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Full Length Research Paper

Symbiotic effectiveness of acid-tolerant *Bradyrhizobium* strains with soybean in low pH soil

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Eight acid tolerant strains of *Bradyrhizobium* isolated from soybean plants grown on acid soils in Madhya Pradesh, India, were examined for their ability to survive in soil and YEMB at low pH levels. All the tested isolates survived in acidic (pH 4 -6) conditions and their survival capacity was higher in soil than in nutrient medium at same levels of low pH. Variation among different strains showed that there is potential to improve strain performance under stress conditions. Further, symbiotic effectiveness of these strains was determined under the polyhouse conditions in sterilized soil (pH 4.5). Highest and lowest symbiotic characters, dry matter production and nitrogen improvement per plant were observed in PSR001 and NSR008 inoculated plants, respectively. All the examined isolates showed considerable variability in their symbiotic performance. The strain found to be more tolerant to stress were more effective N_2 fixers in symbiosis with soybean in acid-soil conditions.

Key words: Symbiotic effectiveness, *Bradyrhizobium* strains, pH, soybean.

INTRODUCTION

Soybean (*Glycine max*) has played a significant contribution to yellow revolution in India (Chauhan and Joshi, 2005), and as a food plant it forms a part of routine diet of the people (Tiwari, 1999). Soybean depends on their symbionts for a large part of their nitrogen requirements for growth and increased dry matter production. The soybean symbionts are classified into slow-growers, *Bradyrhizobium* spp. and fast-growers, *Sinorhizobium* spp. (van Berkum and Eardly, 1998). Soil acidity restricts production of soybean 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 (Taylor et al., 1991; Bayoumi et al., 1995; 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 (Ozawa et al., 1999; Da and Deng, 2003). There is no history of inoculation of acid tolerant strains in acidic soil in this country. A wide variation in the tolerance to acid-soil conditions have been reported among *Bradyrhizobium* strains of many agriculturally important legumes from various countries (Ayanaba et al., 1983; Graham et al., 1994; van Rossum et al., 1994; Bayoumi et al., 1995; Ozawa et al., 1999; Raza et al., 2001) and in India (Appunu et al., 2005). Keeping in view the role of acid-tolerant strain, we report here the survival of *Bradyrhizobium* in acidic conditions and their symbiotic effectiveness in low pH soils.

MATERIALS AND METHODS

Rhizobial Isolates

High acid tolerant *Bradyrhizobium* strains, ASR002, ASR003, PSR007, PSR011, TSR001, TSR004, NSR005 and NSR008 isolated from nodules of acid and non-acid soil grown plants

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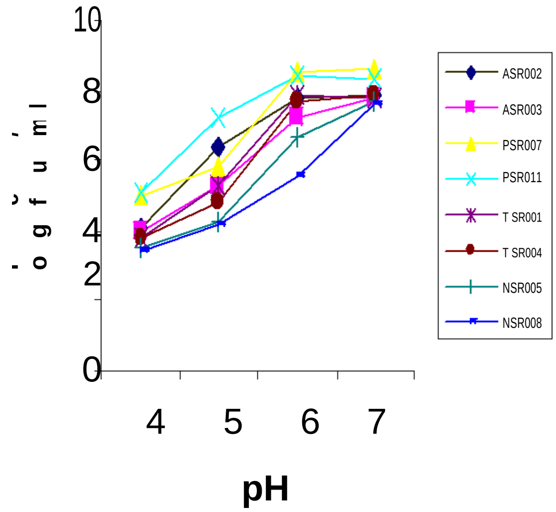

Figure 1. Effect of different pH values on the growth and survival of various *Bradyrhizobium* isolates in soil. Values are means of three samples. LSD = 0.43 ($P < 0.05$) for the interaction between rhizobial strains and pH.

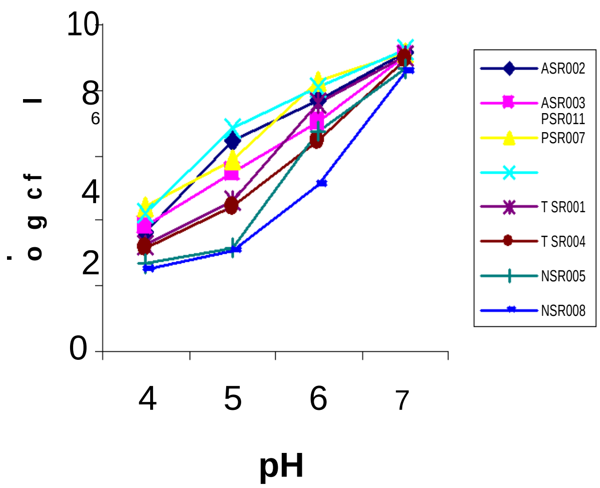

Figure 2. Effect of different pH values on the growth and survival of various *Bradyrhizobium* isolates in YEMB. Values are means of three samples. LSD=0.57 ($P < 0.05$) for the interaction between rhizobial strains and pH.

(Appunu et al., 2005) were used. All strains were maintained on yeast extract mannitol medium (Vincent, 1970) and transferred to fresh slant every month.

