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Tolerance of Soybean Rhizobia to Soil Acidity [1]

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ABSTRAK

Beberapa bakteria pembentuk nodul tidak tahan kepada tanah asid. Satu kajian telah dilakukan untuk menentukan kesesuaian Rhizobium japonicum *kepada keasidan tanah dan daripada itu menjadi pengikat N_2 yang berkesan dalam kacang soya (Glycine max (1.) Merr) yang ditanam pada tanah asid. Nodul-nodul telah didapatkan daripada pokok kacang soya yang ditanam di ladang selama sekurang-kurangnya 5 tahun dengan keadaan pH hampir 5, 6 atau 7. Daam eksperimen faktorial bahan-bahan nodul ini telah disuntik kepada kacang soya yang ditanam di tanah yang sama dengan sumber nodul tersebut diambil, tetapi telah diasapkan dengan metil bromida. Aktiviti nitrogenase (penurunan $C_2H_2 - C_2H_4$) dan berat tumbuhan telah digunakan sebagai penilaian bagi kesan perlakuan. Hasilnya mungkin menunjukkan bahan bakteria pembentuk nodul tidak boleh mensesuaikan diri kepada pH tanah. Pokok-pokok kacang soya yang hidup pada pH 6 adalah yang paling baik sama ada bahan inokulum diambil dari tanah ber pH 5, 6 atau 7. Di dalam tanah berasid inokulum dari tanah ber pH 5 tidak menunjukkan hasil yang lebih baik jika dibandingkan dengan inokulum-inokulum dari tanah yang mengandungi pH 6 atau 7.*

ABSTRACT

Some nodule bacteria do not tolerate acid soils. A study was initiated to determine if Rhizobium japonicum *could adapt to soil acidity and therefore become more effective N_2-fixers in association with soybeans (Glycine max L. Merr.) grown in acidic soils. Nodules were obtained from soybeans grown in fields where the crop had been planted for at least 5 years in soil with pH near 5, 6 or 7. The nodular material was used for inoculation of soybeans in a factorial experiment grown in methyl bromide fumigated soil obtained from the same fields as the inoculum. Nitrogenase activity ($C_2H_2 - C_2H_4$ reduction) and plant weight were used for evaluating the treatment effects. The results seem to indicate that the nodular bacteria did not adapt to a given soil pH. Soybeans grown in soil at pH 6 grew best regardless of whether inoculum was from plants grown at pH 5, 6 or 7. In acid soil, inoculum from soils at pH 5 was no better than those from pH 6 or 7.*

INTRODUCTION

Tolerance of rhizobia and legumes to different soil environments is critical for symbiotic N_2-fixation. Strains of rhizobia have been shown to differ in their abilities to tolerate acid soils, and in some strains *(Vigna unguiculata)* tolerance to acidity may be associated with

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symbiotic effectiveness in acid soils (Keyser *et al.*, 1979). The purpose of this research was to determine symbiotic effectiveness of *Rhizobium japonicum* from soybean nodules grown under different soil pH conditions when inoculated factorially to soybeans *Glycine max* (L. Merr.) grown in soil from the same sites. It was hypothesized that each soil environment may be populated with an indigenous strain of rhizobia that would show superior adaptation characteristics to that specific soil environment when compared with introduced isolates indigenous to other soil environments.

MATERIALS AND METHODS

Cecil soils (Typic Hapludults) were obtained from three field sites where soybeans (cv. 'Wright') had been grown continuously for at least five years with lime variables the only difference in treatment. Soil pH (1 : 1 soil : water) of each field was 5, 6 and 7). When the soil was later brought into the greenhouse, the pH was 5.3, 6.2 and 6.8. Since original soil pH determinations were 5, 6 and 7, hereafter reference will be made to these values.

The experiment was established in the greenhouse using the three soils of differing pH. No additional fertilizer was applied as the soils had been adequately fertilized with P and K while in the field. Treatments were factorially arranged with four replications in a randomized block design. Soils were screened and 7.2 kg (dry wgt.) placed into 20 cm diameter drained plastic pots. Pots and soil were fumigated with methyl bromide, and soybeans planted ten days after fumigation. One week after emergence plants were thinned to four per pot.

Soybean seeds (cv. 'Wright') and nodules were surface sterilized with a 5-minute exposure to 1 : 3 v/v sodium hypochlorite (5.25%) to water followed by the addition of 95% ethyl alcohol (5 min.), and then rinsed five times in deionized sterile water. The seeds were inoculated with rhizobia from crushed nodular material that was previously obtained from soybean fields with soil pH values of 5, 6 and 7. Numbers of rhizobia applied per seed were not determined, but the rate exceeded that required for effective inoculation. To prevent contamination of pots during watering, sterile (8 mm diameter) glass tubes were inserted into the middle of each planted pot. Heat sterilized sand (24 hours at 200°C) was mixed with a paraffin: benzene solution (1 : 100 w/v), allowed to dry, and added to the surface of each pot to 2 cm depth according to Vincent (1970). Sterile water was added by gravity flow via sterile rubber tubing leading from the water source. The flow rate was controlled by 20 gauge needles inserted through a rubber septum placed into the glass tube of each pot.

Plants were sampled after 7 weeks when plants were in the bloom stage. The roots and some adhering soil were removed from the pots and placed into 0.94 l jars with lids fitted with rubber septa. Fifty ml of air was evacuated from each jar and replaced with 50 ml acetylene. After 1 hour incubation, 10 ml of gas was removed from each jar and injected into 10 ml vacutainers. For C_2H_4 analysis, one ml of gas from the vacutainers was injected into a Varian model 2400 gas chromatograph equipped with a flame ionization detector, using a 3 mm I.D. column packed with Porapak R (80 – 100 mesh). The injection chamber, oven and detector temperatures were 120, 60 and 150°C, respectively. The helium carrier gas flow rate was 50 ml/min.

