

# Effects of Phosphate-Solubilizing Bacteria, Rock Phosphate and Chemical Fertilizers on Maize-Wheat Cropping Cycle and Economics



Gurdeep KAUR and Mondem Sudhakara REDDY\*

*Department of Biotechnology, Thapar University, Patiala 147004 (India)*

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## ABSTRACT

A two-year field study was conducted to test the effects of two phosphate-solubilizing bacteria (PSB), *Pantoea cypripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5), inoculated singly or together with rock phosphate (RP) fertilization on maize and wheat cropping cycle by comparing with chemical P fertilizer (diammonium phosphate, DAP), mainly in the crop yield, soil fertility and economic returns. Inoculation of PSB together with RP fertilization increased the crop growth in terms of shoot height, shoot and root dry biomass, grain yield and total P uptake in both maize and wheat crops compared to the other treatments. Soil fertility in the context of available P, enzyme activities and PSB population in both maize and wheat crops was significantly improved with PSB inoculation together with RP fertilization compared to DAP treatment. The combined use of PSB inoculation and RP fertilization was more economical due to minimal cost and maximum returns. These results suggested that PSB inoculation along with RP fertilization would be an appropriate substitute for chemical phosphate fertilizer application in sustainable agriculture systems.

**Key Words:** diammonium phosphate, *Pantoea cypripedii*, *Pseudomonas plecoglossicida*, soil fertility, sustainable agriculture

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## INTRODUCTION

Phosphorus (P) is one of the most important macronutrients required for plant growth, yield and seed formation. Although most agricultural soils have large amounts of inorganic and organic P, these are immobilized and mostly become unavailable. Hence, very limited concentration of P is available to plants due to P deficient in soils (Adesemoye and Kloepper, 2009). A survey of Indian soils revealed that 98% of soils were deficient in P, because the concentration of available P including in fertile soils was generally not higher than  $10 \mu\text{mol L}^{-1}$  even at pH 6.5 where it is mostly soluble (Gyaneshwar *et al.*, 2002). These low levels of P are due to high reactivity of soluble P with Ca, Fe or Al that leads to precipitation thus lowering the overall use efficiency. Oberson *et al.* (2001) reported that P deficiency decreased agricultural productivity on more than 2 billion hectares worldwide and therefore, agronomic treatments to improve P availability in the soil and to increase P utilization in agriculture are of special importance.

The need for P in agriculture can be satisfied by the application of chemical fertilizers. However, overuse of

fertilizers can not only cause unanticipated environmental impacts but also they are expensive, and have some harmful impacts on the soil structure, composition, microflora and other properties of soil (Reddy *et al.*, 2002; Koliaei *et al.*, 2011). Increasingly high cost of chemical fertilizers has been the major stimulus to search for an alternative, naturally-occurring phosphate source. This kind of fertilizer has caused the reconstruction of soil texture and their productivity (Kim *et al.*, 1998).

Natural phosphate rocks (RP) have been recognized as a valuable alternative for P fertilizers. In India, it is estimated that there are almost 260 million tons of phosphate rock deposits and this material will provide a cheap source of phosphate fertilizer for crop production (FAI, 2002). Unfortunately, RP is not readily available to the plants in soils with a pH > 5.5–6.0. Because of this, extension services are reluctant to recommend it and farmers are hesitant to utilize RP directly. One approach for solubilization of RP in field conditions is the application of phosphate-solubilizing microorganisms (PSM).

PSM has the ability to solubilize unavailable P form to soluble forms through the process of organic

\*Corresponding author. E-mail: msreddy@thapar.edu.

acid production, chelation and ion exchange reactions and make them available to plants (Chung *et al.*, 2005). Biological phosphate fertilizers containing beneficial phosphate-solubilizing bacteria (PSB) increase phosphate solutions by increasing soil acidity or phosphatase enzymes. Therefore, the use of PSB in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble fertilizers in the soils to which they are applied (Chang and Yang, 2009; Banerjee *et al.*, 2010). Use of phosphate bio-fertilizers decreases the detrimental effects of phosphate fertilizers on crop and soil health.

Microbial inoculants are promising components for integrated solutions to agro-environmental problems because they possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Weller, 2007; Adesemoye *et al.*, 2008; Singh and Reddy, 2011). The present study therefore aimed to evaluate the comparative effects of chemical phosphate fertilizer and biological phosphate fertilizers (*i.e.*, PSB) singly or together with rock phosphate fertilizer on the growth and economic returns of maize and wheat crops and the effects of these fertilizers on the physicochemical properties of alkaline soil.

