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Interaction of compost additives with phosphate solubilizing rhizobacteria improved maize production and soil biochemical properties under dryland agriculture

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ABSTRACT

Dry land soils from the Indo-Gangetic plains are inherently poor in organic matter content and phosphorus (P). Amendment of these soils with P-enriched compost, together with P solubilizing rhizobacterial (PSR) inoculation has been suggested to improve plant growth and P uptake. We performed 3 × 2 factorial experiment using compost i.e. control, raw saw dust P compost (RPC) and acidified saw dust P compost (APC), and PSR inoculation as factors. The pot study investigated the effects of compost and PSR on maize growth, yield, P nutrition and soil biochemical properties. PSR inoculation with APC enhanced shoot length, cob diameter, grain yield and plant P uptake compared to control. APC amendment, either alone or in combination with PSR, also improved root growth and caused extension of rhizosphere by increasing the root length density of the maize plants. Significant improvement in soil biochemical properties i.e. dehydrogenase (DHA), β-glucosidase (BGA), urease (UA) activity, dissolved organic carbon (DOC), soil organic carbon (SOC) and microbial biomass carbon (MBC) are due to the additive effect of compost amendments. A substantial increase in P availability and associated biochemical attributes reflect the significance of substrate quality, structural stability and optimized pH conditions of added compost that may have enhanced PSR performance, specifically with APC. Moreover, increased carboxylate secretions and PSR abundance in the root zone, lowering of pH and increase in rhizosphere fungal parameters, suggest PSR and APC to be synergetic driving of increased P availability for maize. We conclude that efficiency of P enriched compost could be increased with suitable compost additive in the presence of PSR in dry land soil.

1. Introduction

Cereal crops contribute to 40% of the energy and protein components in the human diet and their growth covers about 70% of total agriculture land area of the world (McDonald and Nicol, 2005; Dunwell, 2013). Of these cereal crops, in terms of human nutrition, energy and protein source; maize (*Zea mays* L.) is the third most important food crop. Additionally, maize crop is increasingly being used to produce biofuels. Taken together, it has been projected that as a food supply the global demand for maize will exceed that for wheat or rice by 2020 (Pingali, 2001). As the demand for food crops will keep growing, maize crop is foreseen to be rather important and perhaps crucial in the future food security for an anticipated additional 2.3 billion people by 2050.

Phosphorus (P) is an essential element for plant growth and productivity, receiving increased attention owing to current and projected future demands on global agricultural production (Richardson and Simpson, 2011). Low P availability limits crop production on global

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scale (Khan and Joergensen, 2009). One unique characteristic of P is its slow diffusion and high fixation in soil (Shen et al., 2011). Hence, it warrants increasingly higher application of P fertilizers in these soils to meet the optimum P requirement for improving plant productivity. On a global scale, over 15 million tons of P fertilizer is used every year, aiming to enhance P availability and increase plant productivity. About 80 percent of the added inorganic P rapidly becomes unavailable to plants as P becomes precipitated and adsorbed to Ca, Fe, Al or other soil components (Gyaneshwar et al., 2002; Syers et al., 2011). This imbalance between P addition and P availability is very critical for plant P uptake (Shen et al., 2013).

Because of the tight coupling between soil organic matter (SOM) and the P pools in dryland soils of Indo-Gangetic region, SOM depletion ultimately limits the agricultural productivity (Manna et al., 2001). Addition of organic amendments (i.e. compost) can increase the nutritional status of the soil, as well as improve porosity, structural stability, moisture content, and biological activity in the soil (Wang et al., 2013). Organic amendments enhance microbial activity that tend to increase P availability in dryland soil (Khan and Joergensen, 2009).

Phosphate solubilizing rhizobacteria (PSR) are considered to play an important role therein, as they release microbial metabolites in the root zone of host plant (Mehta et al., 2015). PSR can increase P availability to the plant by the release of organic acids and phosphatases, which enhance solubility of various inorganic P forms in soil (Dobbelaere et al., 2003). The functional role of these bacteria in dryland soils strongly depends on their rhizosphere competence and growth. The quality of the substrate in soils amended with organic amendments is crucial for the functioning of PSR (Singh et al., 2013).

The use of bulking agents as a bedding material is necessary to provide structural support and maintain optimum free air space during composting. Sawdust, a widely used bulking agent, can regulate the moisture content and increase the porosity of composting material. The pH of sawdust is neutral and hence has no effect on the pH of the compost type for which it is used as a bulking agent. Especially in alkaline-calcareous soils this might be a drawback as the availability of P increases with decreasing soil pH. However, we lack understanding on the effects of acidifying sawdust on PSR activity and concomitant P solubilization. Therefore, we compared the effects of two bulking material, acidified or raw saw dust, on compost quality and performance. Additionally, we examined their effects on plant growth and nutrition with and without PSR inoculation. We hypothesized that (1) soils amended with acidified sawdust bulked P-enriched compost (APC) would have higher soil P availability and hence plant growth than soil amended with raw sawdust bulked P-enriched compost (RPC), and that (2) this effect would even be more enhanced when soils are additionally inoculated with PSR (APC + PSR).

