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Research Paper

Modification of root properties with phosphate solubilizing bacteria and arbuscular mycorrhiza to reduce rock phosphate application in soybean-wheat cropping system

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ABSTRACT

Inoculation of phosphate solubilizing bacteria (PSB) and phosphorus mobilizing arbuscular mycorrhiza (AM) with low quality rock phosphate (RP) can be an alternative source to one of the costliest phosphatic fertilizers in India, i.e. single superphosphate, by enhancing phosphorus influx and modification of root properties. Co-inoculation of PSB and AM may play a pivotal role to reduce phosphorus application through RP. Root properties and grain yield of irrigated soybean-wheat cropping system were evaluated with two levels of RP {100 and 50% recommended P (1.0 RP and 0.5 RP)} and different combinations of PSB and AM with 0.5 RP (0.5 RP + PSB, 0.5 RP + AM and 0.5 RP + PSB + AM) versus 100% recommended P application through soluble single superphosphate (1.0 SP) in the Indo-Gangetic plains. The P influx under 0.5 RP + PSB + AM and 1.0 SP were statistically at par with each other and the former treatment provided 0.6 and 3.2% higher value than the later in soybean and wheat, respectively. The root surface area density (RSAD) of soybean and wheat under 0.5 RP + PSB + AM (13.71 and 6.16 $m^2\,m^{-3}$, respectively) and 1.0 SP (13.70 and 6.37 $m^2\,m^{-3}$, respectively) were non-significant and almost equal with each other. The values of root cation exchange capacity and other root properties under 0.5 RP + PSB + AM and 1.0 SP were also non-significant. The improved root properties under 0.5 RP + PSB + AM provided statistically at par grain yield with 1.0 SP for both soybean and wheat crops. The yield under 0.5 RP + PSB + AM of soybean crop was 3.4% higher than 1.0 SP. The net returns US$$^{-1}$ invested was significantly higher under 0.5 RP + PSB + AM compared to 1.0 SP for both soybean and wheat crops. Co-inoculation of PSB and AM with 50% of recommended P through RP could be recommended for better root properties and profitable grain yield of soybean-wheat cropping system in the Indo-Gangetic alluvial plains.

1. Introduction

The roots play the primary role in the uptake of nutrients. Root growth and development are highly plastic and depend on many soil factors like nutrient availability, soil pH, soil temperature, bulk density, moisture content, etc. (Iman et al., 2006). The importance of root systems combined with the inherent difficulty of studying them has led to root being described as 'the hidden half'. Additionally, root mass, which is easier to measure than root morphology (root length, surface area, volume and radius) has been used to compare root systems (Mahanta et al., 2014). But, root mass measurements are not indicative of the total absorptive area of the root system and alteration of root system architecture can happen without a change in total root biomass (Iman et al., 2006). Alteration of root morphology and other root properties greatly influence the nutrient uptake.

Phosphorus (P) is one of the most important nutrients affecting root development (Kuang et al., 2005; Fageria and Moreira, 2011). High-grade P ore deposits are the source of phosphatic fertilizers, which are

Abbreviations: AHC, agglomerative hierarchical clustering; AM, arbuscular mycorrhiza; B:C ratio, benefit cost ratio (Net returns per US$ invested); CFU, colony forming unit; DAS, days after sowing; P, phosphorus; P Influx, Phosphorus influx; PCA, principal component analysis; PSB, phosphate solubilizing bacteria; PU, phosphorus uptake; PUE, P uptake efficiency; RL, root length; RLD, root length density; Root CEC, root cation exchange capacity; RP, rock phosphate; RSA, root surface area; RSAD, root surface area density; RV, root volume; RVD, root volume density; SP, single superphosphate; SRI, sphere of root influence; S-W system, soybean-wheat cropping system; t, mean root age; 0.5 RP, 50% of recommended P through rock phosphate; 0.5 RP + PSB, inoculation of seed with PSB along with application of 50% of recommended P through rock phosphate; 0.5 RP + AM, application of arbuscular mycorrhiza along with 50% of recommended P through rock phosphate; 0.5 RP + PSB + AM, inoculation of phosphate solubilizing bacteria along with application of arbuscular mycorrhiza and 50% of recommended P through rock phosphate; 1.0 RP, 100% of recommended P through rock phosphate; 1.0 SP, 100% recommended P application through soluble single superphosphate

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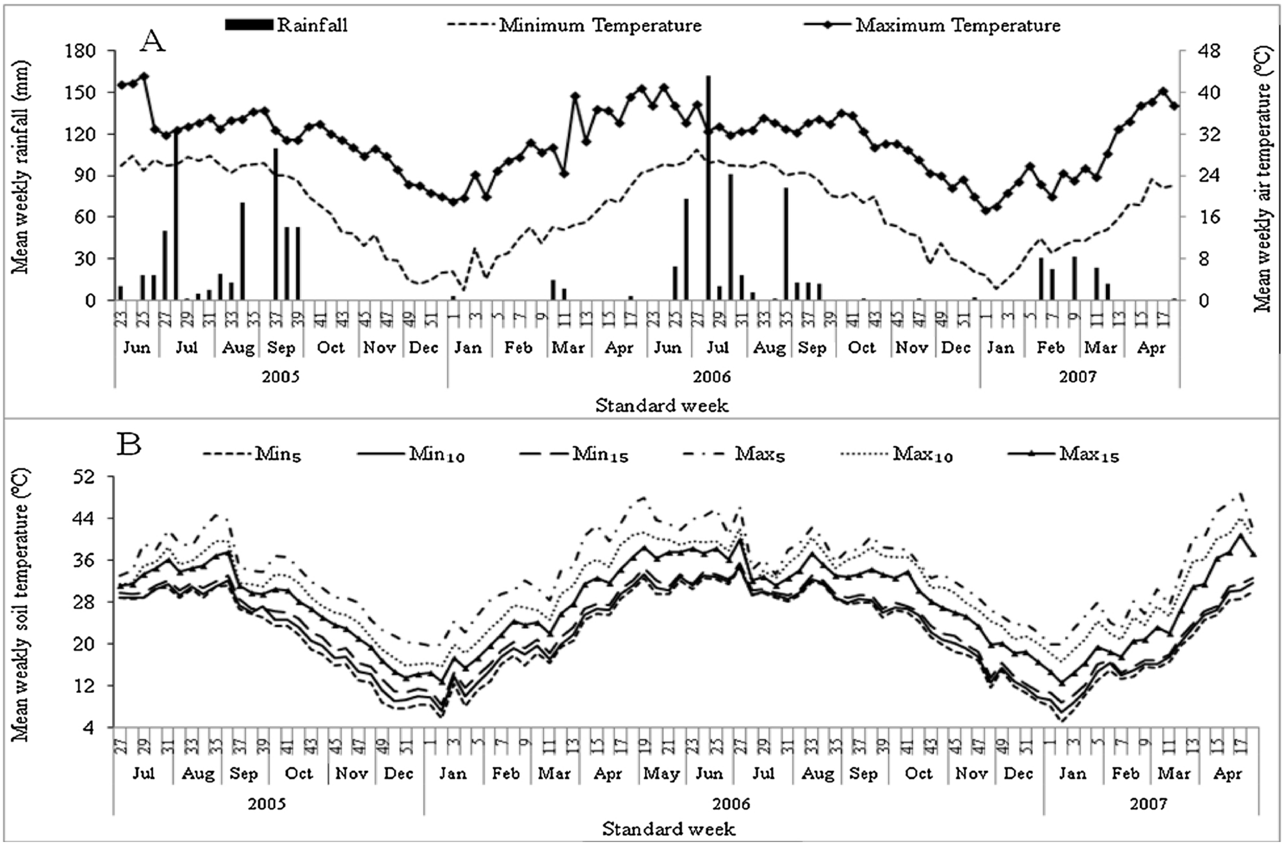

Fig. 1. A. Weekly total rainfall and mean maximum and minimum air temperature during the total experimental period. Fig. 1B. Mean weekly maximum and minimum soil temperature from different depths during the total experimental period.

limited and non-renewable also. Studies claim at current rates of extraction, global commercial phosphate reserves will be depleted in 50–100 years. The remaining potential reserves are of lower quality (Cordell et al., 2009). However, India has about 305 million tons of reserve of indigenous rock phosphate (RP), which is not suitable for manufacture of phosphatic fertilizer due to low content and reactivity of P. But, they perform poorly when applied directly to the neutral and alkaline soil, although it can effectively replace water soluble phosphates in acid soils (Mahanta and Rai, 2008; FAI, 2016). To make lower quality RP effective in the neutral and alkaline soils, it is being converted into water soluble form by mixing with soluble single superphosphate (SP) fertilizer or by partial acidulation with mineral acids, for which sulphur is imported. In all these cases, the underlying principle is to supply acid for conversion of insoluble P of RP into soluble P. In this context, biofertilizers like phosphate solubilizing bacteria (PSB) can be proved effective. The PSB releases organic acids in the rhizosphere which decreases the rhizosphere pH and helps in dissolution of insoluble P. If the Indian origin low quality RP can be utilized for direct application with PSB, it can be a great boon, as 42 per cent of total consumption of P fertilizer in India is imported from other countries (FAI, 2016).

The concentration of phosphate (Pi) in the soil solution is often low (2–10 μM) due to its fixation in soil (Mahanta et al., 2014). Further, the supply of Pi to the root surface by diffusion is slow, and hence, higher application rate of phosphatic fertilizer is recommended. Excess soluble P fertilizer added to crops may run off from the soil into surface waters, resulting in P enrichment of water body, with a consequent loss of habitats and decline in biodiversity. Hence, enhancing the P efficiency is the major criteria. The role of arbuscular mycorrhiza (AM) in the acquisition of P from the soil through its hyphae and enhancing P uptake have been well documented (Cely et al., 2016; Kodre et al., 2017). Hence, dual inoculation of PSB and AM may enhance phosphorus use efficiency through solubilization and mobilization of P, respectively (Mahanta et al., 2014). The root growth is highly dependent upon P nutrition. Hence, improved P use efficiency with inoculation of PSB and AM may influence root properties or vice versa (Kuang et al., 2005; Fageria and Moreira, 2011; Alori et al., 2017; Cortivo et al., 2017).

