Indian Journal of Agricultural Sciences **81** (3): 271–3, March 2011

Impact of phosphorus-solubilizing cold-resistant mutant strain of *Pseudomonas fluorescens* on growth of high-yielding rice (*Oryza sativa*) varieties in highly alkaline soil*

JYOTI SINGH[1], ALOK SHUKLA[2] and REETA GOEL[3]

GB Pant University of Agriculture and Technology, Pantnagar, Uttrakhand 263 145

Received: 24 September 2009; Revised accepted: 18 December 2010

Key words: Biocontrol, Biofertilizing, Phosphorus, *Pseudomonas fluorescens*, Rice

The rhizosphere is inhabited by actively growing microbial population that immensely affect the root and plant biology. Some of these micro-organisms are plant pathogens, whereas others are beneficial. Among the beneficial micro-organisms is a subset of bacteria, referred to as plant growth-promoting bacteria PGPB. A potential bacteria species belonging to this group is *Pseudomonas fluorescens*, the plant growth-promoting abilities of *P. fluorescens* are attributed to release of siderophores (Goel and Katiyar 2004), production of antibiotics (Anita and Rajendran 2006, Gnanamanickam *et al.* 2006), production of lytic enzymes (Bhaskaran *et al.* 2004, Kandan *et al.* 2004), induction of systemic resistance, production of plant growth promoting factors and phosphorus solubilization, *P. fluorescens* increases the availability of phosphorus from insoluble and unavailable sources by the production of alipahatic and aromatic acids, such as oxalic acid and malic acid and production of enzymes, like phytases and phospholipases (Goel and Katiyar 2003).

In this particular study we evaluated the effect of phosphatic biofertilizers on rice cultivation in highly alkaline soils. A pot culture experiment was carried out during 2007–08 in the net house of the College of Basic Sciences and Humanities of the University with three rice (*Oryza sativa* L.) varieties, viz 'NDR359', 'PA6444', 'Pusa Basmati1', two treatments with 12 replications of each. Soil was mixed with farmyard manure in the ratio 1:1 and then 72 pots were filled with 5kg soil in each pot. Following the usual agronomical practices of rice transplanting, 21-days-old seedlings of all the three varieties were used for the experiment. The seedlings were dipped in cold resistant mutant strain of *P. fluorescens* maintained in KB (King's B) broth for 4 hr and were then transplanted in the pots with an average of 5plants/pot (+Ps), with a total of 36 pots. The CRM strain of *P. fluorescens* was obtained from Department of Microbiology of the University, Pantnagar. As control, 36 pots were planted with 21-days-old seedlings with no *P. fluorescens* treatment (-Ps), and every other condition was same. Various growth parameters, like plant height, total number of leaves and total number of tillers were recorded at regular time intervals. Soil for each treatment was tested for *p*H, and available phosphorus content. For determining plant available P in neutral-alkaline soils, the Olsen's method (Cole *et al.* 1954) was used. Statistical analysis of the data was carried out using Analysis of variance (ANOVA) technique for complete randomized design.

At the end of the vegetative phase, all the rice varieties without *P. fluorescens* treatment showed better growth (as

Table 1 Plant height (cm) of three high-yielding rice varieties with and without treatment of *Pseudomonas fluorescens* during *kharif* 2007 at 45, 60, and 75 DAS (days after sowing)

Variety	Plant height (from soil base to uppermost leaf base)					
	45 DAS		60 DAS		75DAS	
	+ Ps	–Ps	+Ps	–Ps	+Ps	–Ps
'NDR359'	11.10	16.61	13.55	22.19	18.13	26.92
'PA6444'	10.02	13.05	14.19	20.40	17.15	22.80
'Pusa Basmati 1'	10.68	13.09	13.5	18.19	17.85	22.98
Mean	10.6	14.24	13.75	20.26	17.71	24.23
SEm (V)	0.449		0.613		0.631	
SEm2 (T)	0.367		0.501		0.515	
SEm3 (V×T)	0.635		0.867		0.892	
CD (P=0.05)						
Variety (V)	1.26		1.73		1.78	
Treatment (T)	1.03		1.41		1.45	
V×T	1.79		2.45		2.52	

*Short note

[1]Research Fellow (e mail: singhjyoti22@gmail.com), SES, JNU, New Delhi 110 067;

Senior Research Officer (e mail: aloks99@yahoo.com), Department of Plant Physiology; [3]Professor and Head (e mail: rg55@rediffmail.com), Department of Microbiology

Table 2 Number of green leaves/hill in three high-yielding rice varieties with *Pseudomonas fluorescens* treatment and without treatment during *kharif* 2007 at 45, 60 and 75 DAS (days after sowing)