2. Materials and methods

2.1. Isolation of PSR

Rhizosphere soil and root samples were collected from the cotton plant stands, located at Chak Jamia Abad (Lat. 31°.36′N; Long. 72°.43′E) of Chiniot district in Punjab, Pakistan. Healthy cotton plants, at boll formation, were uprooted randomly from different locations of field plot. Soil and root samples were transferred immediately to the laboratory and stored at 4 °C for PSR isolation. A potential PSR, AK-35, was isolated by preparing a soil-water (10g × 90 mL) suspension in an Erlenmeyer flask and shaken at 150 rpm for 35 min. Soil water suspension was diluted 10 times followed by plating of 100 µL sample solution on Pikovskaya (PVK) (1948) agar. The chemical composition of PVK growth medium was: 10 g $(NH_4)_2SO_4$ 0.5 g NaCl, 0.2 g KCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.1 g $MnSO_4{\cdot}7H_2O$, 0.5 g $FeSO_4{\cdot}7H_2O$, 0.5 g yeast extract and 15 g of agar powder in 1L distilled water. In addition, 5.0 g of tricalcium phosphate (TCP) was also added as insoluble P source. TCP was sterilized by autoclaving prior to its use. Other sterile ingredients were then aseptically mixed after autoclaving. Sterile broth medium was used as a control treatment. The experiment was replicated three times. Potential PSR was selected primarily based on the formation of clear halo zones on TCP amended PVK media after 5 days of incubation at 30 °C, and plant growth promoting characteristics exhibited in various biochemical assays (See Supplementary Table SP1). Selected PSR was purified with repeated culturing and kept at −20 °C in broth medium containing 20% (w/v) glycerol before it was used for further experiment. Selected PSR AK-35 was identified by determination of 16S rRNA gene sequences as described previously by Zaheer et al. (2016). Partial 16S rRNA gene sequence of selected PSR (AK-35) was compared with known nucleotide sequences in the EzTaxon-e database. Bacterial strain AK-35 showed 99.76% resemblance with *Enterobacter kobei* CIP 105566^T. 16S rRNA gene sequence of strain AK-35 was deposited at EMBL with accession number LK936515.

2.2. Quantitative P solubilizing activity

For quantitative estimation of P solubilization, triplicate cultures of PSR were inoculated into PVK broth containing rock phosphate (RP, 5 g L^{-1}) and incubated at 28 ± 2 °C in a shaking incubator (150 rpm) for 7 days. Rock phosphate supplemented PVK broth is a more reliable and precise indicator of bacterial P solubilization as compared to the plate type and tricalcium phosphate (TCP) assays (Bashan et al., 2013). Sterile uninoculated medium served as control. After 7 days, the broths were centrifuged at 11000 rpm for 20 min to obtain cell free supernatant. Triplicate aliquots of the supernatant were harvested and transferred into clean, dry, acid washed test tubes. The amount of soluble P in the filtrate was determined spectrophotometrically (λ = 882 nm) using molybdate blue method (Murphy and Riley, 1962). For organic acids analysis, the cell-free supernatant was passed through 0.2 µm nylon filters (Millipore, USA) and 20 µL of filtrate was injected to HPLC equipped with Turbochrom software (Perkin Elmer, USA) and C-18 column at a flow rate of 0.6 mL min^{-1} using 30:1:70 (v/v/v) methanol: acetic acid: water as mobile phase. Signals were detected at 210 nm. Organic acid standards were prepared from gluconic, succinic, oxalic, ascorbic and tartaric acid (Sigma-Aldrich). The detection of organic acids produced by the selected PSR and rock phosphate solubilization activity is presented in Supplementary Table SP1.

2.3. Compost preparation

Phosphorus enriched compost was produced from vegetable, fruit and kitchen waste, collected from various commercial markets and residential areas of Faisalabad city (Lat. 31°25′N, Long 73°04′E). The sorted compostable waste material was air dried for 5 days, followed by oven-drying at 60 °C for 24 h and then ground into finer particles (< 2.0 mm). Saw dust was used as a bulking material in a 1:10 ratio. Moisture content was kept approximately at 40% of bulking material-compost feedstock during the composting process. Bulking material (i.e. sawdust) was added in two forms: raw sawdust, and acidified sawdust. The latter was prepared by treating raw sawdust with an acidic salt solution ($KHSO_4$) as a pH neutralizer. The acidic-treatment process was as follows: (1) immersing the raw sawdust in 1% $KHSO_4$ solution for 24 h (adjusted pH value to 5.5 with H_2SO_4); (2) taking out the sawdust without leaching, followed by natural air-drying (< 40 °C) to remove water. The moisture content of acidified saw dust and raw saw dust was measured by oven drying them at 105 °C for 24 h. This allowed us to make sure that the final moisture content of the acidified sawdust was similar to the moisture content of the raw sawdust.

The compost-sawdust mixtures were composted in a locally fabricated compost reactor (40 × 30 × 30 cm) made of glossy tin sheets. The compost mixtures were aerated for 15 min after every hour at a flow rate of 30 L min^{-1}. Such aeration rate is adequate for maintaining sufficient oxygen supply, optimum biological activity and, also provide necessary temperature control during composting process. Humidity of compost mixtures was started with initial value close to 45% then