Although several studies have documented the effect of soluble phosphatic fertilizer with PSB and AM on root morphology and other root properties of different crops (Sheng et al., 2012; Mahanta et al., 2014), but no information is available on comparative influence of lower application rate of insoluble rock phosphate with PSB and AM vis-à-vis soluble phosphatic fertilizer on root properties of soybean [*Glycine max* (L.) Merr.] and wheat (*Triticum aestivum* L. emend. Fiori and Paol.). The hypothesis of the study was that inoculation of PSB and AM with 50% recommended P through RP would help in improving P influx and root properties compared to without inoculation, and finally provide similar root properties and grain yield as application of 100% recommended P through SP. Hence, inoculation of PSB and AM with 50% recommended P through RP vis-à-vis 100% recommended P through SP has been investigated for root cation exchange capacity, P influx, P uptake and other root properties of soybean and wheat in Indo-Gangetic plains and finally the effect on grain yield.

2. Materials and methods

2.1. Experimental site

The experimental site was situated in Indian Agricultural Research Institute, New Delhi, India (Latitude: 28°38′ N, Longitude: 77° 09′ E and Altitude: 228.61 m above mean sea level). It has a semiarid, subtropical climate with hot dry summers and cold winters. The mean maximum temperature during the hottest month of July is about 38.9 °C, while the mean minimum temperature in the coldest month of January is as low as 6.3 °C. The normal onset period of monsoon is in the third week of June. The mean annual rainfall is 614 mm, three-fourth of which is received during July to September and the remaining one-fourth between October and June. The soil is sandy loam in texture, deep percolating and well drained, hypothermic family of the Typic Ustochrept (old alluvium). The soil had the following characteristics in 0-0.15 m depth: pH 8.36 (1:2.5 soil:water suspension), easily oxidizable ($K_2Cr_2O_7$ + H_2SO_4) organic C 5.8 g kg^{-1}, alkaline $KMnO_4$ oxidizable N 66.7 mg kg^{-1}, 0.5 M $NaHCO_3$ extractable P 6.2 mg kg^{-1} and 1.0 N NH_4OAc exchangeable K 62.6 mg kg^{-1} soil.

Weekly total rainfall and mean weekly maximum and minimum air temperature were recorded throughout the experimental period (Fig. 1A). Mean weekly maximum and minimum soil temperatures measured at different depths (5, 10 and 15 cm) during the experimental period are presented in Fig. 1B.

2.2. Experimental design and treatments

The field experiment was conducted during 2005–2007 and the experiment included two crops per year, i.e. soybean (July-October) and wheat (November-April). The treatments were distributed in a randomized complete block design with three replications in a fixed plot size of 5 m × 4.5 m. For the purpose of this study, only seven selected treatments (phosphorus management practices) were considered. These seven treatments were: 1.0 SP (recommended application rate of P through single superphosphate), 1.0 RP {recommended application rate of P through rock phosphate (RP)}, 0.5 RP (half the recommended application rate of P through RP), 0.5 RP + Phosphate solubilizing bacteria (PSB), 0.5 RP + Arbuscular mycorrhiza (AM), 0.5 RP + PSB + AM and the un-amended control. The treatment control means, there was no application of phosphorus, but the recommended level of nitrogen and potassium were applied. The recommended application rate of P for soybean and wheat were 34.9 and 26.2 kg P ha^{-1}, respectively.

A biofertilizer is a substance which contains living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). The different biofertilizers have been used under this experiment as common application and as per the requirement of treatments. *Bradyrhizobium japonicum* inoculant was common for all the treatments in soybean crop. *Bradyrhizobium japonicum* was available in a charcoal:soil (3:1 ratio) based carrier formulation. The inoculant carrier containing around 10^8 *B. japonicum* cells g^{-1} was obtained from Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi and the inoculation rate was 10 g carrier kg^{-1} seed. 10% *jaggery* (evaporated raw sugarcane juice) solution was prepared by boiling. After proper cooling of *jaggery* solution the inoculant carrier was added to form slurry. The slurry was then mixed with the seed until it was evenly coated. The coated seed was left to dry in the shade for 30 min and used for sowing. The recommended rates of N (30 kg N ha^{-1} for soybean and 120 kg N ha^{-1} for wheat) and K (33.2 kg ha^{-1} for both soybean and wheat) were applied uniformly to all the plots. In wheat, the nitrogen was applied in two splits, half at sowing and the remaining half after first irrigation (30 days after sowing), while whole amount of the recommended N was applied during sowing for soybean. Fertilizers used were urea (46% N) for N and muriate of potash (KCl-50% K) for K. The inoculum species used for PSB and AM were *Pseudomonas striata* and *Glomus fasciculatum*, respectively and were obtained from Division of Microbiology, ICAR-IARI, New Delhi. PSB and AM were used as seed inoculation and field application, respectively. The carrier of PSB was sterilized charcoal and soil (3:1 ratio). 10% *jaggery* solution was prepared by boiling to be used as sticker for seed inoculation with *P. striata*. After proper cooling of *jaggery*, *P. striata* culture was thoroughly mixed with it. The seed was heaped on a clean polythene sheet. The inoculant slurry was poured on seed and was mixed uniformly. The inoculated seed was air-dried in shade and used for sowing. The PSB was inoculated at the rate of 500 g carrier ha^{-1} and the population count was 10^8 cells g^{-1} of the carrier. The host plant used to produce the AM inoculum was *Chloris gayana*. The carrier used for AM was soil. The AM carrier was mixed thoroughly with slightly moistened soil and applied in the rows during sowing below the seed with the help of metallic tube attached to hand plough. AM was applied as 5 kg carrier ha^{-1} and the spore count was 30 spores g^{-1} carrier. Single superphosphate (69.9 g kg^{-1} water-soluble P) and Mussoorie rock phosphate (total 87.3 g kg^{-1} P) were applied as per requirement of the treatment. The phosphatic fertilizers were placed in band below the seed row zone. The Low-grade rock phosphate used for this experiment was collected from Rajasthan State Mines and Minerals Ltd., Udaipur, Rajasthan, India. The ground rock phosphate had a particle size of 100 mesh. It falls under low category having 8.73% total P, 6.53% total Ca, 5.61% total Mg, and micronutrient contents of 1000, 665, 22.8 and 43.1 mg kg^{-1} of total Fe, Mn, Cu and Zn, respectively.

2.3. Agronomic practices

Soybean and wheat cultivars were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Soybean cultivar “PK 1042” was sown (80 kg seeds ha^{-1}) in the third week of July each year. The seeds were manually sown in rows 0.45 m apart at a depth of about 50 mm. After seeding, a light roller was dragged to cover the seeds. Full doses of N, P and K in soybean were incorporated before sowing. Hand weeding was also done to manage the weeds and plant protection measures were applied as needed to control the diseases and pests. Crops were harvested manually 50 mm above the ground at physiological maturity in the second week of October using sickles. After soybean harvest, wheat was sown in the third and fourth week of November in the first and second year, respectively. Wheat cultivar “HD 2643” was sown by hand (100 kg seeds ha^{-1}) in rows 0.225 m apart to a depth of 50 mm. Hand weeding was also done to manage the weeds. Wheat was harvested at 50 mm above the soil surface in the fourth week of April and straw was removed from the plots. Both the crops were cultivated under irrigated conditions.

2.4. Economics

Economic analysis of the data was done based on the prevailing cost of inputs/operations and price of produce. The cost of cultivation for growing crops involved the expenditure towards land preparation, seed and sowing, fertilizer and bio-fertilizer and their application, irrigation, inter-cultivation, spraying for pest control, harvesting, threshing and cleaning and land cost. Gross returns were worked out based on the price of main produce (grain) and by-product (straw) of the crops as follows: US $202 and $204 t^{-1} of soybean grain in the year 2005 and 2006, respectively; US $8 and $10 t^{-1} of soybean stover in the year 2005 and 2006, respectively; US $130 and $150 t^{-1} of wheat grain in the year 2005–06 and 2006-07, respectively; US $25 and $28 t^{-1} of wheat straw in the year 2005–06 and 2006-07, respectively. Net returns were estimated by deducting the total cost of cultivation from the gross returns, and the net returns per US$ invested (benefit cost ratio − B:C ratio) was estimated by dividing the net returns with the cost of cultivation.

2.5. P uptake (PU)

$$PU = (\text{Shoot DW} \times \text{shoot P}) + (\text{Root DW} \times \text{root P})$$

where, shoot DW and root DW represent dry weight of shoot and root, respectively. Shoot P and root P represent P concentration in shoot and root, respectively. The root and shoot P concentration was estimated by Vanadomolybdate yellow colour method (Jackson, 1973). For soybean crop, nodule dry weight and P content in nodule were considered for estimation of P uptake.

2.6. Rhizosphere studies

2.6.1. Rhizosphere soil

The root system, together with adhering soil was carefully removed from the soil. Then shaking of the root system was done to remove the bulk soil and only the soil adhered to the root was considered as rhizosphere soil (Mahanta et al., 2014). Then the rhizospheric soil pH was measured in a 1:2.5 soil:water suspension after shaking for 30 min (Jackson, 1973). The available P (0.5 M $NaHCO_3$ extractable P at pH 8.5) (Olsen et al., 1954) and available N (distillation with alkaline $KMnO_4$ and trapped in boric acid as NH_3) (Subbiah and Asija, 1956) were estimated. The population count of PSB in the rhizosphere of soybean and wheat at flowering stage from different phosphorus management practices were enumerated by the soil dilution plate method. Pikovskaya Agar was used for recording the population counts of total PSB (Mahanta et al., 2017).

2.6.2. Root studies

Root studies with respect to root length, root surface area, root volume and root radius were carried out during both the years in two stages i.e. at 40 and 80 days after sowing (DAS) for soybean and 45 and 90 DAS for wheat crop. The second sampling coincided with flowering stage of respective crops. The root growth reaches maximum during flowering stage and then decline. After the harvest of above ground parts of the plant, roots were sampled by the core (150 mm height, 80 mm diameter). Five root sampling cores were used on the second rows from both sides in each plot on each sampling date. The sampling tube was centered over the plant and a sample was taken to a depth of 150 mm. The roots were obtained by gradually loosening the soil in cores that were immersed in water. The remaining soil adhered to roots were washed properly by putting roots on the container with sieves of several mesh sizes to prevent loss of fine roots during washing. Roots were immediately taken in plastic sealable bag and kept in the refrigerator set at 4 °C until analysis. Then these root samples were dried in oven at 70 °C until constant weight and the dry weight was recorded.

