Indian Journal of Agricultural Sciences **80** (12): 1105–8, December 2010

Nodulation pattern and resident rhizobial population of field-grown pea (*Pisum sativum*) from acid soils of Assam*

DHRUBA JYOTI NATH [1], BIBHA OZAH[2], RAJEN BARUAH[3] and DIPTY KUMAR BORAH[4]

Assam Agricultural University, Jorhat 785 013

Received: 30 December 2009; Revised accepted: 14 September 2010

Key words: Field pea, Nodule number, Nodule dry weight, MPN, Nodule score

The significance of root nodule-forming bacteria collectively known as rhizobia, as potential microbial inoculants has been convincingly established. Besides nitrogen (N) fixation, rhizobia have been increasingly associated with secretion of plant growth-promoting substances, solubilization of organic and inorganic phosphate (Antoun *et al.* 1998) and antagonistic action against pathogen (Huang and Erickson 2007, Siddiqui *et al.* 2007). However, the survival, growth and persistence of rhizobia largely depend on the edaphic, biotic and abiotic factors. Soil acidity complex of arable land provides an array of constrains for size and effectiveness of rhizobial population owing to excess availability of aluminum and manganese to toxic levels and characterized by deficiency and poor availability of phosphorus, calcium, magnesium and molybdenum (Slattery *et al.* 2001).

Out of 25 million ha of acid soils (*p*H <5.5) of India, 54% concentrated in the north-eastern region. In this region, pulse legumes are grown in 1.64 million ha of which 76.83% are located in Assam, but the pulse productivity (542 kg/ha) is much below the present national average (658 kg/ha). *Rhizobium* strain selection programme are based on the evaluation of several parameters related to the symbiosis, of which root nodulation represents a major component. Assam soil are moderately to strongly acidic with low cation exchange capacity (CEC) and has poor base saturation. The average P fertility index is low to medium in Al and Fe-rich alluvial soils of Assam (Vadivelu *et al.* 2004).

For increasing pulse production in acid soils of Assam, it appears necessary to define natural variation of resident rhizobial population and to identify the situation where response from inoculation is likely to be obtained.

Thirteen pea (*Pisum sativum* L.) growing sites representing 5 agro-climatic zones of Assam were selected during 2005–07 for collection of root nodules and rhizosphere soil (Table 1). At flowering and initial pod formation stage, 5 plants from each field were excavated randomly, root washed and nodules on them were counted. A whole clod containing the plant roots was dug out and separated nodules and roots by breaking the clod carefully in the field. The separated nodules then oven-dried and weight recorded (Table 2). One gram of fresh nodules from each site was kept separately for isolation of rhizobium. The rhizosphere soils were used for most probable number count and determination of soil chemical properties. The classical serial dilution technique was used for isolation and enumeration of rhizobia in nodules (0.5 g) from each site, using differential Yeast Extract Mannitol Agar media containing Congo Red following the method illustrated by Vincent (1970). White translucent colonies were counted. The population size of rhizobia in nodules of field grown pea is expressed on dry weight basis. The single colony was purified by repeated streaking on yeast extract mannitol agar plates and maintained in yeast extract mannitol slant.

The resident rhizobial populations of each site were determined by most probable number plant infection tests (Brockwell 1963). Initial 10-fold dilutions, followed by 6 further dilutions were made of each rhizosphere. One millilitre of each dilution was used to inoculate in 3 replicate to derive the most probable number estimate. Fieldpea was used as test plant. The chemical properties of rhizosphere soils were analyzed as per the procedures described by Jackson (1973).

Symbiotic effectiveness of the isolated purified rhizobia were assessed using pea (*Pisum sativum* L.) in a sterile potting mix composed of 50:50(v/v) soil : river sand contained in plastic pots (400 g capacity). Each pot contained 2 seedlings [surface sterilized pea seeds germinated on water agar (1.5%) plate] which were inoculated 2 days after sowing in triplicate, followed by watering with quarter strength N-free nutrient solution. Actively growing individual isolates from yeast

---

*Short note

[1] Senior Scientist (email:ndhrubajyoti@yahoo.co.in), [2] Research Associate (e mail: bibha63@ gmail.com), [3] Professor (e mail: rbaruah123@gmail.com), [4] Professor and Head (e mail: dkborah @yahoo.com.), Department of Soil Science

Table 1 Agro-climatic zones, cropping history and chemical properties of pea rhizosphere