Survival of rhizobial isolates at various low pH values

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 (YEMB) were sterilized at 121°C for 15 min. The pH level of the soil sample (pH 4.5) and medium (pH 6.8) was modified to obtain the required values of 4, 5, 6 and 7 by using 1 N HCl or NaCl and no changes in pH were observed after autoclaving. The rhizobial culture 0.1 ml (about 10^8 rhizobial cells) used as standard inoculum introduced into soil mixture (2.5 g of soil plus 1 ml of distilled water) and to YEM broth. Flasks were kept on a rotary incubator shaker (150 rev. min^{-1}) at 28°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

Surface sterilized bold and healthy acid tolerant 'KB 79' soybean seeds were coated with *Bradyrhizobium* (Vincent, 1970). Treated seeds were grown in earthern pots containing sterilized soil [pH 4.5; organic carbon (0.93); CEC (12.8 cmol (+) Kg^{-1}); EC (<0.20dSm^{-1}); exchangeable Al (cmol (+) Kg^{-1}); total N (0.09)] under polyhouse conditions. Each pot was maintained with one healthy soybean plant and untreated seeds were served as control. 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 16,000-2000 lux. Plants were harvested after 5 weeks of sowing and data pertaining to symbiotic and vegetative characters were recorded as described previously (Appunu et al., 2005).

Data was analyzed using completely randomized design. From analysis of variance LSD was used to make comparisons among the means at p (0.05) level of significance.

RESULTS AND DISCUSSION

The *Bradyrhizobium* strains were characterized with respect to their survival and growth response to low pH levels. The results indicated that the isolates were tolerant to extreme low pH since they could survive and grew in the low pH, even at 4.0 (Figures 1 and 2). The rhizobial population showed varied and higher survival capacity in acid soils than in YEM broth at same low pH level. It may be that attachment of rhizobial with cations/anions or organic molecules in the soil are one of the reasons for higher survival rate in soil than in YEM broth. *Rhizobium* strains that survived in the acid soil cannot grow on a nutrient medium with a pH as low as that of the soil from which the strains were isolated (Asanuma and Ayanaba, 1990). Strains of a given species vary widely in their pH tolerance (Zahran et al., 1999). The fast growing *Rhizobium* strains have generally been considered less tolerant to acid pH than slow growing strains of *Bradyrhizobium* strains (Graham, 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).

The effect of inoculation of eight acid tolerant *Bradyrhizobium* isolates on soybean cv. KB 79 is presented in Table 1. All strains formed nodules on the tested cultivar 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 PSR011. However, inoculation of other strains viz., TSR001, TSR004, PSR007 and ASR003 also resulted in

Table 1. Symbiotic effectiveness of acid-tolerant *Bradyrhizobium* strains on soybean cultivar KB79.

Treatment	Total no. of nodules	Dry wt. of nodules (g)	Nitrogenase activity of nodules*	Total plant dry wt. (g)	N content of plant (%)
ASR002	$24.21^{c}\pm2.47$	$0.17^{b}\pm0.01$	$7.90^{b}\pm0.48$	$5.01^{b}\pm0.21$	1.8
ASR003	$29.03^{b}\pm1.97$	$0.15^{c}\pm0.02$	$8.03^{b}\pm0.62$	$5.28^{b}\pm0.17$	2.0
PSR007	$31.10^{b}\pm2.28$	$0.15^{c}\pm0.02$	$9.37^{a}\pm0.33$	$5.67^{a}\pm0.19$	2.0
PSR011	$45.54^{a}\pm1.51$	$0.20^{a}\pm0.03$	$10.31^{a}\pm0.22$	$6.03^{a}\pm0.20$	2.2
TSR001	$39.88^{a}\pm1.32$	$0.18^{b}\pm0.01$	$9.71^{a}\pm0.58$	$5.59^{a}\pm0.11$	2.1
TSR004	$34.01^{b}\pm2.07$	$0.21^{a}\pm0.02$	$7.85^{b}\pm0.69$	$4.85^{c}\pm0.18$	1.8
NSR005	$19.32^{d}\pm2.15$	$0.12^{d}\pm0.01$	$4.91^{c}\pm0.52$	$4.73^{c}\pm0.17$	1.7
NSR008	$18.95^{d}\pm2.52$	$0.09^{e}\pm0.03$	$4.35^{c}\pm0.41$	$4.55^{c}\pm0.12$	1.7
Control	0.00	0.00	0.00	$4.35^{c}\pm0.14$	1.6

*Values are given in µ mol C_2H_2 reduced h^{-1} $plant^{-1}$.
Data are means of five replicates ± SE.
Same letters are not significantly different at LSD P<0.05 level.

significantly high nodule number and dry weight in that order. In comparison to control, an increase of 4.60-38.62% in total plant dry weight was observed with strain inoculated treatments. Maximum enhancement in total plant dry weight (38.62%) was noticed with the strain PSR011, followed by PSR007 (30.34%), TSR011 (28.58%), ASR003 (21.37%) and ASR002 (15.17%). Percent increase of nitrogen content was also observed by the inoculation of strains. Highest nitrogen content (2.20%) per plant was estimated in strain PSR011 inoculated plants, while lowest (1.70%) was with NSR005 and NSR008 inoculated plants, which was 37.50 and 6.25% higher than that of control plants, respectively.

This study shows that inoculation of acid-tolerant strains leads to good nodulation, dry matter accumulation and nitrogen content improvements of soybean in acid soils. These strains 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). In most of the 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.

In conclusion, four *Bradyrhizobium* strains PSR007, PSR011, ASR002 and ASR003 exhibited high survival and 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.

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