## MATERIALS AND METHODS

### *Bacterial strains*

*Pantoea cyripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5) isolated from the rhizospheric soil of *Stevia rebaudiana* growing in an organically managed farm at Pojewal, Punjab, India were used in this study. The organic farm used in this study was a field where no synthetic fertilizers had been used during the last 10 years. Animal manure, vermi-compost and green manure were used to maintain the soil fertility. Both bacterial strains were maintained on Pikovskaya's medium (PKV) (Pikovskaya, 1948) supplemented with 1 g kg<sup>-1</sup> tri-calcium phosphate. The composition of P-KV medium with pH of 7.0 was as follows: 10.0 g L<sup>-1</sup> glucose, 0.5 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2 g L<sup>-1</sup> KCl, 1 × 10<sup>-2</sup> g L<sup>-1</sup> Mg<sub>2</sub>SO<sub>4</sub>·7H<sub>2</sub>O, 1 × 10<sup>-4</sup> g L<sup>-1</sup> Mn<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O, 1 × 10<sup>-4</sup> g L<sup>-1</sup> Fe<sub>2</sub>SO<sub>4</sub>·7H<sub>2</sub>O, and 0.50 g L<sup>-1</sup> yeast extract. These isolates were selected based on their activities for plant growth promotion and crop yield in our previous study (Kaur and Reddy, 2013).

### *Field experiment*

A two-year field experiment was conducted in the field of Thapar University, Patiala, India. This site was situated at 30.30° N latitude and 76.38° E longitude.

The region possessed tropical hot and dry climatic conditions, characterized by very hot and dry in summer and very cold in winter. The soil in the experimental site belongs to Typic Ustifluvents, sandy loam in texture with the following characteristics: pH 8.27 (alkaline soil), electric conductivity 0.17 mS cm<sup>-1</sup>, organic carbon 3.3 g kg<sup>-1</sup>, available P 3.90 mg kg<sup>-1</sup>, and total P 251 mg kg<sup>-1</sup>. The experimental field was not used for agricultural purposes since last 5 years and hence no chemical fertilizers were applied to this site. During the first year of field study, maize variety DKC-9106 was sown at a rate of 20 kg ha<sup>-1</sup> and cultivated in the rainy season (July 2011). Field trials were conducted in a completely randomized block design, consisting of 7 treatments: control, PSB-3, PSB-5, RP, RP + PSB-3, RP + PSB-5, and diammonium phosphate (chemical P fertilizer). Each treatment plot was of 4 m × 4 m (16 m<sup>2</sup>) size and 3 replicate plots were maintained for each treatment. Rock phosphate was amended in respective plots at the rate of 59 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> before sowing during maize cropping. Diammonium phosphate (DAP) was added at a rate of 59 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in chemical P fertilizer treatment at the time of sowing. Urea was added at the rate of 272 kg ha<sup>-1</sup> as per agronomic practices. In chemical fertilizer treatments, where DAP was used at the rate of 59 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, the urea dose was reduced by 47 kg ha<sup>-1</sup> because DAP already contained 18% nitrogen (N). Bacterial strains were inoculated as seed treatment. Both bacterial strains were grown in Pikovskaya's broth at 30 °C on a rotary shaker (130 r min<sup>-1</sup>) for 48 h. The bacterial cultures were centrifuged at 10000 r min<sup>-1</sup> for 3 min followed by washing the cells with sterile distilled water. The pellet was suspended in sterile distilled water. For seed inoculation, the seeds were sterilized first by dipping in 95% ethanol for 3 min followed by 3% sodium hypochlorite for 5 min, and subsequently washed with sterile distilled water followed by treatment with slurry containing 40% gum arabic, 10% sugar solution and bacterial suspension. Seeds treated with 40% gum arabic and 10% sugar solution that did not contain bacterial suspension served as a control. At the time of sowing, size of inoculum per maize seed was 2.5 × 10<sup>5</sup>–3.0 × 10<sup>5</sup> colony-forming units (CFU). All the plots were irrigated once before the sowing to ensure proper germination of seeds and then regularly during crop growth as per agronomic practices. The crop was harvested in October 2011 and the field was left empty for one year.

Wheat variety PBW-621 was sown at a rate of 99 kg ha<sup>-1</sup> in the same field in winter season (November

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