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Full Length Research Paper

Influence of integrated phosphorus supply and plant growth promoting rhizobacteria on growth, nodulation, yield and nutrient uptake in *Phaseolus vulgaris*

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To guarantee a sufficient phosphorus supply for plants, a rapid and permanent mobilization of phosphorus from the labile phosphorus fractions is necessary, because phosphorus concentrations in soil solution are generally low. Several plant growth-promoting rhizobacteria (PGPR) have shown potential to enhance phosphorus solublization and nodulation of legumes when co-inoculated with *Rhizobium*. This investigation was undertaken to assess the feasibility and compatibility of two mineral phosphorus fertilizers; diammonium phosphate (DAP), triple super phosphate (TSP), poultry manure (PM) and two PGPR strains on the growth, nodulation, yield, nutrient uptake and protein content of common bean (*Phaseolus vulgaris*) under deficient phosphorus supply. Integrated application of mineral phosphorus (P), PM and PGPR significantly increased shoot height, shoot fresh weight, shoot dry weight and leaf chlorophyll content by 67, 160, 51 and 106%, respectively, while increase in root length, root fresh weight and root dry weight was 79, 161, and 187%, respectively, over unfertilized control without PGPR application. Integrated use of different P sources and PGPR also increased number of nodules per plant, nodule fresh weight and nodule dry weight by 158, 107 and 168% over the control. Treatment with PGPR significantly increased number of pods per plant and grain yield by 224 and 96%, respectively over the control. Co-inoculation with *Bradyrhizobium* sp. strain MN-S and *Agrobacterium* sp. strain Ca-18 demonstrated two-fold increase in the proportion of nitrogen (N) and P uptake as well as protein content of the common bean grain was increases by 48%. Therefore, application of PGPR with low P fertilizer rates and PM could be a viable supplementary strategy for maximum benefits in terms of cost of production and sustaining productivity.

Key words: Phaseolus vulgaris L., plant growth promoting rhizobacteria (PGPR), nodulation, yield, phosphorus deficiency, protein.

INTRODUCTION

The total phosphorus (P) percentage in soil accounts approximately for 0.04 to 0.10%, but only 1.00 to 2.50% of which can be absorbed by plants, as most of the phosphorus in soils exists in forms unavailable for uptake by plants (Lin, 1990). The low availability of phosphorus nutrition in soils has become the "limiting factor" for plant and root growth (Borch et al., 1999; Kanako et al., 2004) especially after plants have gained sufficient nitrogen nutrition (Woolmanse and Duncan, 1980). Some 17.5 million

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tons of phosphorus is processed annually from world reserves of rock phosphates, of which approximately 85% is used in the production of fertilizers (Cordell et al., 2009a).

However, reserves of rock- phosphorus are finite with an estimated depletion of quality sources expected to occur within the next 50 to 80 years (Isherwood, 2000). Indeed, world commodity markets have already faced rapid and sharp increase in the price of phosphate rock in recent years that is an approximately 7-fold increase in the period between March 2007 and 2008 (Cordell et al., 2009b).

The need to use renewable forms of energy and reduce costs of fertilizing crops has revived the use of organic fertilizers worldwide (Ramesh et al., 2009). A possible way for recovering poor quality soils is to add manure and compost to improve soil health and quality, thereby enhancing biogeochemical nutrient cycles (Emmerling et al., 2000; Niklasch and Joergensen, 2001; Dutta et al., 2003). To evaluate the full benefits of organic fertilization, more knowledge about its effect on nutrient availability is required. This is complicated, since the transformation of organic compounds and nutrient release is a complex process, depending on many factors such as the stability of organic substances (Gutser et al., 2005), climatic conditions (Dorado et al., 2003), soil properties (Huffman et al., 1996), type of cropping system (Van den Bossche et al., 2005) and interaction with mineral fertilizers (Kaur et al., 2005). Furthermore, the composition of organic fertilizers differs greatly, which influences the contents of organic and inorganic phosphate as well (Traore et al., 1999). Organic matter supply to the soil is one of the most important factors for increasing the productivity in plant, with organic phosphate as a significant part of the soil phosphorus cycle contributing to phosphorus nutrition of plants (Tarafdar and Claassen, 2003; Richardson et al., 2005). Organic fertilizers have equivalent or even better effects on crop yields than phosphorus from mineral sources (Sharpley, 1996).

Amendment with organic residues may influence phosphorus dynamics in soils by means of competition between low-molecular-weight organic acids and phosphates for sorption sites that usually favors adsorption of organic acids and delays phosphorus adsorption (Staunton and Leprince, 1996; Geelhoed et al., 1999). On the other hand, the rate and form of phosphate precipitates can change in the presence of organic compounds. Inskeep and Silvertooth (1988) reported that organic acids inhibit the precipitation of hydroxyapatite and favor the formation of thermodynamically less stable di-Ca-phosphate-dihydrate (Grossl and Inskeep, 1991). Braschi et al. (2003) reported that different rates of organic matter addition increased extractable phosphorus at different soil-moisture regimes by inhibiting phosphorus insolubilization, whereas the adsorption process was not affected.