2.6.3. Root length, surface area, volume and average radius

Root length, surface area, volume and average radius were measured using a Hewelett Packard scanner controlled by Win-RHIZO Programme V. 2002C Software (Regent Instruments Inc. Ltd., Quebec, Canada). Here roots were placed in the plexiglass trays (0.2 m x 0.3 m) with 5–10 mm deep water layer. To minimize overlapping, the roots were spread over the tray and the scanning was performed. After scanning, the analysis of the image was done by the programme itself.

2.6.4. Root length density (RLD)

Root length density was calculated as the ratio of the root length (RL, km) to the root sampling core volume (V, m^3) (Myers et al., 2007).

$$\text{RLD} \quad (\text{km}/_{\text{m}^3}) = \frac{\text{RL}}{\text{V}}$$

2.6.5. Root surface area density (RSAD)

Root surface area density was calculated as the ratio of the root surface area (RSA, m^2) to the root sampling core volume (V, m^3).

$$\text{RSAD} \quad (\text{m}^2/_{\text{m}^3}) = \frac{\text{RSA}}{\text{V}}$$

2.6.6. Root volume density (RVD)

Root volume density was calculated as the ratio of the root volume (RV, mm^3) to the root sampling core volume (V, m^3).

$$\text{RV} \quad (\text{m}^3/_{\text{m}^3}) = \frac{\text{RV}}{\text{V}}$$

2.6.7. Sphere of root influence (SRI)

Sphere of root influence (SRI) gives information on average distance of a root to the nearest neighbour. It is the average distance between two roots as located inside the soil of a crop. Indirectly, it indicates the number of root hairs present in a particular volume of soil. When the sphere of influence will be reduced, it means the roots are closer to each other. The formula for sphere of root influence (SRI) as given by Klepper and Rickman (1990) is:

$$\text{SRI(mm)} = \frac{4}{\sqrt{\text{RLD}}}$$

2.6.8. Mean root age (t)

Mean root age (t) was calculated assuming exponential root growth (Fohse et al., 1988).

$$\text{Mean root age (t)} = \frac{t_2 - t_1}{1\text{n}(L_2) - 1\text{n}(L_1)}$$

where, t is time, L is root length and the subscripts refer to the first and second observation. The first observation was taken on 40 and 45 DAS for soybean and wheat crop, and the second observation was taken on 80 and 90 DAS for soybean and wheat crop, respectively.

2.6.9. Root cation exchange capacity

The root samples at flowering stage were powdered, sieved through 1 mm sieve and cation exchange capacity of root was then determined by adding HCl to ground root and washing through KCl, which was titrated by KOH to bring back to neutral pH (7.0) (Crooke, 1964).

2.7. Phosphorus influx (P influx)

Phosphorus influx into the roots during the two inter-sampling periods (40–80 DAS and 45–90 DAS for soybean and wheat, respectively) was calculated by the formula (Vandamme et al., 2013):

$$\text{P influx} = \frac{(\text{PU}_2 - \text{PU}_1) \times \ln\left(\frac{\text{RSA}_2}{\text{RSA}_1}\right)}{(t_2\text{-}t_1) \times (\text{RSA}_2\text{-RSA}_1)}$$

where, PU refers to total P uptake in plant, t to plant age and RSA to root surface area. The subscripts refer to the first and second observations of respective crops.

2.8. P uptake efficiency

Fohse et al. (1988) showed that the P uptake efficiency (PUE) was related to the nutrient concentration of the shoots as follows:

$$\text{PUE(\%)} = \text{I}\times \frac{\text{RL}}{\text{SW}} \times \text{T}\times 100$$

where, I = P uptake rate per unit of root length, RL/SW = root-shoot ratio, RL = root length, SW = shoot dry weight and T = the average period of time the roots absorb P.

For most plants, the concentration of the absorbing roots is greatest in the upper part of the root zone and near the base of the plant. The distribution of active roots in soil is approximately triangular in shape (Reddi and Reddy, 1997), the greatest concentration being near the soil surface. The usual extraction pattern shows that about 40% comes from the upper quarter of the root zone, 30% from the second quarter, 20% from the third quarter and 10% from the bottom quarter. The root zone depth of soybean and wheat are 0.9 and 1.2 m, respectively (Reddi and Reddy, 1997; Mahanta et al., 2014). The RLD, RSAD, RVD, SRI, mean root age, P uptake per root length, P uptake efficiency and P influx were estimated by converting from 0.15 m root depth under direct observation to 1.2 and 0.9 m for soybean and wheat, respectively with consideration of four quarters of the root zone.

2.9. Statistical analysis

Statistical analysis of the data was done using analysis of variance (ANOVA) technique and following SAS 9.4 software (SAS Institute, Cary, North Carolina, USA). Significant differences ($p < 0.05$) among means of experimental results were evaluated by ANOVA and means were compared by Tukey's Honest Significant Difference (HSD) Test. Correlations between various parameters were done by using statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL). For multifactorial comparison, principal component analysis (PCA) was used to display the correlation between the various parameters. Multifactorial analysis was carried out using the XLSTAT 2010 software (ADDINSOFT, New York, NY 10001, USA). Contrast analysis for comparison of selected phosphorus management combinations was also done.

3. Results and discussion

3.1. Rock phosphate and biofertilizer on grain yield and economics

There was significant increase in mean grain yield of soybean and wheat due to application of different phosphorus sources and biofertilizers (Table 1) in comparison with the control plot. The highest grain yield of soybean was observed in the plots received with 0.5 RP + PSB + AM treatment, which was 3.4% higher than 1.0 SP treatment. However, in case of wheat, the highest yield was recorded in the plots under application of 1.0 SP. The yield observed under 0.5 RP + PSB + AM plots was almost the same as 1.0 SP for wheat crop. The grain yield of 0.5 RP + PSB + AM and 1.0 SP were statistically at par with each other for both soybean and wheat crops. Interestingly, the highest grain yield was not achieved with application of 1.0 SP for soybean, although, the highest level of P (34.9 kg P ha^{-1}) in soluble form was applied under 1.0 SP treatment, in comparison with only 17.5 kg P ha^{-1} under 0.5 RP + PSB + AM treatment. It clearly indicated that P acquisition is more important than its amount and availability for higher grain yield of crops. The higher grain yield under 0.5 RP + PSB + AM and 1.0 SP treatments might be due to significantly higher P uptake compared to other treatments (Table 3). The AM might have enhanced the acquisition of P and finally the uptake under 0.5 RP + PSB + AM (Mackay et al., 2017; Sun et al., 2017). The increase of grain yield under 0.5 RP + PSB + AM might have been further enhanced due to the release of the plant growth promoting substances from PSB (Mahanta et al., 2014). Hence, co-inoculation of PSB and AM reduced P application through rock phosphate.

Application of 0.5 RP with or without PSB and AM provided 34–78% and 9–32% higher grain yield of soybean and wheat, respectively compared to control plots, which indicated that the grain yield was better in soybean than wheat due to rock phosphate with or without PSB and AM. It was also clarified from the higher probability value in the contrast analysis of “1.0 RP $_{vs}$ 1.0 SP” and “0.5 RP + PSB + AM $_{vs}$ 1.0 SP” for grain yield of soybean than that of wheat (Table 4). This might be due to the release of citric, malonic, malic and succinic acids through soybean roots, which can solubilize P from RP as well as soil and might have provided higher soil-available P (Table 7) to the crop (Lan et al., 2016; Trabelsi et al., 2017; Wei et al., 2017). Again, the dissolution of RP with or without biofertilizers might have been reduced due to low soil and atmospheric temperature during wheat growing season (Fig. 1A and B). The treatment 0.5 RP + PSB increased 19 and 14% grain yield, while 0.5 RP + AM enhanced 14 and 5% for soybean and wheat, respectively compared to 0.5 RP treatment. It indicated that the response of PSB with rock phosphate for grain yield was more than that of AM. It was also clarified from the lower probability value in the contrast analysis of “PSB $_{vs}$ without PSB” than “AM $_{vs}$ without AM” for grain yield of soybean and wheat (Table 4). The performance of AM with RP was poor, because the fungi translocate the available nutrients from labile pool only, but do not possess special mechanism to solubilize RP (Bagyaraj et al., 2015; Mackay et al., 2017). There was 32 and 20% increase in grain yield with inoculation of both biofertilizers (PSB and AM) for soybean and wheat, respectively. PSB might have solubilized RP by excreting organic acids, i.e. citric and gluconic acids (Trabelsi et al., 2017; Wei et al., 2017), and chelating materials in the immediate vicinity of rhizosphere. AM might have further increased P uptake by more exploration of soil volume thereby making ‘positionally unavailable’ nutrients ‘available’. This might have been achieved by decreasing the diffusion distance of phosphate ions and by increasing the surface area for absorption through the thinner hyphae of AM (2–4 μm diameter) than the diameter of root hairs (Mahanta et al., 2014). The performance of RP with or without biofertilizers was better in the second year of both crops than the first year, which was clearly proved from the contrast analysis of “1.0 RP $_{vs}$ 1.0 SP” and “0.5 RP + PSB + AM $_{vs}$ 1.0 SP” (Table 4) and grain yield (Table 1). The greater P availability in the second year might be due to the solubilization of un-dissolved RP left in the first year along with freshly applied in the second year through solubilizing effect of PSB and also through the root exudates of carboxylates and organic acids in case of soybean (Lan et al., 2016; Trabelsi et al., 2017; Wei et al., 2017).

The cost of P input for soybean and wheat crop was 398 and 342% higher with the use of 1.0 SP compared to 0.5 RP + PSB + AM, respectively (Table 2). Plots under 0.5 RP + PSB + AM and 1.0 SP provided the highest gross return of soybean and wheat crop, respectively. The gross return of wheat under 0.5 RP + PSB + AM was very close to 1.0 SP treatment. However, there was no significant difference among 1.0 SP, 0.5 RP + PSB + AM and 0.5 RP + PSB treatments for gross return of both soybean and wheat crops. The highest gross return obtained in the plots under 0.5 RP + PSB + AM and 1.0 SP was due to higher yield of soybean and wheat, respectively (Table 1). Despite the highest gross returns of wheat was recorded from 1.0 SP treated plots, the net returns US^{-1} invested (B:C ratio) was significantly higher in the plots under 0.5 RP + PSB + AM (1.09 and 2.02 for soybean and wheat, respectively) than the former treatment (0.77 and 1.79 for

Table 1
Effect of rock phosphate with biofertilizers and single superphosphate on grain yield (Mg ha^{-1}) of soybean-wheat cropping system.