Plant shoot, root and nodular materials from the above treatments were dried at 70°C for 24 hours (after washing roots), and weighed. Extractable Al was measured by shaking 5 g soil in 40 ml 1 mol l^- KCl for 30 minutes and analyzing by atomic absorbtion.

RESULTS

Soybean plants grown in soil at pH 6 gave higher nitrogenase activity (C_2H_2 reduction) than those grown at pH 5 or 7 (Table 1) regardless of the inoculant used. Non-inoculated plants gave lower mean nitrogenase activity than inoculated plants. Inoculum from field grown plants of pH 5 gave slightly higher nitrogenase activity than inoculum from pH 7 when tested on soybeans grown at pH 6. It was not statistically higher than the inoculum from pH 6. This was not true for soybeans grown at pH 5 or pH 7 (Table 1).

TABLE 1

Mean nitrogenase activity ($C_2H_2 - C_2H_4$ reduction) for soybeans grown for 7 weeks in soil with different reactions and inoculated with nodular material from plants grown in soil with different acidity levels

A. Acetylene reduction by soybeans grown in three soils

Soil pH	Acetylene reduction (μ moles/pl/hr)
5	0.9
6	22.9
7	5.9
LSD (0.05)	3.2

B. Acetylene reduction by soybeans treated with the inoculant from soils with different pH values

Inoculant	Acetylene reduction (μ moles/pl/hr)
From nodules of soil pH 5	13.2
From nodules of soil pH 6	12.0
From nodules of soil pH 7	10.4
Uninoculated	4.0
LSD (0.05)	3.7

C. Mean acetylene reduction for plant x inoculant interaction

Plant x inoculant		Acetylene reduction (μ moles/pl/hr)
Soil pH where plants were grown	Soil pH of inoculant nodules	
*6	5	34.2
6	6	28.8
6	7	22.6
7	6	7.0
7	7	7.0
6	N	6.1
7	N	5.7
7	5	4.0
5	7	1.8
5	5	1.5
5	6	0.4
5	N	0.1
LSD (0.05)		6.4

*Plants grown in pH 6 oil inoculated with nodules from plants grown at pH 5, etc. N refers to non-inoculated.

TABLE 2
Mean shoot, root and nodule dry weights for soybeans grown for 7 weeks with different soil reactions

Soil reaction	Shoot dry wgt. gm/plant	Root dry wgt. gm/plant	Nodule dry wgt. gm/plant
pH 5	1.2	1.0	0.03
pH 6	2.2	1.5	0.4
pH 7	1.6	1.3	0.2
LSD (0.05)	0.3	0.3	0.01

Shoot, root and nodule dry weights indicated that there were significant differences caused by soil pH (Table 2); however, there was no soil pH x inoculant interaction. Dry weights were higher for plants grown in soil at pH 6 than at pH 5 or 7. All plants were nodulated, but nodules of plants from soils with pH 5 were much smaller than the others. Extractable Al for the soils at pH 5, 6 and 7 were 15, 6 and 4 μg^{-1}, respectively.

DISCUSSION

These data fail to confirm adaptation of *R. japonicum* to soil reaction conditions. No evidence is presented that demonstrates greater compatability between soybeans grown in soils with a certain pH and corresponding rhizobia that may have been adapted to these soils. There are a number of factors that affect N_2-fixation by legumes in acid soils (Mulder and Van Veen, 1960). The number of compatible rhizobia in the rhizosphere and the degree of infection of the roots by the bacteria are important factors which are controlled by environmental conditions such as soil pH. Keyser and Munns (1979), using a liquid media rapid screening procedure, concluded that in acid soils, Al toxicity and acidity itself were probably more important in limiting rhizobial growth than Mn toxicity or Ca deficiency. Their laboratory study included 10 strains of *R. japonicum* tolerant of pH 4.8.

Damirgi *et al.* (1967) observed that serogroups 123, 135, 31 and 3 of *R. japonicum* were present in Iowa soils, with 123 and 135 being dominant. Serogroup 123 was found to be the dominant strain in acid soils and 135 in alkaline soils. No relationship between observed distribution of strains and their symbiotic effectiveness was shown. A number of studies have been made on tolerance of rhizobia to acid conditions in liquid and agar culture (2, 9, 10). Munns *et al.* (1979) showed that among 40 rhizobial strains for mungbean (*Vigna radiata* L.), there was a large variation in acid tolerance. A few strains failed to nodulate at pH 5.0, about half were moderately sensitive to pH 5, and the remainder were tolerant with some strains combining high tolerance with high effectiveness. Munns *et al.* (1981) found that with soybeans, unlike other legumes, poor growth in acid soil might not be due to nodulation failure but to poor soybean growth caused by Al toxicity to the host plant. They suggested that efforts to improve acid tolerance should be directed toward the plant and not the bacteria. The relatively high extractable Al found in our test soils with pH 5 is very likely a contributing factor to poor soybean growth in this soil.

The reason for lower growth of soybeans in the pH 7 soil than in the pH 6 soil may be due to Zn deficiency. Payne (1983) found that Zn application improved soybean yield on this soil at the same farm when limed to near pH 7.

We agree with Munns *et al.* (1981) in that soybean rhizobia seem to be much more tolerant of acid soils than the soybean plant.

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