The common bean *(Phaseolus vulgaris* L.) is an important legume for human nutrition and a major protein and calorie source in the world (Anderson, 2003). But its yield remain low to moderate due to the scarce nodulation, high inputs of chemical fertilizers and low technologies applied(Garcia et al., 2004). Common bean is usually considered poor nitrogen (N)-fixing legume; however, its promising potential to fix nitrogen has been shown in several studies (Asadi et al., 2005; Remans et al., 2008). Poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant, particularly the nodulation promiscuity (Michiels et al., 1998), as well as the great sensitivity to other nodulation-limiting factors, such as high rates of N fertilizer used in intensive agriculture, high temperatures and soil dryness (Graham, 1981; Giller and Cadisch, 1995).

Compatibility of *Rhizobium*-plant cultivars can also greatly affect the efficiency of symbiosis established. This variability often limits the nitrogen-fixing performance of soil native rhizobia or use of commercially available inocula. As a result, increased use of fertilizer nitrogen (N) and P to improve crop yield in developing countries is limited by costs, availability and potential negative environmental impact (Hinsinger, 2001).

With the increasing problems associated with the use of synthetic chemicals in agriculture (impacts on health and the environment), there has been an ever-increasing interest in the use of native and non-native beneficial microorganisms to improve plant health and productivity, while ensuring safety for human consumption and protection of the environment. Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Dashti et al., 1998; Remans et al., 2008).

PGPR can positively affect plant growth through different mechanisms such as nitrogen fixation, production of plant growth regulators (Vessey, 2003) and increasing plant water and nutrient uptake (Dey et al., 2004). PGPR have been shown to increase nodulation and nitrogen fixation of legumes leading to increased plant growth. Green-house and field studies with PGPR strains have demonstrated enhanced nodulation and nitrogen fixation in soybean (Dashti et al., 1998), chickpea (Valverde et al., 2006) and common bean (Figueiredo et al., 2008). The effects of native PGPR strains on common bean in Azad Jammu and Kashmir have not been studied earlier. The objectives of this study were to investigate the integrated effect of inorganic P sources, poultry manure and PGPRs applied to common bean on its growth, nodulation, yield, seed energy content and nutrients uptake under field conditions.

