH 3.5-6.5) conditions. Survival capacity of rhizobia was higher in soil than in nutrient medium at low pH 3.5-6.5 levels. Symbiotic effectiveness of these strains under polyhouse conditions in sterilized soil of pH 4.5 recorded the highest and lowest symbiotic characters for dry matter production and nitrogen improvement per plant in CR09 and CR20 inoculated plants, respectively. All the examined isolates showed variability in their symbiotic performances. The strain found to be more tolerant to stress were more effective N₂ fixers in symbiosis with cowpea cv. Paiyur1 under acid-soil conditions. Symbiotic variation among different strains showed that there is potential to improve strain performance under stress conditions.

Key words: Acid tolerant, bradyrhizobia, cowpea, symbiotic effectiveness.

INTRODUCTION

In India, cowpea (Vigna unguiculata L. Walp) is cultivated by commercial and subsistence farmers. They provide a valuable source of protein and thereby sustaining the nutritional balances of low income populations (Singh et al., 1997) and also help in maintaining soil health through biological nitrogen fixation by symbiotic rhizobia. Cowpea depends on their symbionts for a large part of their nitrogen requirements for growth and dry matter production. The cowpea symbionts are classified into slow-growers, Bradyrhizobium spp. and fast-growers, Sinorhizobium spp. (Zhang et al., 2007). Selected symbiotically efficient rhizobia were used as inoculants to increase biologically fixed N₂ under field conditions. The introduced strain must compete with highly adapted indigenous rhizobia for legume nodulation under specific physiological and biological soil conditions. Many biotic and abiotic factors affect the persistence of symbiotically effective introduced rhizobial strain in soil. Soil acidity is the one of the factors which restricts production of cowpea through its impact on nitrogen fixation. The failure of nodulation under acid soil conditions is common, especially in soils of pH less than 5. Soil acidity limits symbiotic nitrogen fixation by limiting Rhizobium survival and persistence in soils, as well as reducing nodulation (Ibekwe et al., 1997). High symbiotic effective rhizobial inoculation is a common practice in agricultural legume production (Catroux et al., 2001) which requires survival and establishment of inoculated rhizobia in the soil environment (Da and Deng, 2003). There is no history of inoculation of acid tolerant strains in acidic soil in India. A wide variation in the tolerance to acid-soil conditions have been reported among Bradyrhizobium strains of many agriculturally important legumes from across the world.
MATERIALS AND METHODS

Bradyrhizobial Isolates

Eight acid tolerant bradyrhizobia strains, CR01, CR05, CR07, CR09, CR12, CR15, CR18 and CR20 were isolated from nodules of indigenous cowpea plants grown in acid soil by following the method of Vincent (1970) and were used in the experiments. All strains were maintained on yeast extract mannitol (YEM) medium and transferred to fresh slant every month.

Growth of bradyrhizobial isolates at low pH levels

Soil pH was estimated by suspending 40 g of air dried soil in 100 ml distilled water, after allowing the suspension to stand for 1 h at room temperature. Soil sample and yeast extract mannitol broth were sterilized in autoclave at 121°C for 15 min (JRIC-39E, Osworld India). The pH level of the soil sample (pH 4.5) and medium (pH 6.8) was modified to obtain the required pH values of 3.5, 4.5, 5.5 and 6.5 by adding 1 N HCl or NaOH and no changes in pH were observed after autoclaving. All the bradyrhizobia isolates were multiplied in YEM broth and 1 ml of multiplied rhizobial culture (about $10^8$ rhizobial cells/ml) used as standard inoculum introduced into soil mixture (2.5 g of soil + 1 ml of distilled water) and to YEM broth of low pH levels and YEM broth with pH 6.8 as control for comparison. Standard volume of inoculum also introduced in the YEM Flasks containing treated soil and YEM broth were kept on a rotary incubator shaker (200 rev. min$^{-1}$) (ACM-22064-I, ACMAS Technology India) at 28 ± 2°C for 7 days. The rhizobial growth was determined (cfu ml$^{-1}$) by a plate count technique (Vincent, 1970) using YEM agar plates.