Treatment[#]	Soybean			Wheat		
	I year	II year	Mean	I year	II year	Mean
Control	1.24 ± 0.14d[†]	1.09 ± 0.11d	1.17 ± 0.05d	3.26 ± 0.17d	3.17 ± 0.27c	3.21 ± 0.21d
1.0 RP	1.61 ± 0.12bc	1.75 ± 0.18bc	1.68 ± 0.07bc	3.65 ± 0.13bc	3.66 ± 0.21b	3.65 ± 0.17bc
0.5 RP	1.52 ± 0.18bc	1.62 ± 0.18c	1.57 ± 0.09c	3.50 ± 0.19 cd	3.53 ± 0.21bc	3.51 ± 0.09 cd
0.5 RP + PSB	1.81 ± 0.17ab	1.93 ± 0.17ab	1.87 ± 0.05ab	3.97 ± 0.15ab	4.05 ± 0.21ab	4.01 ± 0.18ab
0.5 RP + AM	1.71 ± 0.12b	1.86 ± 0.16b	1.79 ± 0.02b	3.67 ± 0.18bc	3.72 ± 0.08b	3.69 ± 0.12bc
0.5 RP + PSB + AM	1.96 ± 0.13a	2.18 ± 0.18a	2.07 ± 0.16a	4.16 ± 0.22ab	4.31 ± 0.24a	4.23 ± 0.20a
1.0 SP	1.98 ± 0.14a	2.03 ± 0.13ab	2.00 ± 0.12a	4.27 ± 0.12a	4.34 ± 0.37a	4.30 ± 0.23a

[#] See materials and methods section for treatment details.
[†] Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.

Table 2
Effect of rock phosphate with biofertilizers and single superphosphate on economics of soybean-wheat cropping system.

Treatment[#]	Soybean			Wheat			S-W system[‡]
	Cost of P input (US $ ha^{-1})	Gross return (US $ ha^{-1})	B:C ratio	Cost of P input (US $ ha^{-1})	Gross return (US $ ha^{-1})	B:C ratio	B:C ratio
Control	0.00	300 ± 11e[†]	0.26 ± 0.05d	0.00	666 ± 36e	1.43 ± 0.13d	0.89 ± 0.07d
1.0 RP	11.45	424 ± 15bc	0.70 ± 0.06c	8.59	748 ± 28c	1.65 ± 0.10c	1.20 ± 0.03c
0.5 RP	5.73	396 ± 22 cd	0.63 ± 0.09c	4.30	724 ± 14 cd	1.60 ± 0.05 cd	1.15 ± 0.06c
0.5 RP + PSB	6.86	469 ± 12ab	0.91 ± 0.05ab	5.43	811 ± 26ab	1.91 ± 0.09ab	1.44 ± 0.03ab
0.5 RP + AM	8.00	448 ± 5bc	0.82 ± 0.02bc	6.57	752 ± 17bc	1.68 ± 0.06bc	1.28 ± 0.04bc
0.5 RP + PSB + AM	9.14	517 ± 36a	1.09 ± 0.15a	7.70	849 ± 29ab	2.02 ± 0.10a	1.58 ± 0.12a
1.0 SP	45.45	501 ± 29ab	0.77 ± 0.10bc	34.09	859 ± 44a	1.79 ± 0.14b	1.30 ± 0.10bc

[#] See materials and methods section for treatment details.
[†] Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.
[‡] Soybean-wheat cropping system.

Table 3
Effect of rock phosphate with biofertilizers and single superphosphate on phosphorus uptake of soybean and wheat in the Indo-Gangetic plains (mean of two years).

Treatment[#]	Phosphorus uptake (mg plant^{-1}) at flowering stage	
	Soybean	Wheat
Control	61 ± 6e[*]	7.8 ± 0.5d
1.0 RP	91 ± 5 cd	12.6 ± 1.0c
0.5 RP	76 ± 2de	10.5 ± 0.5 cd
0.5 RP + PSB	119 ± 11b	17.4 ± 0.7b
0.5 RP + AM	102 ± 5bc	13.3 ± 1.7c
0.5 RP + PSB + AM	155 ± 18a	20.2 ± 1.3ab
1.0 SP	150 ± 7a	21.4 ± 0.6a

[#] See materials and methods section for treatment details.
[*] Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.

soybean and wheat, respectively) for both soybean and wheat crops as well as soybean-wheat cropping system. The lower cost of inputs (RP and biofertilizers) enhanced B:C ratio under 0.5 RP + PSB + AM compared to 1.0 SP treated plots. Hence, 0.5 RP + PSB + AM was economically more sustainable than 1.0 SP treatment.

3.2. Rock phosphate and biofertilizer on P uptake and concentration

The highest P uptake at flowering stage was recorded with the treatment of 0.5 RP + PSB + AM (155 mg plant^{-1}) in soybean crop, while that in wheat crop was recorded under 1.0 SP plots (21.4 mg plant^{-1}) (Table 3). But, the P uptake under 0.5 RP + PSB + AM and 1.0 SP plots were non-significant for both crops. The higher P uptake under 1.0 SP and 0.5 RP + PSB + AM plots at flowering stage was due to the higher rhizospheric available P (Table 7) and relatively higher value of P concentration in different plant parts (Table 5) and better root properties of both crops under this plot (Figs. 2 A–D and 3 A–D; Table 6). The P uptake by plant was not directly proportional to the rhizospheric available P status at flowering stage (Tables 3 and 7). It is worth mentioning that inoculation with AM dramatically enhanced the P uptake to the range of 30–33 and 16–27% in soybean and wheat, respectively. The highest root, shoot and nodule P concentration at flowering stage of soybean was observed under 0.5 RP + PSB + AM plots (3.84, 3.64 and 5.12 mg g^{-1} for root, shoot and nodule, respectively), while those of wheat at flowering stage were estimated under 1.0 SP plots (1.90 and 3.35 mg g^{-1} for root and shoot, respectively) (Table 5). The P concentration in different plant parts of wheat under 0.5 RP + PSB + AM plots were statistically at par with 1.0 SP plots. Similar trends were also recorded for P concentration in root and shoot of both crops, at 40/45 days after sowing stage. The higher P concentration during flowering stage under 0.5 RP + PSB + AM might be due to the higher P influx and uptake efficiency (Table 6; Figs. 2 B and 3 B).

3.3. Rock phosphate and biofertilizer on P influx and P uptake efficiency

The phosphorus influx to plant system through root surface from soil under 0.5 RP + PSB + AM treatment was highest for both soybean (117.8 mg P m^{-2} root surface area day^{-1}) and wheat (37.9 mg P m^{-2} root surface area day^{-1}) crops and the increases were 9.2 and 0.2% higher than 1.0 SP plots, respectively (Table 6). The P influx under 0.5 RP + PSB + AM was significantly higher than 1.0 SP plots for soybean, while they were non-significant for wheat. The P influx depends upon available P concentration in rhizospheric soil and P uptake efficiency (PUE) of the crop. Ironically, 0.5 RP + PSB + AM plots provided higher P influx than 1.0 SP plots, although the rhizospheric available P status was lower (Table 7) under the former treatment. The higher P influx under 0.5 RP + PSB + AM might have been sustained with higher PUE through the higher P acquisition by the AM infection (Mahanta et al., 2014). The phosphate released by PSB might have taken up by AM and the result was the synergistic interaction that improved P acquisition (Barea et al., 2005; Zhang et al., 2016; Mackay et al., 2017). Further, PSB was stimulated in the root zone of the AM inoculated plants, to provide more soluble P under 0.5 RP + PSB + AM plots (Mahanta et al., 2014; Zhang et al., 2016). The higher P influx was also because of the lower sphere of root influence (SRI) and higher root CEC and better root morphology (Table 6; Fig. 2A–D; Fig. 3A–D). The lower P influx/absorbance with higher soluble P under 1.0 SP may run off from the soil into water body after the harvest of the crop. This will enrich water body with P. Continuous P enrichment may cause severe environmental degradation as loss of habitats of different fauna and decline in biodiversity. Further, manufacture of SP will emit more greenhouse gases, which will accelerate climate change.

The PUE of the plant is the ability of the root system to acquire P from soil and accumulate it in the shoots (Bhadoria et al., 2002). The highest PUE was recorded with the application of 0.5 RP + PSB + AM treatment in both soybean (0.48%) and wheat (1.32%) crops. The PUE depends on the capability of roots to absorb P and the active lifetime of roots (Fohse et al., 1988). The younger roots are more active than older roots, which indicate that lower root age will supply more nutrients. In case of soybean, 0.5 RP + PSB + AM treated plots recorded lower mean root age (Table 6) and might have enhanced absorption of P, which might have helped in enhancing PUE in these plots. The AM might have mobilized P and enhanced the absorption of P and PUE under 0.5 RP + PSB + AM plots of both crops.

3.4. Influence of P management on root CEC

The root CEC under 0.5 RP + PSB + AM (237 mmol kg^{-1}) and 1.0

Table 4
Probability level of significance for contrast analysis of grain yield, P influx, rhizospheric soil properties and root properties of soybean and wheat.