MATERIALS AND METHODS

The study site

This study was carried out on an experimental field at the Research

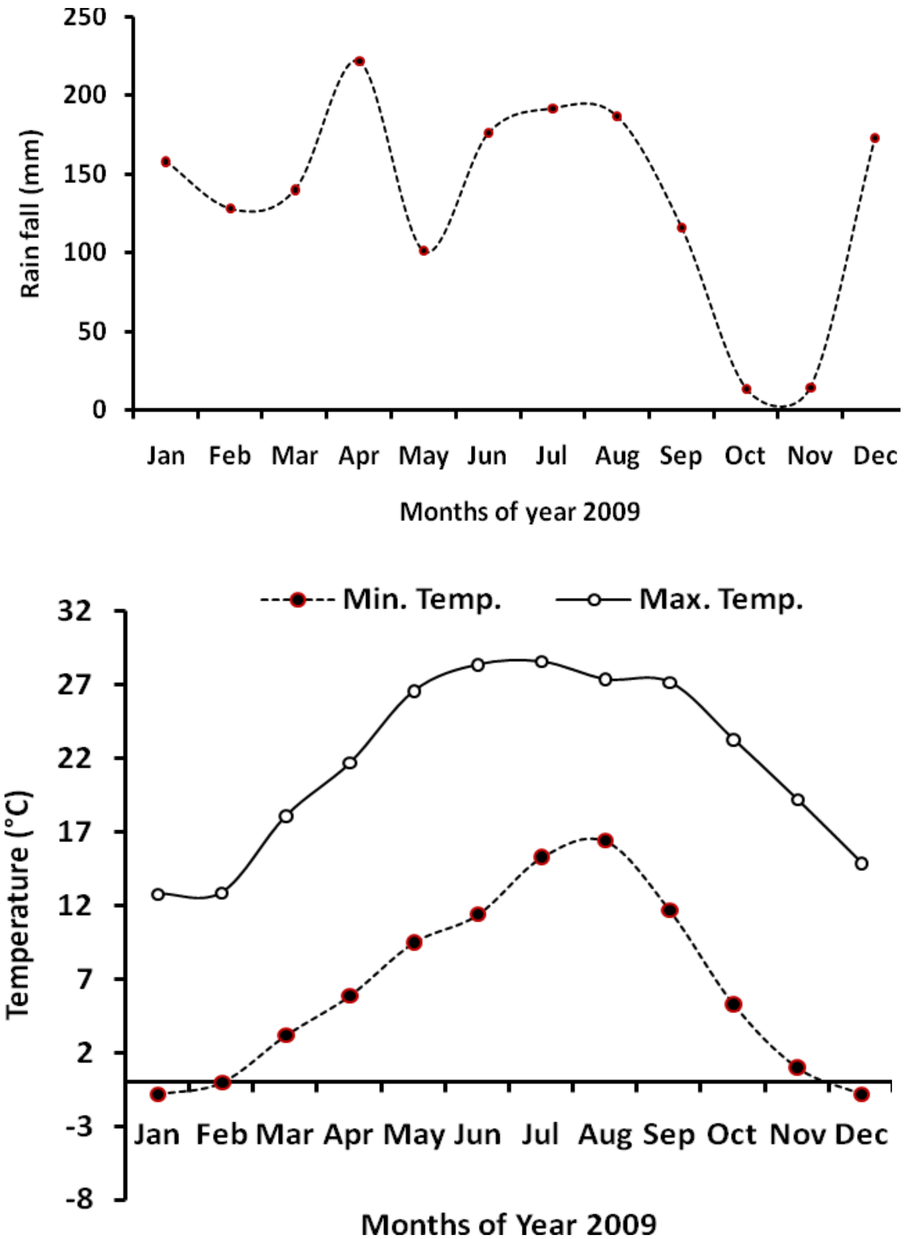

Figure 1. Mean annual rainfall, minimum and maximum temperature at Rawalakot, Azad Jammu and Kashmir during the study year 2009.

farm, Faculty of Agriculture, University of Azad Jammu and Kashmir (UAJK) Rawalakot AJK (33 to 36°N latitude and 73 to 75°E longitude) during the year 2009. The study area (Rawalakot) lies between the altitudes of 1800 to 2000 m above sea level in the northeast of Pakistan under the foothills of great Himalayas at Poonch district, AJK, Pakistan.

The study area is characterized by annual rainfall ranging from 500 to 2000 mm (depending on season), most of which is irregular and falls as intense storms during the monsoon and in winter. Mean annual temperature is about 28°C (maximum) in summer, while winter is fairly cold with -6°C. The monthly precipitation and temperature of the experimental area are presented in Figure 1. Generally, the soils are coarse silty, medium textured, mesic typic hapludolls acidic soil with mountain/hill and slopy relief and parent material residum-colluvium from shales (Ali et al., 2006).

Field operation, experiment description and treatments

Before actual experiment, soil samples from the experimental field were collected for physical and chemical characteristics (Table 1). A field of 36 × 12 m^2 was selected where maize-wheat was practiced previously since the last five years. For proper seed bed preparation, field was ploughed thoroughly twice with tractor and

Table 1. Physical and chemical properties of soil used for cultivation of common bean.

Soil parameter	Value
Organic C (g kg^{-1})	4.5
Total N (g kg-1)	0. 28
Available P (mg kg^{-1})	5.14
K (mg kg^{-1})	123
ECe (dSm-^{1})	0.62
pH	6.63
CEC (C mol kg^{-1})	17.8
Sand (g kg^{-1})	265
Silt (g kg^{-1})	476
Clay (g kg^{-1})	259
Bulk density (mg m^{-3})	1.12
Porosity (%)	51

Table 2. Chemical characteristics of the poultry manure used in the study.

Nutrient element	Value
C (%)	36.6
N (%)	2.21
P (%)	2.01
K (%)	2.84
Ca (%)	2.27
Mg (%)	0.29
Fe (mg kg^{-1})	927
Zn (mg kg^{-1})	107
Mn (mg kg^{-1})	216
pH	6.88
Dry matter (%)	48
Moisture (%)	52

left as such for next two weeks. The individual plots were prepared according to the treatments and the net plot size was 3 × 3 m^2 which was kept according to the size of the field. The plot was properly leveled for even and efficient fertilizer/water distribution. The experiment was laid out in a randomized complete block design (RCBD) with three replications.

The treatments comprised of two different sources of P that is, inorganic fertilizer as (i) DAP (diammonium phosphate, $(NH_4)_2$ HPO_4, 46% P_2O_5 and 18% N); (ii) TSP (Triple super phosphate, Ca $(H_2PO_4)_2H_2O$, 36% P_2O_5), and organic as poultry manure (PM), respectively and a mixture of PGPRs, *Bradyrhizobium* sp. strain MN-S and *Agrobacterium* sp. strain Ca-18 along with a control. Altogether, a total of eight treatments with three replications were established in the experiment. Phosphorus was applied at recommended rate (60 kg ha^{-1}) from either source at or equivalent basis. The treatments were, T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculation) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculation). Poultry manure was applied on the basis of P content at recommended rate equivalent to 60 kg P ha^{-1} 15 days before sowing.

The chemical composition of poultry manure (PM) is presented in Table 2. A mixture of promising PGPRs *Bradyrhizobium* sp. strain MN-S and *Agrobacterium* sp. strain Ca-18 was obtained from National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. Urea was used as N source and full dose of N fertilizer at 90 kg ha^{-1} was applied by broadcast method at the time of sowing. Similarly, a basal dose of potassium was applied to all plots including control at the time of sowing at 40 kg K_2O ha^{-1} as sulphate of potash (SOP), by broadcast method. All the fertilizers were well mixed into the soil.

Common bean *(P. vulgaris* L.) variety "Mothi", was used in the experiment and seeds were collected from Agriculture Office Havali, Azad Jammu and Kashmir. Bean was sown in lines on May 02, 2009 and seed were inoculated where required. After germination, the distance between the plants was maintained at 40 cm, while the row to row distance was 60 cm and total of four rows per plot were established. All standard local cultural practices were followed when required throughout the growth period and was harvested on August 28, 2009.