Symbiotic efficiency of rhizobial isolates

Genetically pure and healthy seeds of cowpea cv. Paiyur1 were surface sterilized as explained by Appunu et al. (2005). In brief, seeds were surface-sterilized with acidified mercuric chloride (0.2 % w/v) for 3-5 minutes and 70% ethyl alcohol for 1-2 minutes and then thoroughly rinsed with sterile distilled water for 4-5 times. Sterilized seeds were coated with bradyrhizobia as detailed by Vincent (1970). The untreated seeds were served as control. Seeds were grown in earthern pots containing sterilized soil [pH 4.5; organic carbon (0.78); CEC (11.8 cmol (+) Kg$^{-1}$); EC (<0.23 dSm$^{-1}$); exchangeable Al (cmol (+) Kg$^{-1}$); total N (0.10)] under polyhouse conditions. Each pot was maintained with one healthy seedling. Each treatment was replicated five times. Plants were supplied with water at appropriate times and were maintained to grow till 5 weeks in polyhouse having adequate temperature 30-35 °C, humidity 70-80% and light intensity 1,600-2000 lux. Plants were harvested after five weeks of sowing and data pertaining to symbiotic and vegetative characters were recorded as described previously (Appunu et al., 2005).

Data were subjected to analysis of variance, and means were classified using Duncan’s multiple-range test at the 0.05 probability level.

RESULTS AND DISCUSSION

The bradyrhizobia isolates were characterized to their growth response to low levels of pH 3.5 - 6.5. The results showed that these isolates were tolerant to extreme acidic conditions since they could survive and grow in the low pH, even at 3.5 (Figures 1 and 2). The rhizobia population showed higher level of survival capacity in acid soils than in nutrient broth.
at low levels of pH 3.5 - 5.5. It could be explained that attachment of rhizobial with cations/anions or organic molecules in the soil are one of the reasons for higher growth rate in soil than in nutrient broth. These results are in agreement with those reported earlier that *Rhizobium* strains which survived in the acid soil cannot grow on a nutrient medium with pH as low as that of the soil (Asanuma and Ayanaba, 1990). Rhizobial strains of a given species vary widely in their pH tolerance (Zahran et al., 1999; Appunu et al., 2005). The fast growing *Rhizobium* strains have generally been considered less tolerant to acid pH than slow growing strains of *Bradyrhizobium* (Graham et al., 1994). However, Mpepereki et al. (1997) reported that both fast- and slow-growing *Bradyrhizobium* strains of *Vigna unguiculata* are tolerant to pH values as low as 4.0. Rhizobia adopt various mechanisms to survive in the acid soil conditions (Zahran et al., 1999).

**Figure 01:** Effect of different level of acidic conditions (pH levels) on the growth of bradyrhizobial isolates of cowpea in soil.

**Figure 02:** Effect of different level of acidic conditions (pH levels) on the growth of bradyrhizobial isolates of cowpea in yeast extract mannitol broth.
The effect of inoculation of eight acid tolerant bradyrhizobia isolates on cowpea cv. Paiyur1 is presented in Table 1. All strains formed nodules and a significant variation was noticed in nodule number, dry weight and nitrogenase activity, dry matter production and nitrogen accumulation per plant. Highest nodule frequency, dry weight and nitrogenase activity were found in the plant inoculated with the strain CR09. However, inoculation of strains CR01, CR05, CR07, CR09 and CR12 also resulted in significantly high dry weight. In comparison to control, an increase of 5.74-39.08% in total plant dry weight was observed with strain inoculated treatments. Maximum enhancement in total plant dry matter (39.08%) was noticed with the strain CR09, followed by CR07 (30.34%), CR12 (28.04%), CR05 (19.77%) and CR01 (14.71%). Percent increase of nitrogen content was also observed by the inoculation of strains. The highest nitrogen content (2.40%) per plant was estimated in strain CR09 inoculated plants, while the lowest (1.90%) was with CR18 and CR20 inoculated plants, which was 33.33 and 5.55% higher than that of control plants, respectively.