Site of the agro-climatic zone	Cropping history	Chemical properties of pea rhizosphere				
		*p*H	OC%	Mineralizable N (mg/kg)	Bray's I P_2O_5(mg/kg)	1 n NH_4OAC extractable K_2O (mg/kg)
Dergaon (PR$_{\mathrm{De}}$)*	*Bao* rice–pea as relay crop	6.5	0.56	128.80	18.89	111.60
Jorhat (PR$_{\mathrm{Jo}}$)	Pea alone (flood during June –July)	5.1	0.98	123.20	11.45	102.84
Kamargaon (PR$_{\mathrm{Ka}}$)	*Ahu* rice – pea (flood during June –July)	5.7	1.19	145.60	17.17	234.72
Majuli (PR$_{\mathrm{Ma}}$)	*Sali* rice – pea (flood during June –July)	5.0	1.10	168.00	10.32	166.08
Tinsukia (PR$_{\mathrm{Ti}}$)	*Ahu* rice- pea as relay crop	6.5	0.98	173.60	22.89	74.63
Hatisung (PR$_{\mathrm{Ha}}$)	*Sali* rice- pea	4.8	1.27	145.60	13.74	139.80
Borbhujia (PR$_{\mathrm{Bo}}$)	*Ahu* rice-pea as relay crop	6.0	1.38	190.40	21.18	113.51
Odalguri (PR$_{\mathrm{Od}}$)	Vegetable-pea (heavy use of pesticides)	4.8	0.87	84.00	15.45	9+0.84
Rangia (PR$_{\mathrm{Ra}}$)	*Sali* rice-pea as relay crop	6.3	1.58	184.80	22.89	86.17
Cachar (PR$_{\mathrm{Ca}}$)	*Sali* rice-pea	5.0	1.26	145.60	19.46	240.48
Baraibazar (PR$_{\mathrm{Ba}}$)	Vegetable – pea (flood during June–July)	6.5	0.90	179.20	22.89	166.08
N.Lakhimpur (PR$_{\mathrm{Nl}}$)	*Sali* rice– pea	5.0	1.62	207.20	17.17	82.08
Dhemaji (PR$_{\mathrm{Dh}}$)	Pea alone (flood during June–July)	4.8	1.19	212.80	10.30	234.72

*****PR, Pea *Rhizobium*

Table 2 Nodulation status, rhizobial population in nodule, MPN and symbiotic effectiveness of field-grown pea

Rhizobial isolates	Nodulation status of field-grown pea		Rhizobium population		Symbiotic effectiveness
	Nodule/plant	Nodules dry wt. (mg/ plant)	Log cfu /g dry nodule	MPN count	Mean nodule score
PR De	56.0 (± 11.40)	271 (± 19.72)	09.9	1 470	2.8
PR Jo	27.0 (± 11.72)	134 (± 18.76)	10.2	230	2.3
PR Ka	45.6 (± 16.63)	159 (± 52.33)	10.6	92	3.0
PR Ma	13.0 (± 06.89)	173 (± 69.84)	09.7	14700	3.3
PR Ti	22.8 (± 12.59)	556 (± 98.64)	11.0	9190	2.2
PR Ha	31.0 (± 19.20)	115 (± 37.22)	10.6	42	1.5
PR Bo	77.8 (± 31.99)	319 (± 92.63)	08.8	2300	3.2
PR Od	13.2 (± 03.27)	60 (± 25.87)	09.7	9	2.2
PR Ra	90.4 (± 18.87)	261 (± 51.32)	10.1	918	2.0
PR Ca	10.6 (± 02.50)	298 (± 80.50)	10.0	1470	2.3
PR Ba	80.4 (± 32.03)	553 (±102.09)	07.5	147	2.5
PR Nl	13.4 (± 04.03)	66 (± 28.15)	08.8	424	0.2
PR Dh	75.0 (± 16.63)	321 (± 93.40)	09.9	23	2.5
Control					0.0
Sem±	10.55	42.30			0.9
CD (*P*=0.05)	21.23	85.13			1.7

* Nodule score: No nodule, 0; < 5 white nodule, 1; > 5 white nodule, 2; <5 red nodule, 3; >5 red nodule, 4. Data in parentheses are standard deviation

extract mannitol broth were inoculated @ 5 ml (10^8 cfu/ml) in each pot. Control pots were prepared where broth without culture was added @ 5 ml for comparisons. However each uninoculated control received nutrient solution containing N as KNO_3 @ 0.05%. Plants were grown in a net house in ambient conditions and watered with quarter strength sterile N-free nutrient solution as required. Single plants were maintained after 10 days of growth. Plants were harvested 21 days after inoculation and scored for nodulation based on the number and colour of nodules on roots.