Measurements

A set of five healthy and vigorous plants in the central rows of each plot was randomly selected at flowering for nodulation potential (number of nodules, nodule fresh weight and nodule dry weight). Plants were carefully uprooted with the help of spade by digging soil core of 50 cm to both side and 100 cm in depth. Plants were placed into plastic buckets of water to remove adhering soil. Thereafter, plant shoots were removed. The roots were brought into the laboratory, followed by washing of roots under running water with a screen underneath to catch the detached nodules. The nodules were separated from the roots, counted and stored dried in a desicator for recording total number of nodules, nodules fresh weight and nodules dry weight per plant was recorded. Care was taken to avoid damage to nodules.

For the measurement of morphological characteristics, plants were carefully uprooted (as done for nodulation) just before pod filling stage. Different growth characteristics of crop like shoot length, shoot fresh weight and shoot dry weight; root length, root fresh weight and root dry weight were recorded. In the laboratory, plants were separated into shoot and root by cutting from the first unable node. The roots were washed with tap water. Shoot length and root length was measured with the help of a meter. Root and shoot fresh and dry weight $plant^{-1}$ was taken after oven drying at 70°C till constant weight.

Chlorophyll content readings were taken with a handheld dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot, 10 younger fully expanded leaves per plot were used when the plants were at flowering stage. The instrument stored and automatically averaged these readings to generate one reading per plot. Shelling percentage was calculated by dividing the grains weight of ten pods with total weight of ten pods multiplied with 100. Pod number was counted per plant and recorded.

Yield attributes were determined at full maturity from randomly selected ten plants from two central rows. Numbers of pods per plant were counted before picking. Pods were removed from the plants and threshed by hand. Grain yield was corrected for 13% moisture content after determining humidity level with a grain moisture tester. Total above ground dry matter yield was the sum of selected plants after sun drying in the field for more than 10 days. Harvest index was calculated by dividing the grain yield over total biomass yield multiplied with 100.

Biochemical analysis of plant and seed samples

Plant material (shoot plus leaf) and seed was dried in a forced-draft oven at about 70°C until constant weight and ground to pass a 1 mm sieve with an ED-5 Wiley mill (Arthur H. Thomas Co.). The ground material was analyzed for N and P concentration. Total nitrogen in ground material of shoot and seed was estimated by Kjeldhal digestion, distillation and titration method (Bremner and Mulvaney, 1982). Grain protein content was determined using the formula: protein concentration = N (%) × 6.25 (Nelson and Sommers, 1973).

Phosphorus content in shoot and seed was estimated by wet digestion with a 2:1 mixture of nitric acid (HNO_3) and Perchloric acid ($HClO_4$). The P content was then determined by the vanado-molybdate yellow color at 440 nm using spectrophotometer (Murphy and Riley, 1962). Total N and P accumulation (uptake) was calculated from dry matter accumulation and N and P concentration in shoot plus seed.

Statistical analysis

For the determination of significant effect of treatments on the growth, nodulation and yield of crop, analysis of variance (ANOVA) and least significant difference (LSD ≤ 0.05) tests among means were conducted for each character using a MSTAT-C statistical analysis package (Steel et al., 1997).

RESULTS

Agro-morphological characteristics

Growth

Application of different P sources along with PGPRs resulted in significant difference for all morphological parameters that is, shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight and leaf chlorophyll content. Shoot length was significantly increased for all the treatments with P fertilization and inoculation over control (Table 3). The relative increase in shoot length was 41 to 67% over the uninoculated control without P addition.

Application of DAP + PM + PGPRs resulted in significantly highest shoot length 128 cm, than the control with 76.5 cm. In rest of treatments, the combination of inorganic P fertilizers with PM showed greater plant height than their sole application but less than where PGPRs was also applied. All the treatments showed significant increase in shoot fresh weight compared with the control (Table 3). In general, at equivalent rates of application, DAP + PM + PGPRs resulted in higher shoot fresh weight followed by plants treated with TSP + PM + PGPRs.

The increases in shoot fresh weight with the P fertilization and inoculation were 36 to 160% compared with the control. Likewise, shoot dry weight was also greatest 34.7 g in treatment supplied with the DAP + PM + PGPRs over the control with 23 g. Inoculated plants significantly developed a greater root length and root mass of common bean than plants without inoculation (Table 3). Out of the seven tested treatments, all induced greater root length and a greater root mass. Increases ranged from 41 to 83% for root length, from 25 to 161% for root fresh weight and 29 to 181% for root dry weight over control. Combine application of DAP + PM + PGPRs showed highest results for all root growth parameters over rest of treatments. Application of PGPRs in combination with DAP and PM, resulted in highest

Table 3. Effect of different inorganic P sources, PM and PGPRs on the growth components of shoot and root in common bean.