Table 01: Symbiotic effectiveness of acid-tolerant bradyrhizobial isolates on cowpea cv. Paiyur1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total no. of nodules</th>
<th>Dry weight of nodules (g)</th>
<th>Nodules nitrogenase activity (µmol g⁻¹ plant⁻¹)</th>
<th>Total dry matter production (g)</th>
<th>N content improvement of plant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR01</td>
<td>27.21c</td>
<td>0.19b</td>
<td>7.95b</td>
<td>4.99b</td>
<td>2.0</td>
</tr>
<tr>
<td>CR05</td>
<td>32.03b</td>
<td>0.17c</td>
<td>8.08b</td>
<td>5.21b</td>
<td>2.2</td>
</tr>
<tr>
<td>CR07</td>
<td>34.10b</td>
<td>0.17c</td>
<td>9.42a</td>
<td>5.67a</td>
<td>2.2</td>
</tr>
<tr>
<td>CR09</td>
<td>48.54a</td>
<td>0.22a</td>
<td>10.36a</td>
<td>6.05a</td>
<td>2.4</td>
</tr>
<tr>
<td>CR12</td>
<td>42.88a</td>
<td>0.20b</td>
<td>9.76a</td>
<td>5.57a</td>
<td>2.3</td>
</tr>
<tr>
<td>CR15</td>
<td>37.01b</td>
<td>0.23a</td>
<td>7.91b</td>
<td>4.85c</td>
<td>2.0</td>
</tr>
<tr>
<td>CR18</td>
<td>22.32d</td>
<td>0.14d</td>
<td>4.96c</td>
<td>4.73c</td>
<td>1.9</td>
</tr>
<tr>
<td>CR20</td>
<td>21.95d</td>
<td>0.11e</td>
<td>4.41c</td>
<td>4.55c</td>
<td>1.9</td>
</tr>
<tr>
<td>Control</td>
<td>0.00e</td>
<td>0.00f</td>
<td>0.00d</td>
<td>4.35c</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Those not followed by common superscript letters differ significantly at the LSD probability of <0.05

This study showed that inoculation of acid-tolerant isolates CR01, CR05, CR07, CR09 and CR12 leads to good nodulation, dry matter accumulation and improvement of nitrogen content cowpea. These isolates exhibited great diversity in their symbiotic performance and a few of them accumulated considerably high total plant dry weight and percent nitrogen content. Differential symbiotic performance of Bradyrhizobium isolates has already been reported (Zhang et al., 2002; Meghvanshi et al., 2005; Appunu et al., 2008). In most cases, pH sensitive stage in nodulation occurs early in the infection process and that Rhizobium attachment to root hairs is one of the stages affected by acidic conditions in soils. Only one of the symbionts needed to be acid tolerant for good nodulation to be achieved at pH 4.5 (Vargas et al., 1988). van Rossum et al. (1994) reported that inoculation of acid
tolerant Bradyrhizobium strains under acid-soil conditions improves the groundnut vegetative characters and yields. Selection of acid tolerant rhizobia to inoculate legume hosts under acid conditions will ensure the establishment of symbiosis and also successful performance (Correa and Barneix, 1997). However, the success or failure of inoculation depends on the competitive nodulation ability against indigenous bradyrhizobia under natural conditions. Graham (1992) and Carter et al. (1994) reported the existence of positive correlation between acid tolerance in laboratory and competitive nodulation on acidic soils.

**CONCLUSIONS**

In conclusion, four bradyrhizobia isolates CR07, CR09, CR01 and CR05 exhibited high growth at low pH levels and also showed better symbiotic performance in acid soils under laboratory conditions. These isolates could become useful inoculants in acid soils if they are superior in competitiveness under natural ecological conditions in the field.

**REFERENCES**


