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Symbiotic effectiveness of *Bradyrhizobium japonicum* in acid soils can be predicted from their sensitivity to acid soil stress factors in acidic agar media

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ABSTRACT

In acid soil, low pH, reduced availability of nutrients, and toxicity of Al and Mn limit plant growth and the survival and effectiveness of rhizobia. The symbiosis between legumes and rhizobia is particularly sensitive to acid soil stress. A pot experiment evaluated whether *Bradyrhizobium japonicum* strain growth on acidic agar media would predict ability to colonize the rhizosphere and form effective nodules in acidic soils. Three Indonesian strains of *B. japonicum* with similar effectiveness at neutral pH in sand culture but with different tolerance of acid soil stress factors in agar media, and an acid-tolerant commercial strain (CB1809) of comparable effectiveness, were tested in three acid soils using the Al tolerant soybean (*Glycine max* cv PI 416937). At 7 days after inoculation all strains had achieved large rhizosphere populations, but by day 14 the rhizosphere population of the acid-sensitive strain had decreased, while the more acid-tolerant strains increased. The acid-tolerant strains had significantly greater nodulation and symbiotic effectiveness than plants inoculated with the acid-sensitive strain. Laboratory prescreening of *B. japonicum* for acid, Al and Mn tolerance in acid media successfully identified strains which were symbiotically competent in low pH soils.

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1. Introduction

In low pH soils, stresses such as high concentrations of soluble Al and Mn, and reduced availability of Ca, Mg, P and Mo reduce plant growth, and the survival and symbiotic effectiveness of *Bradyrhizobium*. Amelioration of these problems, especially in the subsoil, is difficult and generally not economically feasible (Foy et al., 1974). An alternative approach, which may be more cost effective, is to select varieties (Sartain and Kamprath, 1978; Sapra et al., 1978) and screen *Bradyrhizobium* strains (Taylor et al., 1991) tolerant of acid soil stresses.

Strains differ in their ability to grow on agar media formulated to simulate stresses associated with acid soils. Generally *Sinorhizobium* strains, eg those nodulating *Medicago sativa*, are particularly sensitive to acidity although more tolerant strains have been selected (eg Howieson et al., 1988, 1992; Reeve et al., 1993). Variation in tolerance of acidity *in vitro* has been demonstrated for other rhizobia (reviewed by Dilworth et al., 2000; Poole et al., 2008) such as those nodulating cowpea (*Vigna unguiculata* (L.) Walp.) (Keyser and Munns, 1979; De Carvalho et al., 1981; Hartel and Alexander, 1983), soybean (*Glycine max* (L.) Merr.) (Ayanaba et al., 1983; Hungria et al., 2001; Musiyiwa et al., 2005; Indrasumunar, 1999), beans (*Phaseolus vulgaris* (L.)) (Lowendorf and Alexander, 1983; Karanja and Wood, 1988; Vargas and Graham, 1988; Frey and Blum, 1994), tree legumes (Da Silva and Franco, 1984) and *Mesorhizobium loti* nodulating lotus (*Lotus pedunculatus* Cav.)(Wood et al., 1988; Rickert et al., 2000). For rhizobia strains nodulating soybeans in Brazil there was little correlation between the pH of the soil of their origin and relative acid tolerance *in vitro* (Hungria et al., 2001). Both *in vitro* and in acid soil (pH 5), some *Rhizobium tropici* strains tolerated acid soil related stresses and higher temperatures and were more competitive than other species nodulating beans (eg Frey and Blum, 1994; Graham et al., 1994; Anyango et al., 1995). However, few studies extended their laboratory findings to soil culture to see if *in vitro* response to acid soil related stresses matched symbiotic performance in acid soil. We examine the relationship for bradyrhizobia nodulating soybean.

Conflicting results have been reported for a number of rhizobia on the relationship between *in vitro* acid tolerance and performance in acid soil. With strains of *Rhizobium leguminosarum* bv *trifoli*, Thornton and Davey (1983, 1984) found a positive correlation

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between growth at low pH in laboratory media and nodulation in acid soils, whereas Bromfield and Jones (1980) and Gemell and Roughley (1993) found no such relationship and the acid-tolerant strains, although dominating in the rhizosphere, formed very few nodules in the acid soil from which they were isolated. A similar contrast has been reported for *R. leguminosarum* bv *phaseoli*. Vargas and Graham (1989) found in sand culture that at acid pH the competitive ability in nodulation of the acid-tolerant strain in competition with an ineffective, acid-sensitive strain varied with the cultivar and pH. Earlier, Lowendorf and Alexander (1983) found that a strain of *R. leguminosarum* bv *phaseoli* that was tolerant of acidity *in vitro* survived better in two non-sterile acid soils than an acid insensitive strain but did not survive well in a sandier acid soil even in the rhizosphere of a bean plant.

This paper compares the performance of a number of Indonesian *Bradyrhizobium japonicum* strains which differ in their tolerance of acidity on agar medium, as inocula for soybean in three acid soils. The soils used were selected to present a range of Ca, Al and Mn status and contained no indigenous population of *B. japonicum* capable of nodulating soybean. The hypothesis tested was that strains which grew on a laboratory agar, formulated to impose stresses representative of an acid soil, would survive and nodulate the host plant better in acid soils than an acid-sensitive strain.