Treatment*	Shoot length(cm)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant)	Root length	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})
T1	77^{g}	75^{g}	23^{d}	24.8^{c}	9.5^{c}	2.9^{f}
T2	115^{d}	133^{e}	27^{bcd}	41.5^{ab}	15.9^{b}	5.3^{cd}
T3	108^{f}	127^{e}	26^{bcd}	40.8^{ab}	15.7^{b}	4.5^{de}
T4	110^{ef}	102^{f}	25^{cd}	41.7^{ab}	11.8^{bc}	3.7^{ef}
T5	119^{c}	155^{c}	32^{ab}	34.9^{b}	23.1^{a}	7.0^{ab}
T6	112de	143^{d}	29^{abc}	42.2^{ab}	21.9^{a}	6.5^{bc}
T7	128^{a}	196^{a}	35^{a}	45.5^{a}	24.8^{a}	8.1^{a}
T8	122^{b}	166^{b}	31^{abc}	44.4^{a}	22.6^{a}	7.1^{ab}
LSD (P≤0.05)	2.57	10.33	5.72	9.26	4.46	1.28

*T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated). Means in the same column followed by the same letter do not differ significantly at P ≤ 0.05.

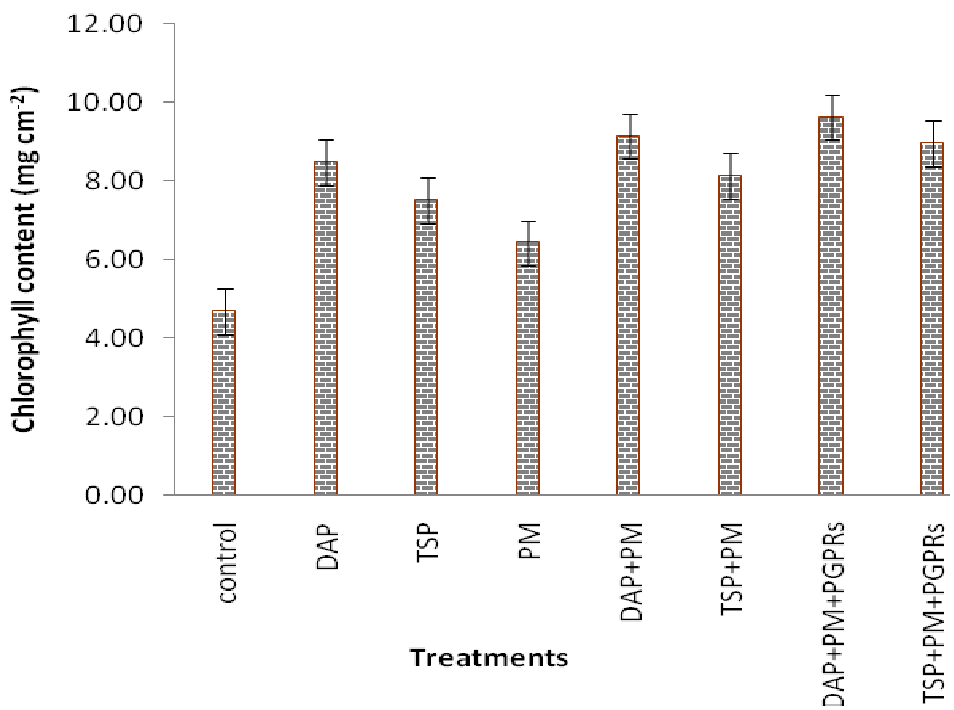

Figure 2. Effect of mineral P fertilizers, PM and PGPRs on chlorophyll content in common bean. Vertical bars bars shows standard error (n = 3).

chlorophyll content of common bean plants as compared to control (Figure 2). Chlorophyll content significantly increased from 4.7 mg cm^2 in the control to 9.6 mg cm^2 with DAP + PM + PGPRs application. Average across treatments, the increase in chlorophyll content due to integrated P application and PGPRs inoculation was 37 to 106% when compared with the control.

Nodulation

The common bean plants developed significantly more root nodules when inoculated with PGPR (Table 4). In the controls without inoculation, 34 nodules per plant was recorded, those significantly increased from 42 to 88 nodules per plant following the application of mineral P

Table 4. Effect of different P sources and PGPRs on the nodulation characteristics in common bean.

Treatment*	Nodule number ($plant^{-1}$)	Nodule fresh weight (g $plant^{-1}$)	Nodule dry weight (g $plant^{-1}$)
T1	34^{e}	1.00^{g}	0.35^{f}
T2	72^{bc}	1.67^{d}	0.55^{d}
T3	60^{d}	1.59^{e}	0.47^{e}
T4	42^{e}	1.22^{f}	0.45^{e}
T5	81^{ab}	1.89^{b}	0.75^{bc}
T6	64^{cd}	1.79^{c}	0.67^{c}
T7	88^{a}	2.08^{a}	0.95^{a}
T8	76^{b}	1.89^{b}	0.75^{b}
LSD (P≤0.05)	1	0.066	0.079

*T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated). Means in the same column followed by the same letter do not differ significantly at P ≤ 0.05.

Table 5. Effect of different P sources and PGPRs on yield and yield components in common bean.