Source	Soybean															
	Grain yield		P uptake		Root CEC		P influx		Available P		pH		PSB		Root surface area density	
	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year
Control $_{vs}$ rest	0.0001	< 0.0001	0.0001	< 0.0001	0.0002	< 0.0001	0.0057	0.0015	< 0.0001	< 0.0001	0.7371	0.9598	< 0.0001	< 0.0001	< 0.0001	0.0002
#1.0 RP $_{vs}$ 1.0 SP	0.0121	0.0610	< 0.0001	0.0005	0.0052	0.0215	0.0109	0.0711	< 0.0001	< 0.0001	0.0251	0.0896	0.1153	0.0156	0.0001	0.0012
0.5 RP $_{vs}$ 0.5 RP + BF	0.0105	0.0044	< 0.0001	0.0001	0.0034	0.0044	0.0049	0.0120	0.0268	0.0009	0.0455	0.0466	< 0.0001	< 0.0001	0.0001	0.0007
PSB $_{vs}$ without PSB	0.0392	0.0332	0.0006	0.0021	0.0223	0.0056	0.0265	0.3431	0.0346	0.0002	0.0224	0.0322	< 0.0001	< 0.0001	0.0007	0.0066
AM $_{vs}$ without AM	0.1444	0.0869	0.0201	0.0360	0.0578	0.0088	0.2148	0.8053	0.8005	0.5192	0.5980	0.4255	0.0399	0.0770	0.0059	0.0892
PSB + AM $_{vs}$ without PSB + AM	0.0040	0.0105	0.0001	0.0002	0.0118	0.0073	0.0070	0.1221	0.0041	0.0004	0.0398	0.0493	< 0.0001	< 0.0001	0.0001	0.0001
0.5 RP + PSB + AM $_{vs}$ 1.0 SP	0.8907	0.2683	0.2683	0.1247	0.7976	0.3556	0.7907	0.2249	< 0.0001	0.0089	0.0003	0.0006	< 0.0001	< 0.0001	0.2223	0.2899
	Wheat															
	Grain yield		P uptake		Root CEC		P influx		Available P		pH		PSB		Root surface area density	
	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year
Control $_{vs}$ rest	0.0002	0.0004	0.0001	< 0.0001	0.0002	0.0009	0.0044	0.0007	< 0.0001	0.0001	0.1539	0.1491	< 0.0001	< 0.0001	0.0009	0.0022
1.0 RP $_{vs}$ 1.0 SP	0.0013	0.0067	0.0005	0.0001	0.0164	0.0314	0.4532	0.0152	< 0.0001	< 0.0001	0.4126	0.5924	0.2123	0.0137	0.0002	0.0038
0.5 RP $_{vs}$ 0.5 RP + BF	0.0040	0.0125	0.0004	0.0001	0.0005	0.1683	0.0412	0.0517	0.0002	0.0003	0.0060	0.0089	< 0.0001	< 0.0001	0.0004	0.0034
PSB $_{vs}$ without PSB	0.0081	0.0269	0.0016	0.0003	0.0029	0.3431	0.0029	0.3431	0.0001	0.0001	0.0029	0.0060	0.0001	< 0.0001	0.0007	0.0138
AM $_{vs}$ without AM	0.2814	0.3754	0.0504	0.1584	0.0164	0.8053	0.0164	0.8053	0.7791	0.7498	0.3903	0.2474	0.0795	0.0561	0.0355	0.0372
PSB + AM $_{vs}$ without PSB + AM	0.0009	0.0211	0.0017	0.0003	0.0044	0.1221	0.0044	0.1221	< 0.0001	0.0001	0.0041	0.0092	< 0.0001	< 0.0001	0.0002	0.0031
0.5 RP + PSB + AM $_{vs}$ 1.0 SP	0.4707	0.8694	0.4478	0.4524	0.8795	0.9018	0.7914	0.8654	< 0.0001	0.0176	0.0156	0.0186	< 0.0001	0.0001	0.4054	0.7107

#RP = Rock phosphate; SP = Single super phosphate; 0.5RP = 50% of recommended P through rock phosphate; BF = Biofertilizer; 0.5 RP + BF = Group of 0.5 RP + PSB, 0.5 RP + AM and 0.5 RP + PSB + AM PSB $_{vs}$ without PSB = 0.5 RP vs 0.5 RP + PSB AM $_{vs}$ without AM = 0.5 RP vs 0.5 RP + AM 0.5 RP + PSB + AM = 50% of recommended P through rock phosphate + PSB + AM; 1.0 SP = 100% recommended rate of P through single superphosphate.

Table 5
Effect of rock phosphate with biofertilizers and single superphosphate on phosphorus concentration in different plant parts of soybean and wheat in the Indo-Gangetic plains (mean of two years).

Treatment[#]	P concentration ($mg\ g^{-1}$) in different plant parts								
	Soybean					Wheat			
	Flowering			40 DAS		Flowering		45 DAS	
	Root	Shoot	Nodule	Root	Shoot	Root	Shoot	Root	Shoot
Control	2.59 ± 0.26d[*]	2.61 ± 0.10d	3.80 ± 0.10c	2.94 ± 0.15d	3.10 ± 0.19d	1.07 ± 0.14c	2.36 ± 0.22d	1.43 ± 0.13c	2.80 ± 0.23c
1.0 RP	3.12 ± 0.03bc	3.05 ± 0.31bcd	4.29 ± 0.09bc	3.27 ± 0.20bcd	3.49 ± 0.14c	1.37 ± 0.11bc	2.73 ± 0.03 cd	1.63 ± 0.08bc	3.14 ± 0.26bc
0.5 RP	2.82 ± 0.15 cd	2.79 ± 0.17 cd	4.00 ± 0.21bc	3.11 ± 0.13 cd	3.29 ± 0.08 cd	1.27 ± 0.19bc	2.50 ± 0.21d	1.58 ± 0.23bc	2.98 ± 0.09bc
0.5 RP + PSB	3.51 ± 0.14ab	3.35 ± 0.09ab	4.71 ± 0.36ab	3.54 ± 0.28abc	3.74 ± 0.11bc	1.63 ± 0.18ab	3.09 ± 0.34abc	1.83 ± 0.05abc	3.43 ± 0.26ab
0.5 RP + AM	3.33 ± 0.23abc	3.18 ± 0.33abc	4.55 ± 0.11ab	3.46 ± 0.22abcd	3.70 ± 0.07bc	1.41 ± 0.13bc	2.78 ± 0.21bcd	1.67 ± 0.04bc	3.25 ± 0.34abc
0.5 RP + PSB + AM	3.84 ± 0.12a	3.64 ± 0.31a	5.12 ± 0.09a	3.70 ± 0.27ab	3.93 ± 0.10ab	1.84 ± 0.22a	3.31 ± 0.32ab	1.84 ± 0.17ab	3.54 ± 0.27ab
1.0 SP	3.83 ± 0.29a	3.59 ± 0.09ab	5.12 ± 0.10a	3.98 ± 0.07a	4.18 ± 0.32a	1.90 ± 0.09a	3.35 ± 0.30a	2.10 ± 0.20a	3.79 ± 0.20a

* Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.
\# See materials and methods section for treatment details.

SP treatments (152 mmol kg^{-1}) were highest for soybean and wheat, respectively (Figs. 2 A and 3 A). The root CEC values of the above two treatments were non-significant and the values under them were significantly higher than rest treatments, except 0.5 RP + PSB and 0.5 RP + AM for soybean crop. It was observed that the root CEC was higher in treatments where rhizospheric P availability was higher (1.0 SP and 0.5 RP + PSB) (Mahanta et al., 2014) and the CEC might have been enhanced by younger (Table 6) and active roots with lower SRI and root surface area density (RSAD). Inoculation of AM further increased the root CEC of 0.5 RP + PSB + AM compared to 0.5 RP + PSB. The increase in root CEC following AM application might be by altering the root cell structure, particularly at the fungus-cell interface through fungus infection. The cytoplasm of the host cell is separated from the hyphae by an interfacial pectic matrix, altering cell wall structure and therefore the higher CEC of AM inoculated roots (Mahanta et al., 2014). Better P availability might have increased the number and the length of root hairs which were more active and possessed more pectic substance and directly increased root CEC (Mahanta et al., 2014).

3.5. P management on root morphology

The lower SRI signifies that the root length is higher and the roots are very closer to each other involving in efficient utilization of phosphorus, due to its immobile nature in soil. The roots under 0.5 RP + PSB + AM plots had the lowest SRI (43.6 and 68.8 mm for soybean and wheat, respectively) for both soybean and wheat crops, which were non-significant with 1.0 SP plots (Table 6). Increase or decrease of total root length, decreased or increased the SRI, respectively (Klepper and Rickman, 1990). Inoculation of PSB and AM maintain the growth of roots by providing enough phosphorus through increasing available P status and acquisition, respectively. However, application of 1.0 SP could not maintain the root length as 0.5 RP + PSB + AM, although having highest available P status (Table 7). It might be due to the lack of better acquisition in comparison to later treatment. The root length in wheat is directly proportional to P nutrition (Postma et al., 2014). Hence, roots under 0.5 RP + PSB + AM plots provided the lowest SRI.

The younger roots are more efficient in acquisition of nutrients and moisture. The lowest mean root age (12.0 days) was observed under 0.5 RP + PSB + AM plot of soybean at flowering stage, which was non-significant with 1.0 SP plots (Table 6). In wheat, there was no significant difference among different treatments. Better nutrition of phosphorus was able to regenerate new finer roots in soybean and the mean root age decreased. In wheat, P nutrition also produced new roots but the production of newer roots was not proportional to the phosphorus nutrition.

The highest root surface area density (RSAD) of soybean (13.7 $m^2\ m^{-3}$) and wheat (6.37 $m^2\ m^{-3}$) was observed under 0.5 RP + PSB + AM and 1.0 SP plots, repectively and these treatments were non-significant with each other for both crops. The highest root volume density of soybean ($179 \times 10^4\ mm^2\ m^{-3}$) and wheat ($91 \times 10^4\ mm^2\ m^{-3}$) were recorded under 1.0 SP plots and the RVD under 0.5 RP + PSB + AM plots was non-significant with it. Root surface area and volume are directly related to P nutrition (Jin et al., 2005; Iman et al., 2006) and hence the highest RSAD and RVD were recorded under the above treatments.

The root radius under the control plots were the thinnest (0.494 mm) among other treatments in soybean crop, while there was no significant difference for wheat crop. The highest root radius was recorded under 1.0 SP plots and that of 0.5 RP + PSB + AM was non-significant with the former. The thinner root under control plots of soybean might have been due to the increased number of root hairs due to P deficiency. This behaviour of soybean root is justified as legume roots typically respond to P deficiency through allocation of more carbon to roots, resulting in increased number of root hairs (Mahanta et al., 2014).