The ANOVA, standard error differences, critical difference and regression study were analyzed statistically (Gomez and Gomez 1984). The most probable number data were transformed to log value prior to regression analysis.

The frequency of nodule number in field grown pea varied significantly (10.6–90.4/plant) across 13 diverse sites. There was also high heterogeneity observed among plants in same sites as reflected in the standard deviation value. Likewise significant variation of nodule dry weight observed between 60 and 566 mg/plant across the sites irrespective of numbers of nodule located (Table 2). The surveyed sites provided us with field evidence of different crop rotation with or without tillage operation and inundation by flood before the field pea were grown (Table 1). There was prolific nodulation in relay cropping as well as in the sites inundated by flood before the crop was sown. The higher nodulation status in these sites may be attributed to the favourable soil conditions fostered by no tillage operation (Kaschuk *et al.* 2006). No correlation was observed in between numbers of nodule and nodule dry weight.

Of the chemical properties of rhizosphere soil assessed, soil *p*H (Table 3) significantly correlated (r= 0.563*, *P*=0.01) with nodule number. Nodule dry weight was influenced by both soil *p*H (r=0.700*) and Brays P(r=0.600*). It is apparent from the survey that soils vary in their ability to produce uniform nodulation primarily as a result of *p*H changes. Nodule number and respective nodule dry weight were reasonably lower in the sites where *p*H below 5.7 except Dhemaji. Relatively higher nodulation in this site may be attributed to high organic carbon (1.19%) accretion after inundation by flood. Soil *p*H, crop rotation, management, tillage operation and flooding may all contribute to the wide variation of nodulation status (Venkateswarlu *et al.* 1997, Slattery *et al.* 2001, Kaschuk *et al.* 2006)

The most probable number of pea *rhizobia* ranged from 9 to 14 700/g soil, highest being observed at Majuli where rice (*Oryza sativa* L.) –pea crop rotation was followed (Table 2). Depending on the legume considered, number of nodulating rhizobia below 50/g soil could be a limiting factor for the symbiotic growth of legumes (Slattery *et al.* 2004). The pea nodulating resident rhizobial populations were consistently largest where relay cropping system has been practised. Venkateswarlu *et al.* (1997) reported that the cropping had more critical influence on the abundance of native rhizobial population than soil or climatic factors. The positive consequence of no-tillage in relay cropping, could be attributed to several factors, such as a lower soil temperature, higher soil moisture content, preservation of soil aggregates and a higher carbon content, favouring soil biomass and many classes of microorganisms, including *rhizobia* (Kaschuk *et al.* 2006). Though sizeable number (>100) of pea rhizobia was present in 9 sites, the number of resident rhizobia varied to a large extent and was poorly explained when comparing different soils. No correlation could be established between nodule number and nodule dry weight with most probable number, despite abundant nodulation. However, the most probable number of pea rhizobia was significantly correlated (r=0.531*) with the change of soil *p*H (Table 3). In the present study it appears that soil *p*H < 5.0, the size of rhizobial population declined severely (most probable number 9–42/g soil) irrespective of nodulation status and the detrimental effects on the native rhizobia are unlikely above this threshold. Small native rhizobial population in these sites (*p*H <5.0) may be ascribed to the deleterious effect exerted by some of acid soil properties. Previous reports have shown close negative correlation between the size of native rhizobial populations and increasing soil acidity (Rice *et al.*1997, Slattery *et al.* 2004). None of the other chemical parameter measured, showed correlation with most probable number.

Table 3 Relationship of nodule number, nodule dry weight and most proble number (MPN) count with *p*H and nodule dry weight with Bray's P.