Treatment*	Pod number ($plant^{-1}$)	Shelling percentage	Seed yield (kg ha^{-1})	Dry matteryield (kg ha-^{1})	Biomass yield (kg ha^{-1})	Harvest index (%)
T1	8^{e}	89	899^{g}	1308^{f}	2207^{f}	41^{bcd}
T2	15^{cd}	91	1425^{d}	1937^{c}	3362^{c}	42^{abc}
T3	15^{cd}	91	1332^{e}	1779^{d}	3111^{d}	43^{ab}
T4	12^{de}	91	1145^{f}	1614^{e}	2759^{e}	41^{bcd}
T5	19^{bc}	88	157^{c}	1921^{cd}	3493^{bc}	45^{a}
T6	18^{bc}	90	1431^{d}	2154^{b}	3585^{b}	40^{cd}
T7	27^{a}	89	1765^{a}	2530^{a}	4295^{a}	41^{bcd}
T8	23^{ab}	89	1629^{b}	2546^{a}	4176^{a}	39^{d}
LSD (P≤0.05)	5.92	NS**	37.82	151.58	133.57	2.99

*T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated). **NS= non significant. Means in the same column followed by the same letter do not differ significantly at P ≤ 0.05.

fertilizer, PM and PGPR that is, double increase in the nodule number. The relative increase in nodule number ranged from 24 to 158%.

Treatment DAP + PM + PGPRs induced the highest nodules per plant (88), followed by DAP + PM (81). Nodules fresh weight and dry weight showed similar increasing trend. Increases ranged from 21 to 107% for nodules fresh weight and 27 to 168% for nodules dry weight over the uninoculated control without P addition. Similar to nodule numbers, nodule fresh weight and dry weight differed considerably among different P inputs with PGPR application and were extremely high with treatment DAP + PM + PGPRs, followed by the integration of TSP + PM + PGPRs and DAP + PM, respectively.

Yield and yield attributes

Yield and yield components of common bean, showed similar trend that recorded for growth parameters (Table 5). Application of PGPR along with mineral P fertilizers and PM significantly increased number of pods per plant, seed yield, dry matter yield, total biomass yield and harvest index over the uninoculated control without P fertilization. However, shelling percentage showed no significant effect for different treatments.

The relative increase in yield characteristics ranged from 40 to 224% for number of pods, 27 to 96% for grain yield, 23 to 95% for dry matter yield, 25 to 95% for total biomass yield and up to 11% for harvest index. Increments in yield attributes varied considerably among

Table 6. Effect of different P sources and PGPRs on shoot P, seed P and total plant P in common bean.

Treatment*	Shoot P (%)	Seed P (%)	Total plant P (%)
T1	0.29[e]	0.46[f]	0.75[f]
T2	0.33[d]	0.58[d]	0.91[d]
T3	0.33[d]	0.55[de]	0.88[d]
T4	0.30[e]	0.5 [e]	0.82[e]
T5	0.39[b]	0.66[b]	1.05[b]
T6	0.36[cd]	0.62[c]	0.98[c]
T7	0.44[a]	0.70[a]	1.14[a]
T8	0.37[bc]	0.66[ab]	1.03[b]
LSD (P≤0.05)	0.03	0.034	0.42

*T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated).Means in the same column followed by the same letter do not differ significantly at P ≤ 0.05.

Table 7. Effect of different P sources and PGPRs on shoot N, seed N, total plant N and protein content in common bean.

Treatment*	Shoot N (%)	Seed N (%)	Total plant N (%)	Seed protein content (%)
T1	1.31 [f]	3.12 [f]	4.43 [f]	20 [f]
T2	2.15 [bc]	3.92 bcd	6.07 [bc]	25 [bcd]
T3	1.81 [de]	3.61 [de]	5.42 [de]	23 [de]
T4	1.69 [e]	3.47 [e]	5.16 [e]	22 [ef]
T5	2.18 [bc]	4.03 [bc]	6.21 [bc]	25 [bc]
T6	2.00 [cd]	3.78[cde]	5.77 [cd]	24 [cde]
T7	2.54 [a]	4.62 [a]	7.15 [a]	29 [a]
T8	2.31 [ab]	4.23 [b]	6.54 [b]	26 [b]
LSD (P≤0.05)	0.23	0.34	0.56	2.17

*T_1 = without fertilization (P_0); T_2 = DAP at 60 kg P_2O_5 ha^{-1}; T_3 = TSP at 60 kg P_2O_5 ha^{-1}; T_4 = PM equivalent to 60 kg P_2O_5 ha^{-1}; T_5 = DAP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_6 = TSP + PM at 60 kg P_2O_5 ha^{-1} (50:50 ratio); T_7 = DAP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated) and T_8 = TSP + PM + PGPRs at 60 kg P_2O_5 ha^{-1} (50:50 ratio + inoculated). Means in the same column followed by the same letter do not differ significantly at $P \leq 0.05$.

the different P inputs and their combination and were extremely high with treatment DAP + PM + PGPRs, followed by the integration of either TSP + PM + PGPRs or DAP + PM. The highest number of pods, seed yield, dry matter yield, total biomass yield and harvest index was recorded for DAP + PM + PGPRs over rest of treatments.