Variety	Number of green leaves/hill					
	45 DAS		60 DAS		75DAS	
	+ Ps	–Ps	+Ps	–Ps	+Ps	–Ps
'NDR359'	4.83	5.33	6.16	7.66	7.75	12.83
'PA6444'	5.16	5.58	8.25	9.16	12.66	16.83
'Pusa Basmati 1'	5.00	5.50	8.00	9.33	13.16	14.75
Mean	5	5.47	7.47	8.72	11.19	14.8
SEm (V)	0.112		0.199		0.423	
SEm (T)	0.915		0.162		0.345	
SEm (V×T)	0.158		0.281		0.598	
CD (*P*=0.05)						
Variety (V)	0.31		0.56		1.19	
Treatment (T)	0.25		0.45		0.97	
V×T	0.44		0.79		1.69	

Table 3 Tillers/hill in three high-yielding rice varieties with *Pseudomonas fluorescens* treatment and without treatment during *kharif* 2007 at 60 and 75 days after sowing

Variety	Number of tillers/hill			
	60DAS		75DAS	
	+ Ps	–Ps	+Ps	–Ps
'NDR359'	1.00	1.75	2.41	4.58
'PA6444'	1.66	2.33	4.75	6.33
'Pusa Basmati1'	2.25	2.41	5.16	6.00
Mean	1.64	2.16	4.11	5.64
SEm (V)	0.139		0.217	
SEm (T)	0.114		0.177	
SEm (V×T)	0.197		0.307	
CD (*P*=0.05)				
Variety (V)	0.39		0.61	
Treatment (T)	0.32		0.5	
V×T	0.56		0.87	

shown in Tables 1, 2, 3). As per mean values variety 'NDR359' (-Ps) attained the maximum height (26.92 cm) and 'PA6444' (+Ps) had the minimum height (17.15 cm), although the difference was not significant. Maximum green leaves/hill (16.83) and maximum tillers/hill (6.33) were recorded in 'PA6444' (–Ps). On the other hand lowest green leaves/hill and tillers/hill were recorded in 'NDR359' (+Ps), ie 7.55 and 2.41, respectively. The number of green leaves/ hill in the varieties without treatment was significantly greater than that in varieties with *Pseudomonas fluorescens* treatment. The average effect of treatment on the growth parameters was more pronounced than that of varietal differences. Soil samples taken from pots of the three varieties with or without *P. fluorescens* treatment had alkaline *p*H (Fig 1). All the 3 *P. fluorescens* treated varieties had greater *p*H as compared to their respective untreated ones, variety 'NDR359' (+Ps) had the highest pH (8.4), variety 'PA 6444' (–Ps) had the lowest *p*H (8.12). The *p*H of soil before transplanting was 8.19. A *p*H of 8.3 or above is generally very high for most of the plants. Phosphorus immobilization occurs in Ca phosphates due to excessive liming, excessive use of N fertilizers with insufficient phosphorus application (Dobermann and Fairhust 2000). Phosphorus is particularly important in early growth stages, plants deficient in phosphorus show stunted growth, are small in size and often show reduced number of green leaves. Availability of phosphorus also affects tillering in case of rice (Abel *et al*. 2002). Soil sample taken from 'Pusa Basmati 1' (–Ps) had the lowest amount available phosphorus (26.75 kg/ha), whereas soil from 'NDR359' (+Ps) had the highest amount of phosphorus content (32.78 kg/ha) (Fig 2). The phosphorus content of soils from all the *P. fluorescens* treated varieties was greater than that of untreated ones. The amount of available phosphorus in the soil before transplanting was 21.66 kg/ha.

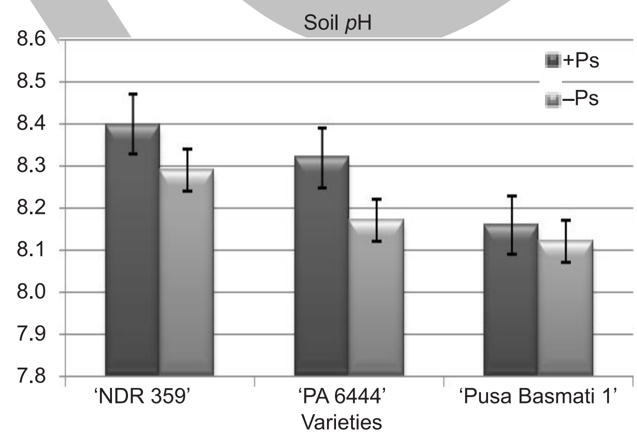

Fig 1 Soil *p*H profile of rice varieties with *Pseudomonas fluorescens* treatment and without treatment, respectively