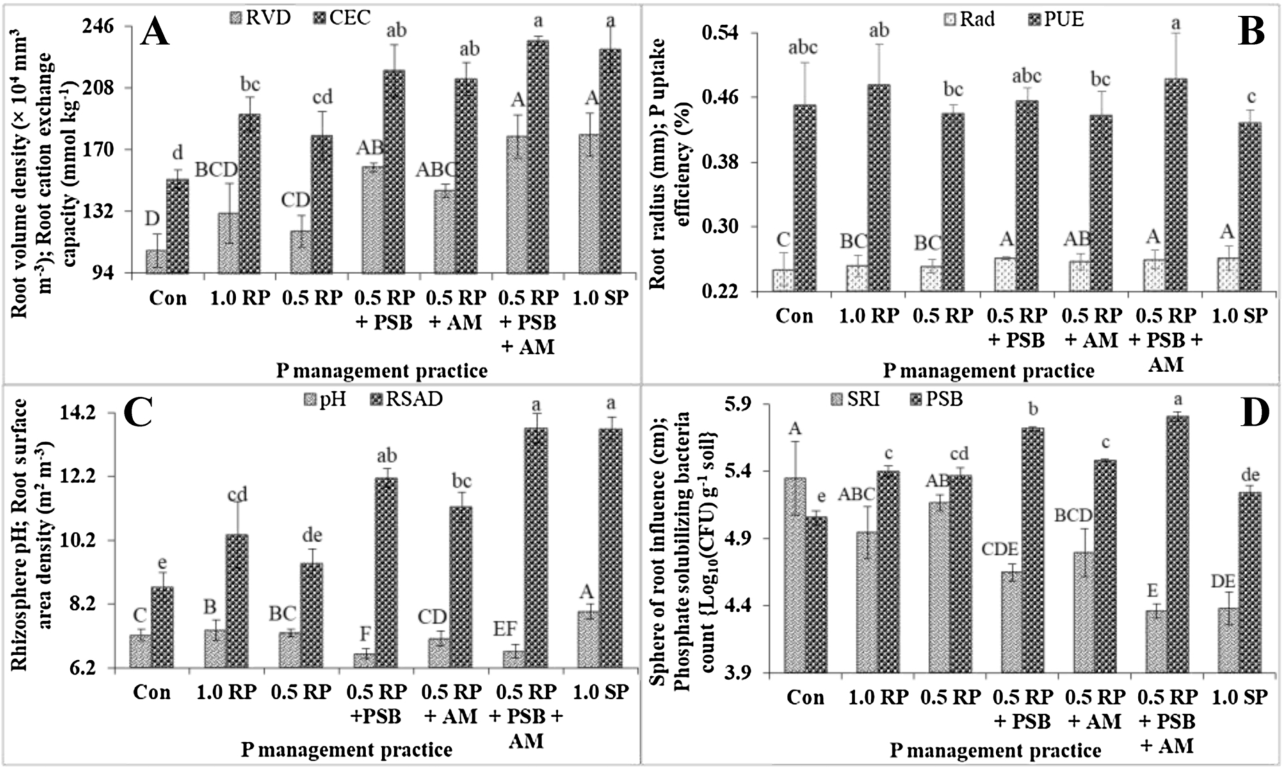

Fig. 2. Phosphorus uptake efficiency, root and rhizospheric soil properties (mean of two years) at flowering stage of soybean under different phosphorus management practices (Fig. 2A. RVD = root volume density; CEC = root cation exchange capacity; Fig. 2B. Rad = root radius; PUE = P uptake efficiency; Fig. 2C. pH = rhizospheric soil pH; RSAD = root surface area density; Fig. 2D. SRI = sphere of root influence; PSB = rhizospheric soil phosphate solubilizing bacteria count). Bars with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different. Error bars represent standard deviation. See materials and methods section for treatment details.

3.6. P management on rhizospheric P, N, pH and phosphate solubilizing bacteria

The available P in the rhizosphere under 0.5 RP + PSB + AM and 0.5 RP + PSB provided significantly higher value than the plots under 0.5 RP at all stages of both soybean and wheat crops. The P availability in soils is reported to be the maximum at the pH value of 6.5 (Havlin et al., 2011). The pH values under above two treatments were nearer to the optimum P availability through the secretion of organic acids by PSB (Trabelsi et al., 2017; Wei et al., 2017). Due to the above reason, the available P of rhizosphere under 0.5 RP + PSB and 0.5 RP + PSB + AM treatments were higher than 0.5 RP. The available P in the rhizosphere under 1.0 SP plots at different stages was significantly higher than all treatments for both soybean and wheat crops, except at flowering stage under 0.5 RP + PSB and 0.5 RP + PSB + AM treatments of second year soybean crop. The increase of rhizospheric available P under 1.0 SP plots at different stages of both soybean and wheat crop was 4.0–17.1 mg kg^{-1} higher than 0.5 RP + PSB + AM plots. The higher available P under 1.0 SP was due to the presence of 91% water soluble P in single superphosphate fertilizer (SP) compared to 0% in RP (FAI, 2016). Further, 50% more amount of P was added to soil under 1.0 SP plot compared to the treatment of 0.5 RP with and without biofertilizer. The greater P availability in the second year soybean under 0.5 RP + PSB and 0.5 RP + PSB + AM might be due to the solubilization of un-dissolved RP left in the first year along with freshly applied in the second year through solubilizing effect of PSB and also through the root exudates of carboxylates and organic acids from soybean crop (Lan et al., 2016; Trabelsi et al., 2017; Wei et al., 2017). The significantly higher population of PSB under 0.5 RP + PSB + AM and 0.5 RP + PSB than other treatments might have also favoured for

Table 6
Effect of rock phosphate with biofertilizers and single superphosphate on root properties of soybean and wheat (mean of two years).

Treatment[#]	Soybean			Wheat		
	P influx (mg day^{-1} m^{-2})	Sphere of root influence at flowering stage (mm)	Mean root age between 40 DAS and flowering stage (day)	P influx (mg day^{-1} m^{-2})	Sphere of root influence at flowering stage (mm)	Mean root age between 45 DAS and flowering stage (day)
Control	63.1 ± 8.8d[*]	53.5 ± 2.7a	16.0 ± 0.4a	21.7 ± 1.6c	83.1 ± 0.4a	17.9 ± 0.4a
1.0 RP	82.7 ± 5.9 cd	49.4 ± 1.9abc	14.4 ± 0.8b	31.1 ± 2.5ab	79.0 ± 1.6ab	19.0 ± 0.1a
0.5 RP	72.5 ± 1.8 cd	51.7 ± 0.6ab	15.3 ± 0.1ab	27.4 ± 0.4bc	81.0 ± 1.3ab	18.7 ± 0.4a
0.5 RP +PSB	96.9 ± 9.5abc	46.5 ± 0.6cde	12.8 ± 0.5 cd	35.3 ± 1.3ab	70.3 ± 1.1 cd	18.8 ± 1.1a
0.5 RP + AM	85.8 ± 5.2bcd	47.9 ± 1.8bcd	13.4 ± 0.3c	29.6 ± 4.0abc	75.4 ± 1.8bc	19.1 ± 0.6a
0.5 RP + PSB + AM	117.8 ± 16.4a	43.6 ± 0.5e	12.0 ± 1.1d	37.9 ± 3.1a	68.8 ± 1.9 cd	19.4 ± 0.6a
1.0 SP	108.7 ± 7.5ab	43.8 ± 1.2e	12.1 ± 1.2d	37.7 ± 3.3a	67.4 ± 3.0d	19.8 ± 1.4a

* Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.
[#] See materials and methods section for treatment details.

Table 7
Effect of rock phosphate with biofertilizers and single superphosphate on rhizosphere available P status (mg kg^{-1}) at different stages of soybean and wheat.

Treatment[#]	Soybean				Wheat			
	I year		II year		I year		II year	
	40 DAS	Flowering	40 DAS	Flowering	45 DAS	Flowering	45 DAS	Flowering
Control	6.0 ± 0.8d[*]	5.3 ± 1.9d	4.4 ± 0.6e	3.9 ± 1.7d	5.4 ± 0.9d	4.8 ± 0.7d	5.1 ± 0.7d	4.5 ± 0.6d
1.0 RP	12.2 ± 1.5 cd	16.8 ± 2.3bc	15.2 ± 2.2 cd	21.3 ± 5.5bc	8.8 ± 1.2d	9.9 ± 1.5c	9.0 ± 1.4d	10.4 ± 2.1 cd
0.5 RP	8.9 ± 1.4d	12.4 ± 2.4c	8.4 ± 1.4de	15.5 ± 1.5 cd	5.8 ± 0.9d	6.2 ± 0.8 cd	5.9 ± 0.9d	8.0 ± 2.7 cd
0.5 RP +PSB	15.8 ± 2.2bc	19.4 ± 2.6b	22.2 ± 3.1bc	36.6 ± 6.2a	17.0 ± 2.4bc	18.1 ± 2.7b	28.4 ± 3.9b	28.3 ± 4.6b
0.5 RP + AM	10.6 ± 1.3 cd	13.1 ± 3.0c	12.4 ± 2.0d	18.2 ± 3.1 cd	10.1 ± 1.7 cd	6.8 ± 1.1 cd	10.6 ± 1.9 cd	9.1 ± 1.4 cd
0.5 RP + PSB + AM	18.1 ± 2.2b	22.9 ± 3.7b	27.5 ± 3.1b	35.1 ± 6.3ab	19.2 ± 1.9b	19.8 ± 3.1b	31.4 ± 4.1b	27.2 ± 5.0b
1.0 SP	57.3 ± 8.4a	43.1 ± 6.4a	62.6 ± 9.1a	47.8 ± 6.2a	42.6 ± 6.4a	33.9 ± 5.3a	44.4 ± 6.2a	36.5 ± 6.6a

* Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.
\# See materials and methods section for treatment details.

higher rhizospheric available P (Figs.2 B and 3 C).

There was significant increase in the available N in the rhizosphere under both soybean and wheat crops at different stages due to application of different phosphorus sources and biofertilizers (Table 8) compared to the control plot, except at 40 DAS of first year soybean, although same amount of N was applied to all treatments. The highest available N was estimated under 1.0 SP plots at all stages during both years in both crops, except at the flowering stage of second year soybean and at 45 DAS of second year wheat crop, where the soil under 0.5 RP + PSB + AM recorded the best. The available N status at different stages of both crops during both years under 1.0 SP and 0.5 RP + PSB + AM plots were almost equal and statistically at par to each other. The available N status under these plots were significantly higher than 0.5 RP treatment for both crops during both years at all stages, except at 40 DAS of first year soybean. The experiment clearly indicated that the variation in rhizospheric available N was due to the different P management practices. It has been already established that the better managed P fix more N_2 in legume crops than poorly managed (Sa and Israel, 1991; Tang et al., 2001). Hence, 1.0 SP and 0.5 RP + PSB + AM treatments might have fixed more N_2 in soybean. The difference in available N might be due to the difference in N_2 fixation in soybean and its residual effect in succeeding wheat crop. The soybean crop at 40 DAS of first year might not have reached the peak of N_2 fixation, which might have been the cause of similar N status for all treatments at this stage. But, the variation of available N at 40 DAS of second year soybean among treatments might be due to the residual effect of first year.