Regression equation	r	R^2
Y_1 = –85.233+23.114 *p*H	0.563*	0.362
Y_2 = –613.869+156.454 *p*H	0.700*	0.491
Y_2 = –103.447 + 20.686 Bray's I P_2O_5 ppm	0.600**	0.360
Y_3 = –1.447+0.731 *p*H	0.531*	0.282

Y_1, Nodule number; Y_2, nodule dry weight; Y_3, Log MPN count

The effectiveness of isolated rhizobia demonstrated significant variation of nodule score (0.2–3.3) among the isolates from 13 different sites (Table 2). Fewer nodules (0.2–1.5) were formed on plants from the isolates of pea crop grown in acid soils (*p*H 4.8–5.0). There were instances of sub optimal nodulation (2.0–2.8) by the isolates from 8 sites, where the resident rhizobial population in soil ranged from 09 to 9190/g soil. None of the isolate could achieve a nodule score of 4.0. Notably, only 3 isolates from Kamargaon, Majuli and Borbhujia that followed relay cropping could attain nodule score of 3.0 or more irrespective of resident rhizobial population. The limited scoring of these naturalized isolated rhizobia may be due to their inactive and poor competitive abilities. It is likely that a number of rhizobia are unable to nodulate legumes and these isolates have been reported to constitute a significant proportion of the total resident rhizobia (Languerre *et al.* 1993). It appears that the presence of resident rhizobial population in soil does not necessarily mean effectiveness of the isolated rhizobia.

SUMMARY

The present assessment identified substantial variation in nodulation status and resident population of pea rhizobia growing in acid soils of Assam. Besides nodule number or the dry weight of nodule, the symbiotic effectiveness of *rhizobia* in nodulation is noteworthy in *rhizobium* strain selection programme. Naturalized isolates from 3 sites showed reasonably better effectiveness in nodulation (nodule score 3 or more), the risk of sub optimal nodulation or nodulation failure is very low. However, inoculation is imperative in other sites to ensure adequate nodulation in these acid soils, since suboptimal nodulation resulted by the isolated rhizobia from most of the sites.

ACKNOWLEDGEMENT

We thank Indian Council of Agricultural Research for the financial assistance for this study through All India Network Project on Soil Biodiversity- Biofertilizer.

REFERENCES

Antoun H, Beauchamp C J, Goussard N, Chabot R and Lalande R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant and Soil* **204**: 57–67.

Brockwell J. 1963. Accuracy of a plant-infection technique for counting populations of *Rhizobium trifolii*. *Applied Microbiology* **11**: 377–83.

Gomez K A and Gomez A A, 1984. *Statistical Procedures for Agricultural Research*, 2 nd edn, 680 pp. John Wiley & Sons, Singapore.

Huang H C and Erickson R S. 2007. Effect of seed treatment with *Rhizobium leguminosarum* on *Pythium* damping-off, seedling height, root nodulation, root biomass, shoots biomass, and seed yield of pea and lentil. *Journal of Phytopathology* **155**: 31–7.

Jackson M L.1973. *Soil Chemical Analysis*, 452pp. Prentice Hall of India Pvt Ltd, New Delhi.

Kaschuk G, Hungria M, Santos J C P and J F Berton-Junior. 2006.Differences in common bean rhizobial populations associated with soil tillage management in southern Brazil. *Soil & Tillage Research* **87**: 205–17

Laguerre G, Bardin M and Amarger N. 1993. Isolation from the soil of symbiotic and nonsymbiotic *Rhizobium leguminosarum* by DNA hybridization. *Canadian Journal of Microbiology* **39**: 1142–9.

Rice W A, Penny D C and Nyborg M.1997. Effects of soil acidity on rhizobia numbers, nodulation and nitrogen fixation by alfalfa and red clover.*Canadian Journal of Soil Science* **57**: 197–203

Siddiqui Z A, Baghel G and Akhtar M S. 2007. Biocontrol of *Meloidogyne javanica* by *Rhizobium* and plant growth-promoting rhizobacteria on lentil. *World Journal of Microbiology and Biotechnology* **23**: 435–41.

Slattery J F, Coventry D R and Slattery W J.2001. Rhizobial ecology as affected by the soil environment. *Australian Journal of Experimental Agriculture* **41**:289–98.

Slattery J F, Pearce D J and Slattery W J.2004. Effects of resident rhizobial communities and soil type on the effective nodulation of pulse legumes. *Soil Biology and Biochemistry* **36**:1339–46.

Vadivelu S, Sen T K, Bhaskar B P, Baruah U, Sarkar D, Maji A K and Gajbhiye K S. 2004. *Soil Series of Assam*, 229p, **101**, National Bureau of Soil Survey and Land Use Planning, Nagpur,

Venkateswarlu B, Had K and Katyal J C.1997. Influence of soil and crop factors on the native rhizobial populations in soils under dryland farming. *Applied Soil Ecology* **7:** 1–10.

Vincent J M. 1970.*A Manual for the Practical Study of Root- Nodule Bacteria*, 164 pp. Blackwell Scientific Publications, Oxford and Edinburgh.