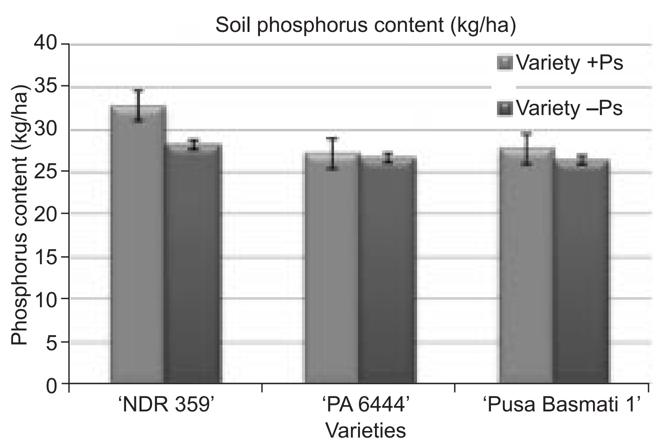

Fig 2 Phosphorus content (kg/ha) of soil taken from rhizosphere of rice varieties with *Pseudomonas fluorescens* treatment and without treatment, respectively.

This study demonstrates that highly alkaline *p*H is a limiting factor for the use of phosphatic biofertilizers in rice cultivation. In the case of *P. fluorescens* treated varieties, the soil *p*H was higher than that of the varieties without treatment. On account of the higher *p*H, the phosphorus present in the soil might have not been available to the *P. fluorescens* treated varieties and this would have been the reason for their reduced growth.

The cold resistant mutant (CRM) *P. fluorescens* strain used is a phosphorus-solubilizing strain. It increases the availability of phosphorus from insoluble and unavailable sources (Goel and Katiyar 2003). The bioinoculant performed its work of solubilizing phosphorus efficiently as higher phosphorus content was found in the soil of treated varieties, but available phosphorus was of no use to the plants as they could not take up that phosphorus from the soil because of higher alkalinity. It can be concluded that phosphatic biofertilizers, like *P. fluorescens* CRM cannot be beneficial in every soil condition, rather they would be beneficial in a defined range of soil *p*H and other properties. The use of biofertilizers would be successful if right bioinoculant will be used for specific soil types and it is very important to go for soil testing before using any bioinoculant.

SUMMARY

A pot culture experiment was carried out during 2007–08 in the net house of College of Basic Sciences and Humanities, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. A cold resistant mutant (CRM) strain of *P. fluorescens* was taken to evaluate its effect on the growth of three high-yielding rice varieties, viz 'NDR359', 'PA6444', 'Pusa Basmati 1' under high *p*H condition. The plants without treatment were significantly better than the ones treated with *P. fluorescens* in terms of morphological features, however the amount of available phosphorus in the soil was greater in case of treated plants, but the plants could not take up that due to high alkaline *p*H. The results indicated that the performance of phosphatic biofertilizers is *p*H dependent and it is recommended to go for soil testing before using biofertilizers.

REFERENCES

Abel S, Ticconi C A and Delatorre C A. 2002. Phosphate sensing in higher plants. *Physiology Plantarum* **115**: 1–8.

Anita B and Rajendran G. 2006. Effect of different methods of application of the plant growth promoting bacterium, *Pseudomonas fluorescens* Migula on the management of *Meloidogyne graminicola* Golden and Birchfield m (1965) infecting rice. *Journal of Biological Control* **20**: 233–6.

Bhaskaran R, Nagarajkumar M and Velazhahan R. 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiological Research* **159**: 73–81

Cole C V, Dean L A, Olsen S R and Watanabe F S. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *U.S. Department of Agriculture Circular*. 939.

Dobermann A and Fairhurst T. 2000. *Rice: Nutrient Disorders and Nutrient Management*, 191 pp. Potash & Phosphate Institute, Potash & Phosphate Institute of Canada and International Rice Research Institute, Singapore and Los Baños.

Gnanamanickam S S, Immanuel J E, Thomashow L and Velusamy P. 2006. Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. *Canadian Journal Microbiology* **52**: 56–65.

Goel R and Katiyar V. 2003. Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens. Microbiological Research* **158**: 163–8.

Goel R and Katiyar V. 2004. Improved plant growth from seed bacterization using siderophore overproducing cold resistant mutant of *Pseudomonas fluorescens*. *Journal of Microbiology and Biotechnology* **14**: 653–7.

Kandan A, Nandakumar R, Radjacommare R and Samiyappan R. 2004. Association of the hydrolytic enzyme chitinase against *Rhizoctonia solani* in rhizobacteria-treated rice plants. *Journal of Phytopathology* **152**: 365–70.