The rhizospheric pH at flowering stage was recorded the lowest in soil under 0.5 RP + PSB plots, followed by 0.5 RP + PSB + AM plots (Figs.2 C and 3 D). The rhizospheric soil under 0.5 RP + PSB + AM recorded 1.25 and 0.65 units lower pH than that observed under 1.0 SP plots in soybean and wheat, respectively. PSBs have been reported to solubilize inorganic forms of RP by excreting a number of organic acids

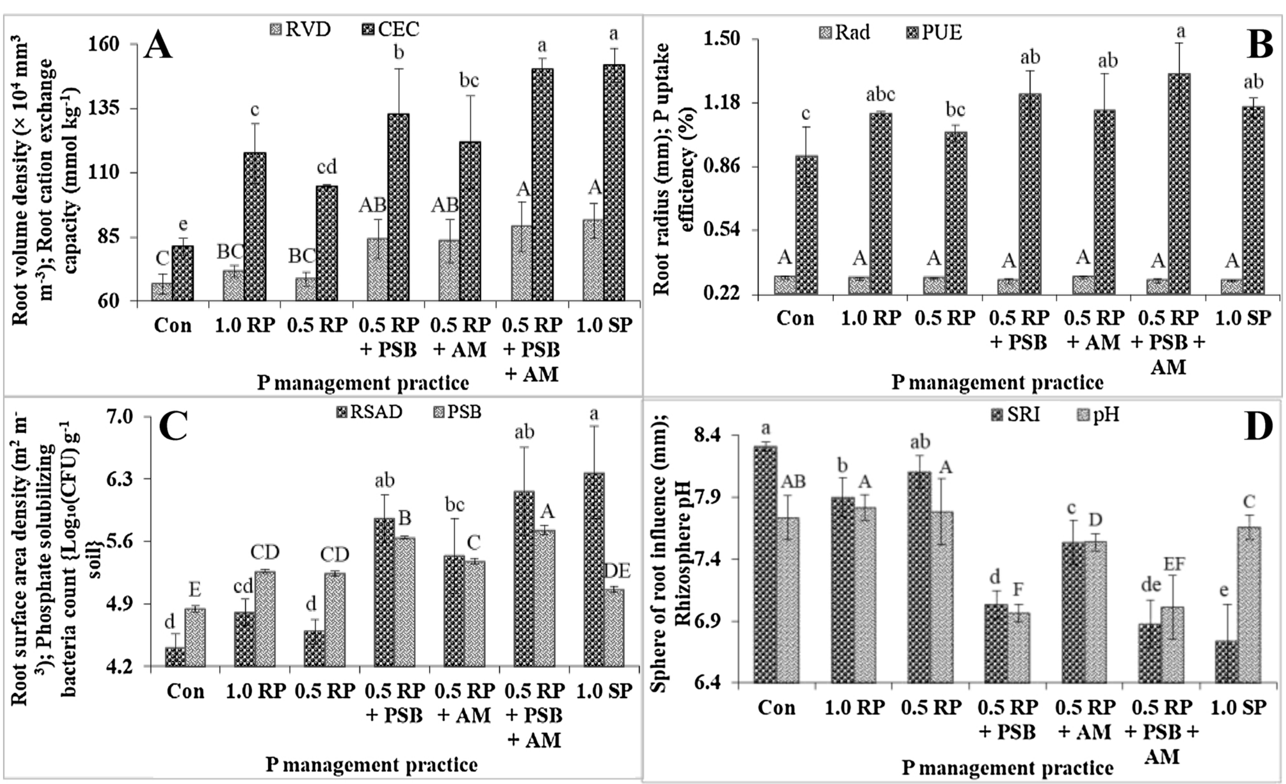

Fig. 3. Phosphorus uptake efficiency, root and rhizospheric soil properties (mean of two years) at flowering stage of wheat under different phosphorus management practices (Fig. 3A. RVD = root volume density; CEC = root cation exchange capacity; Fig. 3B. Rad = root radius; PUE = P uptake efficiency; Fig. 3C. RSAD = root surface area density; PSB = rhizospheric soil phosphate solubilizing bacteria count; Fig. 3D. SRI = sphere of root influence; pH = rhizospheric soil pH). Bars with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different. Error bars represent standard deviation. See materials and methods section for treatment details.

Table 8
Effect of rock phosphate with biofertilizers and single superphosphate on rhizosphere available N status (mg kg^{-1}) at different stages of soybean and wheat.

Treatment[#]	Soybean				Wheat			
	I year		II year		I year		II year	
	40 DAS	Flowering	40 DAS	Flowering	45 DAS	Flowering	45 DAS	Flowering
Control	69.3 ± 4.7a	74.2 ± 6.7c	70.6 ± 7.4b	75.5 ± 7.7c	74.4 ± 7.6b	69.8 ± 1.6b	76.1 ± 10.4c	71.8 ± 4.0c
1.0 RP	70.7 ± 6.8a	79.7 ± 7.9bc	76.0 ± 7.6ab	83.8 ± 9.2bc	79.6 ± 2.8b	74.3 ± 2.6b	84.7 ± 12.5abc	79.4 ± 9.2bc
0.5 RP	70.2 ± 8.3a	76.9 ± 7.2bc	73.6 ± 6.0b	81.3 ± 8.5bc	76.8 ± 5.9b	72.3 ± 12.5b	81.4 ± 9.7bc	77.0 ± 6.6bc
0.5 RP +PSB	74.9 ± 8.9a	85.8 ± 4.6ab	83.9 ± 6.5ab	91.7 ± 9.0ab	87.4 ± 11.3ab	81.8 ± 8.1ab	90.0 ± 10.8abc	84.3 ± 7.1abc
0.5 RP + AM	74.6 ± 5.8a	82.5 ± 6.8ab	78.6 ± 9.8ab	86.8 ± 10.2ab	82.6 ± 4.5ab	77.0 ± 4.9ab	88.0 ± 12.9abc	82.5 ± 6.2bc
0.5 RP + PSB + AM	76.0 ± 7.0a	92.0 ± 4.9a	88.3 ± 7.0a	100.7 ± 9.2a	93.0 ± 12.2a	86.6 ± 13.2a	102.6 ± 17.4a	94.3 ± 9.6a
1.0 SP	78.8 ± 6.4a	93.5 ± 8.8a	88.8 ± 7.5a	96.1 ± 7.9ab	95.0 ± 10.9a	87.3 ± 9.7a	101.9 ± 13.1a	95.8 ± 10.3a

*Values are the mean ± standard deviation of three replications; Means in the same column with different letters are significantly (Tukey's HSD tests, $P < 0.05$) different.
[#] See materials and methods section for treatment details.

Table 9
Correlation coefficients between grain yield with root and soil properties of soybean and wheat.

	PU	Influx	PUE	CEC	Age	SRI	RSAD	RVD	Rad	pH	P	N	PSB	SP	RP	NP
Soybean																
GY	0.935[b]	0.944[b]	0.150	0.986[c]	-0.959[c]	-0.960[c]	0.941[b]	0.924[b]	0.922[b]	-0.099	0.844[a]	0.945[b]	0.659	0.962[c]	0.962[c]	0.949[b]
PU		0.994[c]	0.154	0.957[c]	-0.973[c]	-0.991[c]	0.996[c]	0.995[c]	0.895[b]	-0.038	0.899[b]	0.999[c]	0.606	0.989[c]	0.987[c]	0.992[c]
Influx			0.252	0.959[c]	-0.970[c]	-0.987[c]	0.987[c]	0.985[c]	0.884[b]	-0.124	0.863[a]	0.995[c]	0.681	0.988[c]	0.982[c]	0.983[c]
PUE				0.113	-0.114	-0.136	0.111	0.115	-0.047	-0.578	-0.108	0.153	0.616	0.170	0.130	0.111
CEC					-0.990[c]	-0.983[c]	0.970[c]	0.953[c]	0.952[b]	-0.113	0.847[a]	0.965[c]	0.650	0.984[c]	0.987[c]	0.979[c]
Age						0.993[c]	-0.987[c]	-0.977[c]	-0.960[c]	0.113	-0.869[a]	-0.978[c]	-0.639	-0.993[c]	-0.996[c]	0.992[c]
SRI							-0.997[c]	-0.988[c]	-0.932[b]	0.054	-0.895[b]	-0.992[c]	-0.613	-0.999[c]	-0.999[c]	-0.999[c]
RSAD								0.996[c]	0.925[b]	-0.041	0.904[b]	0.996[c]	0.598	0.994[c]	0.995[c]	0.998[c]
RVD									0.921[b]	-0.056	0.912[b]	0.995[c]	0.606	0.986[c]	0.985[c]	0.989[c]
Rad										-0.137	0.855[a]	0.906[b]	0.602	0.931[b]	0.940[b]	0.929[b]
pH											0.283	-0.069	-0.793[a]	-0.078	-0.058	-0.038
P												0.891[b]	0.303	0.887[b]	0.893[b]	0.894[b]
N													0.631	0.990[c]	0.988[c]	0.992[c]
PSB														0.633	0.612	0.593
SP															0.999[c]	0.997[c]
RP																0.998[c]
Wheat																
GY	0.994[c]	0.985[c]	0.875[b]	0.987[c]	0.883[b]	-0.978[c]	0.967[c]	0.932[b]	-0.843[a]	-0.593	0.929[b]	0.986[c]	0.571	0.993[c]	0.998[c]	
PU		0.974[c]	0.859[a]	0.976[c]	0.865[a]	-0.979[b]	0.970[c]	0.934[b]	-0.843[a]	-0.596	0.939[b]	0.990[c]	0.553	0.997[c]	0.998[c]	
Influx			0.917[b]	0.986[c]	0.876[b]	-0.947[b]	0.924[b]	0.884[b]	-0.842[a]	-0.604	0.882[b]	0.948[b]	0.626	0.973[c]	0.974[c]	
PUE				0.905[b]	0.749	-0.856[a]	0.835[a]	0.825[a]	-0.670	-0.763	0.656	0.842[a]	0.848[a]	0.876[b]	0.857[a]	
CEC					0.929[b]	-0.956[b]	0.948[b]	0.929[b]	-0.766[a]	-0.555	0.870[a]	0.974[c]	0.576	0.977[c]	0.983[c]	
Age						-0.814[a]	0.823[a]	0.828[a]	-0.565	-0.225	0.762[a]	0.885[b]	0.304	0.852[a]	0.884[b]	
SRI							-0.995[c]	-0.972[c]	0.813[a]	0.673	-0.923[b]	-0.970[c]	-0.589	-0.987[c]	-0.976[c]	
RSAD								0.988[c]	-0.764[a]	-0.646	0.909[b]	0.973[c]	0.556	0.978[c]	0.969[c]	
RVD									-0.658	-0.623	0.846[a]	0.956[c]	0.544	0.953[c]	0.941[b]	
Rad										-0.677	-0.904[b]	0.894[b]	0.600	0.925[b]	0.912	
pH											-0.474	-0.560	-0.928[b]	-0.625	-0.575	
P												0.921[b]	0.351	0.933[b]	0.939[b]	
N													0.528	0.986[c]	0.993[c]	
PSB														0.582	0.548	
SP															0.993[c]	
RP																

PU = P uptake at flowering stage of crop; Influx = P influx; PUE = P uptake efficiency; CEC = root CEC at flowering stage of crop; Age = Mean root age; SRI = Sphere of root influence at flowering stage of crop; RSAD = root surface area density at flowering stage of crop; RVD = root volume density at flowering stage of crop; Rad = root radius at flowering stage of crop; pH = rhizosphere soil pH at flowering stage of crop; P = rhizosphere soil available P status at flowering stage of crop; N = rhizosphere soil available N status at flowering stage of crop; PSB = phosphate solubilizing bacteria count at flowering stage of crop; SP = Shoot P concentration at flowering stage of crop; RP = Root P concentration at flowering stage of crop; NP = Nodule P concentration at flowering stage of crop.
[a] Indicate significance at 5% level.
[b] Indicate significance at 1% level.
[c] Indicate significance at 0.1% level.