2. Materials and methods

2.1. Bradyrhizobium strains

In a previous study (Indrasumunar, 1999), 14 strains of *B. japonicum*, classified as either tolerant or sensitive to acid soil stress on agar medium, were tested for their symbiotic effectiveness in sand culture (neutral pH). The *in vitro* selection method involved comparison of the growth of strains in four agar screening media previously developed for selection of acid-tolerant rhizobia. The media differed in the number of acid soil stresses imposed (pH, low calcium (Ca) and phosphorus (P), high aluminium (Al) and manganese (Mn)). Survival of *B. japonicum* in the agar media was then compared with that in two acid soils. A repeat stab inoculation method which provided a declining range of inoculum cell number to 10^3 cells per stab was used to assess the daily growth of the strains on the screening media at 5 pH levels (3.8, 4.2, 4.5, 5.0, and 6.8). These *B. japonicum* strains were part of the Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor collection and were obtained from sites throughout Indonesia. Three strains from this collection, that have different tolerance to acid agar media but equal effectiveness in sand culture, were chosen for this experiment; FCB152 (acid-tolerant), FCB230 (moderately-tolerant) and FCB179 (acid-sensitive). In addition, the acid-tolerant commercial strain CB1809 was included as a reference inoculant.

2.2. Soil characteristics

The A horizon of three acid soils with no previous history of soybean cropping was collected from southeast Queensland. Soils were air-dried and sieved to 2 mm diam before being analyzed for soil pH (1:2.5 H_2O); exchangeable bases extracted by 0.1 M $BaCl_2$/0.1 M NH_4Cl (Gillman and Sumpter, 1986); exchangeable Al and Mn extracted by 1 M KCl (soil:solution = 1:10); extractable Al and Mn extracted by 0.01 M $CaCl_2$ (soil:solution = 1:2) (Hoyt and Nyborg, 1971a,b). Elemental concentrations in the extracts were determined using inductively coupled plasma atomic emission spectroscopy (ICPAES). Ammonium and nitrate extracted by 2 M KCl was measured by steam distillation (Bremner and Keeney, 1965). Destructive harvests were undertaken for plant growth parameters at days 28 and 56 and the soil was sub-sampled at these times for chemical analysis. Chemical characteristics of the soils are presented in Table 1. All three soils were Al toxic, with $CaCl_2$ extractable Al concentration generally exceeding 10 $\mu g\,g^{-1}$. Using this extractant, Bell (1996) reported yield reduction in soybean at 0.7 $\mu g\,g^{-1}$ while Slattery and Coventry (1993) and Slattery et al. (1995) found highly toxic Al levels for wheat to be between 2 and 5 mg/kg. None of the soils were strongly Mn toxic, with all soils showing $CaCl_2$ extractable levels lower than the threshold of 10 $\mu g\,g^{-1}$ suggested by Bell (1996).

The population of naturalized *Bradyrhizobium* capable of nodulating soybean in each of the soils was determined by a soil dilution, plant infection assay using *Glycine ussuriensis* Regel and Maack (Brockwell et al., 1975) as described below.

2.3. Bradyrhizobia colonization and symbiotic effectiveness

Root colonization and symbiotic effectiveness of the *B. japonicum* strains was evaluated utilizing the acid-tolerant soybean cultivar PI 416937. Seeds were sterilized in 90% ethanol for 30 s and 1.2% $HClO_4$ for 4 min, rinsed 7 times with sterile deionized water, then imbibed for 2 h. Five seeds were planted in each pot and inoculated with diluted broth culture (1-mL) of acid-tolerant or acid-sensitive strains at the rate of 10^6 cfu seed^{-1}. Control pots were planted with uninoculated seed. For the root colonization study 10-cm diam white plastic pots (1.0 kg soil) were used, while the effectiveness trial was conducted in 14-cm diam pots (1.2 kg soil). At 14 days after planting seedlings were thinned

Table 1
Some chemical properties of acid soils used in this trial.

Soil/day	pH	Exchangeable cations ($cmol_{(+)}\,kg^{-1}$)						Extractable ($\mu g\,g^{-1}$)			
		Ca	Mg	Na	K	Al	Mn	Al	Mn	NH_4	NO_3
Soil 1											
Day 0	4.62	0.24	0.20	0.16	0.44	1.48	0.014	10.8	6.70	148	13.8
Day 28	4.01	0.23	0.16	0.11	0.17	1.84	0.012	31.2	6.22	16.6	4.9
Day 56	4.52	0.23	0.13	0.08	0.07	1.78	0.010	nd	nd	4.4	2.3
Soil 2											
Day 0	4.58	0.84	0.52	0.07	0.28	1.84	0.009	20.6	4.44	7.7	2.3
Day 28	4.66	0.82	0.51	0.05	0.17	2.00	0.008	20.8	3.98	5.8	3.3
Day 56	4.74	0.71	0.41	0.07	0.11	1.84	0.008	nd	nd	6.3	2.9
Soil 3											
Day 0	4.38	1.63	3.69	1.79	0.53	1.84	0.016	10.0	4.32	10.1	8.8
Day 28	4.44	1.56	3.26	1.49	0.34	1.87	0.016	8.8	3.68	13.5	1.8
Day 56	4.68	1.46	2.77	0.51	0.18	1.92	0.014	nd	nd	13.6	4.0

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