Biochemical characteristics

Nutrient accumulation and uptake

Plants inoculated with PGPRs and different P inputs were chosen for nutrient analysis (N and P) in shoot and seed. Common bean seed had the highest nutrient than shoot, with N content ranging from 3.47 to 4.62% and P contents ranging between 0.53 to 0.70% (Tables 6 and 7). In shoot, the N content ranged between 1.69 to 2.54%, while the P content was 0.30 to 44%. Total plant N and P content of common bean (shoot plus seed) ranged between 5.16 to 7.15% and 0.82 to 1.14%, respectively. Plant N and P increase, differ considerably among different treatments with PGPR inoculation and P sources and were extremely high either with DAP + PM + PGPRs or TSP + PM + PGPRs.

Total N and P uptake by common bean in response to different P inputs and PGPR application is presented in Figure 3. P fertilization and PGPR inoculation significantly increased N uptake and ranged between 67 to 146 kg ha-

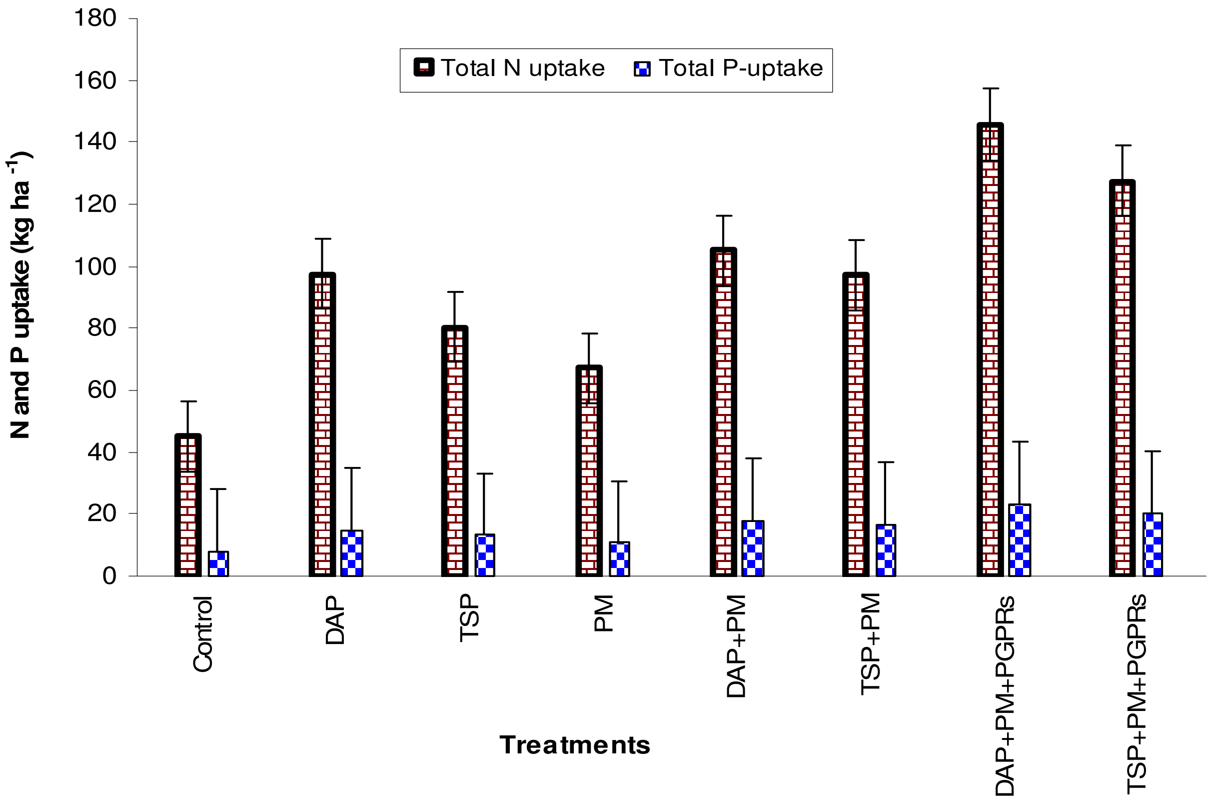

Figure 3. Effect of mineral P fertilizers, PM and PGPRs on total nitrogen and phosphorus uptake in common bean. Vertical bars shows standard error (n = 3).

1 for different treatments, demonstrating 48 to 222% increase over the uninoculated control without P addition. Similarly, plant P uptake was also significantly increased ranging from 10 to 23.4kg ha^{-1} for different P inputs and indicating 37 to 197% increase over the control. Plant N and P uptake increases varied significantly among different P treatments and were extremely higher either with either DAP + PM + PGPRs or TSP + PM + PGPRs over rest of treatments.

Seed protein content

Influence of different P sources and PGPR on common bean seed protein concentrations was measured and presented in Table 7. Seed protein was increased between 11 to 48% for different treatments with P addition and PGPR application. Integrated use of inorganic P + PM + PGPR lead to the highest protein content of 29% (in DAP + PM + PGPR) compared to 20% in the control.