(Trabelsi et al., 2017; Wei et al., 2017). As acids, they have the effect of decreasing pH of soil medium. Hence, the treatments consisting inoculation of PSB have lower pH, closer to the optimum P availability. P stress stimulates acid production in roots of soybean crop (Mahanta et al., 2014; Liao et al., 2006). This is also the reason of lower pH in soybean crop, where SP is not applied.

There was at least 72% greater population of PSB with its inoculation (under the treatment of 0.5 RP + PSB + AM and 0.5 RP + PSB) in the rhizosphere at flowering stage of both soybean and wheat crops (Figs.2 B and 3 C), which was significantly higher than rest of the treatments. Due to the inoculation of PSB, its population level increased manifold under the soil of 0.5 RP + PSB and 0.5 RP + PSB + AM treatments compared to other nutrient sources. Co-inoculation of AM with PSB further significantly enhanced the population of PSB compared to 0.5 RP + PSB treatment. The PSB population under 0.5 RP + PSB + AM treatment was 23 and 267% higher than the rhizospheric

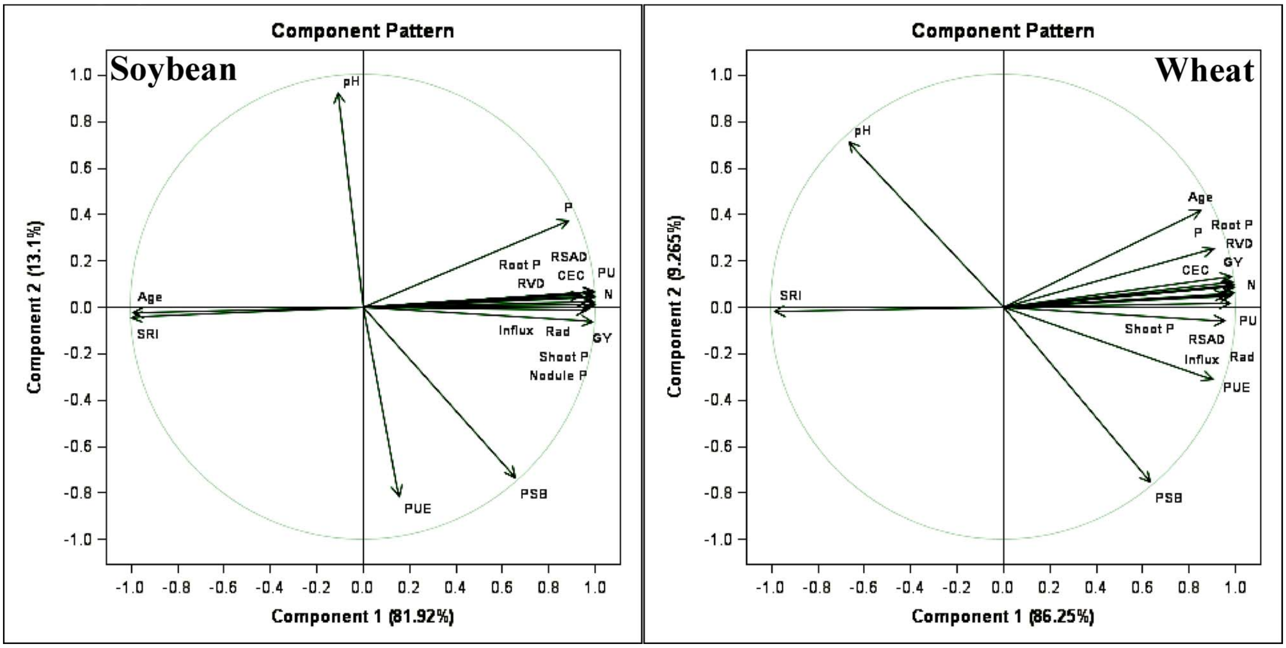

Fig. 4. Multifactorial comparison of root properties with grain yield using principal component analysis (GY = Grain yield; PU = P uptake at flowering stage; Influx = P influx of crop between 40/45 DAS and flowering stage; PUE = P uptake efficiency; CEC = Root cation exchange capacity at flowering stage; RSAD = Root surface area density at flowering stage; RVD = Root volume density at flowering stage; Rad = Root radius at flowering stage; Age = Mean root age; SRI = Sphere of root influence at flowering stage; P = Rhizospheric soil available P at flowering stage; N = Rhizospheric soil available N at flowering stage; pH = Rhizospheric soil pH at flowering stage; Shoot P = P concentration in shoot at flowering stage; Root P = P concentration in root at flowering stage; Nodule P = P concentration in soybean nodule at flowering stage; PSB = Phosphate solubilizing bacteria count at flowering stage).

soil under 0.5 RP + PSB and 1.0 SP plots, respectively. The AM released substantial amount of C to the rhizosphere, triggering PSB growth and activity. In return, the PSB enhanced mineralization of P, increasing P availability for the AM. When additional P was mineralised to increase soil available P, the PSB enhanced AM hyphal growth, and PSB activity was also stimulated by the fungus (Zhang et al., 2016). That's why the population of PSB was significantly higher under 0.5 RP + PSB + AM than 0.5 RP + PSB.

3.7. Contrast analysis and correlation

The contrast analysis for "control vs rest" indicated that P and biofertilizers play a very crucial role for improving root and soil properties and grain yield of soybean and wheat (Table 4). The very low probability of different root and soil properties and grain yield in soybean and wheat for the contrast analysis of "1.0 RP vs 1.0 SP" revealed that rock phosphate alone cannot be used directly for soybean and wheat crops. It is clearly proved from the very low probability in the contrast analysis of "0.5 RP $_{vs}$ 0.5 RP + BF" (group of 0.5 RP + PSB, 0.5 RP + AM and 0.5 RP + PSB + AM treatments), that biofertilizers (PSB and AM) are inevitable with RP application for enhancement of root and soil properties and grain yield of soybean and wheat crops. The contrast analysis "PSB vs without PSB", "PSB + AM vs without PSB + AM" and "0.5 RP + PSB + AM vs 1.0 SP" for rhizospheric pH, rhizospheric available P and PSB under soybean and wheat crops clearly indicated that inoculation of PSB improved these soil properties. The high probability ($P > 0.05$) in the contrast analysis of all root properties and grain yield for "0.5 RP + PSB + AM vs 1.0 SP" indicated that inoculation of PSB and AM with 0.5 RP reduced application of 50% of the recommended P.

A correlation matrix showed significant correlations ($P < 0.05$) between all the different root and soil properties and grain yields, except PUE in soybean (Table 9). Inoculation of PSB played a very significant role for enhancing its population in the rhizosphere and finally reduced the rhizospheric pH considerably, which has been clarified from the negative significant correlation between them.

Principal component analysis (PCA) is a useful statistical technique which had found application in reduction of the original variables in a smaller number of underlying variables (principal component) in order to reveal the interrelationships between the different variables and to find the optimum number of extracted principal components (Mahanta et al., 2015). The PCA comprising two principal components (Component 1 and 2) accounted for 95 and 96% of variance for soybean and wheat crops, respectively.

The longer the line in PCA, the higher is the variance. The variance among the variables in the biplot was almost similar for both soybean and wheat (Fig. 4). The cosine of the angle between the lines approximates the correlation between the variables they represent. The closer the angle to 90 or 270°, the smaller was the correlation. An angle of 0 or 180° reflects a correlation of 1 or −1, respectively (Mahanta et al., 2013). The biplot showed a strong positive relationship between the grain yield with root CEC, root P, shoot P, P influx, P uptake, SRI, RSAD and rhizospheric available N during flowering stage for both crops, while SRI had a strong negative relation with grain yield of both crops (Fig. 4). It was confirmed from the correlation (Table 9) and PCA (Fig. 4) that root CEC, root P, shoot P, SRI, root age, P influx, nodule P, rhizospheric available N, RSAD and P uptake of soybean and root P, P uptake, shoot P, root CEC, rhizospheric available N, P influx, SRI, RSAD, RVD and rhizospheric available P of wheat were very closely correlated with grain yield of soybean and wheat, respectively.

4. Conclusions

The results provided the information that the co-inoculation of PSB and AM with 50% of the recommended P through insoluble rock phosphate (0.5 RP + PSB + AM) markedly improved root CEC, P influx, P uptake, sphere of root influence (SRI) and root surface area density (RSAD) and other root properties of soybean and wheat compared to without inoculation. The P influx, root CEC, P uptake and other root properties under 0.5 RP + PSB + AM were either better or almost equal with the application of recommended P through soluble single superphosphate (1.0 SP). Although, application of 1.0 SP provided considerably higher rhizospheric available P compared to 0.5 RP + PSB + AM, but the highest grain yield of soybean and almost the same yield of wheat as former treatment were recorded under later due to modification of root properties. This indicated that acquisition of P is more important than availability. Further, the net returns US$$^{-1}$ invested under 0.5 RP + PSB + AM treatment was significantly higher than 1.0 SP for soybean-wheat cropping system. The results of this study indicated that dual inoculation of PSB and AM with 50% of recommended P through rock phosphate could be recommended for better root properties, P influx and uptake, higher grain yield and profit of soybean-wheat cropping system in the Indo-Gangetic plains.

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