Correlations

Many measurements in this study were significantly correlated with each other (Figures 4 and 5). Most of the parameters observed during the study have highly significant correlation (positive) with the number of nodules per plant and total P uptake. Dry matter yield, grain yield, total N and total P uptake were all positively and significantly correlated with number of nodules, that is, r^2 = 0.71, 0.95, 0.88 and 0.87, respectively. Similarly, dry matter yield, grain yield, chlorophyll content and protein content, were all positively and significantly correlated with total P uptake, that is, r^2 = 0.92, 0.96, 0.87 and 0.96, respectively.

DISCUSSION

Agro-morphological characteristics

Growth

Common bean is a N_2 fixing crop and was grown in the field where soybean-wheat rotation was cultivated since the last five years. Therefore, P fertilizer were applied instead of N because the experimental field was P deficient having 5.14 mg kg^{-1} P. Results of this study show that the application of P inputs along with PGPR

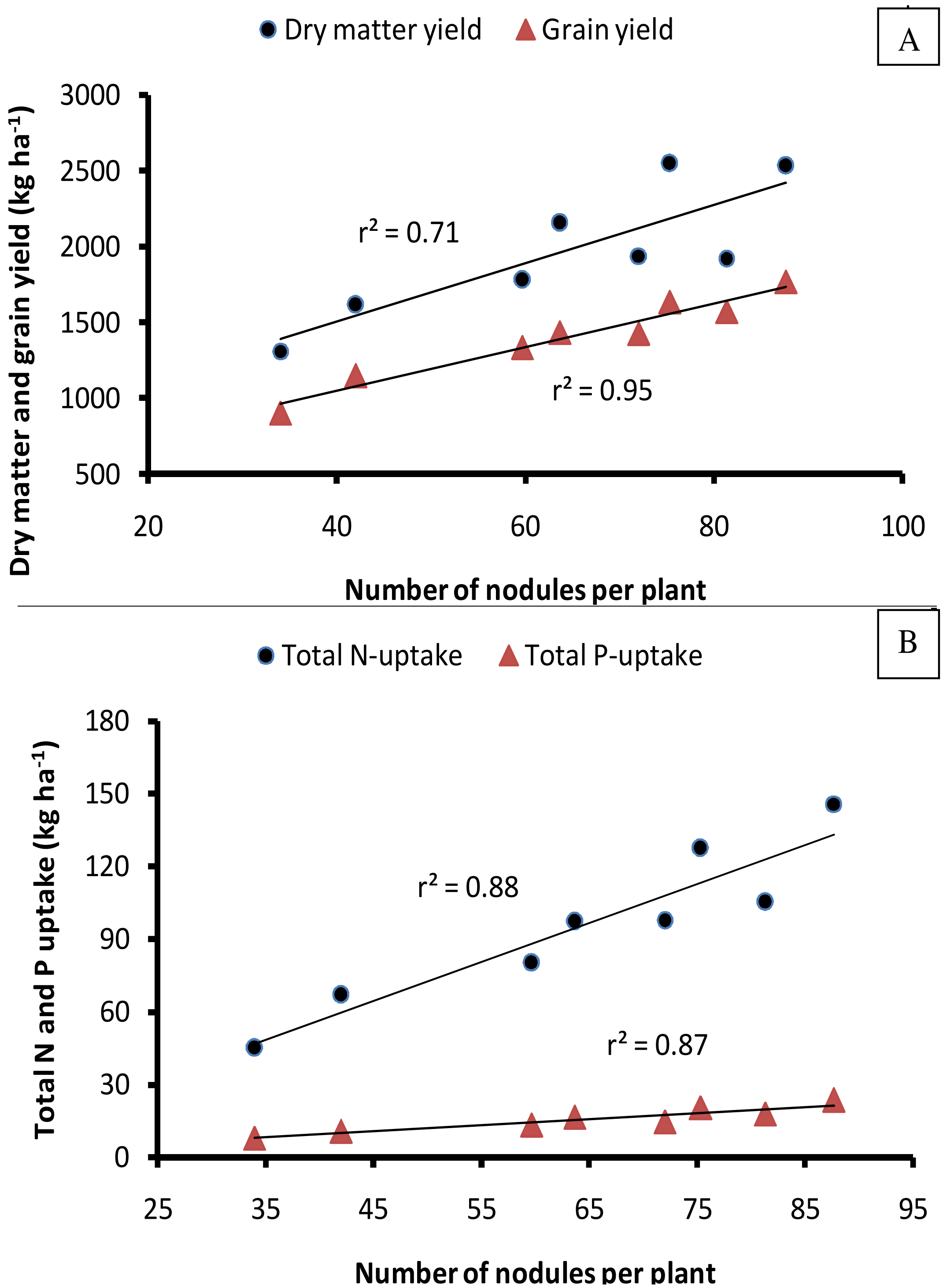

Figure 4. Correlation between (A) number of nodules per plant vs. dry matter yield, number of nodules per plant vs. grain yield and (B) number of nodules per plant vs. total N uptake and number of nodules per plant vs. total P uptake.

results in significant increase in common bean growth characteristics that is, shoot length, shoot fresh weight and shoot dry weight, root length, root fresh weight and root dry weight and leaf chlorophyll content.

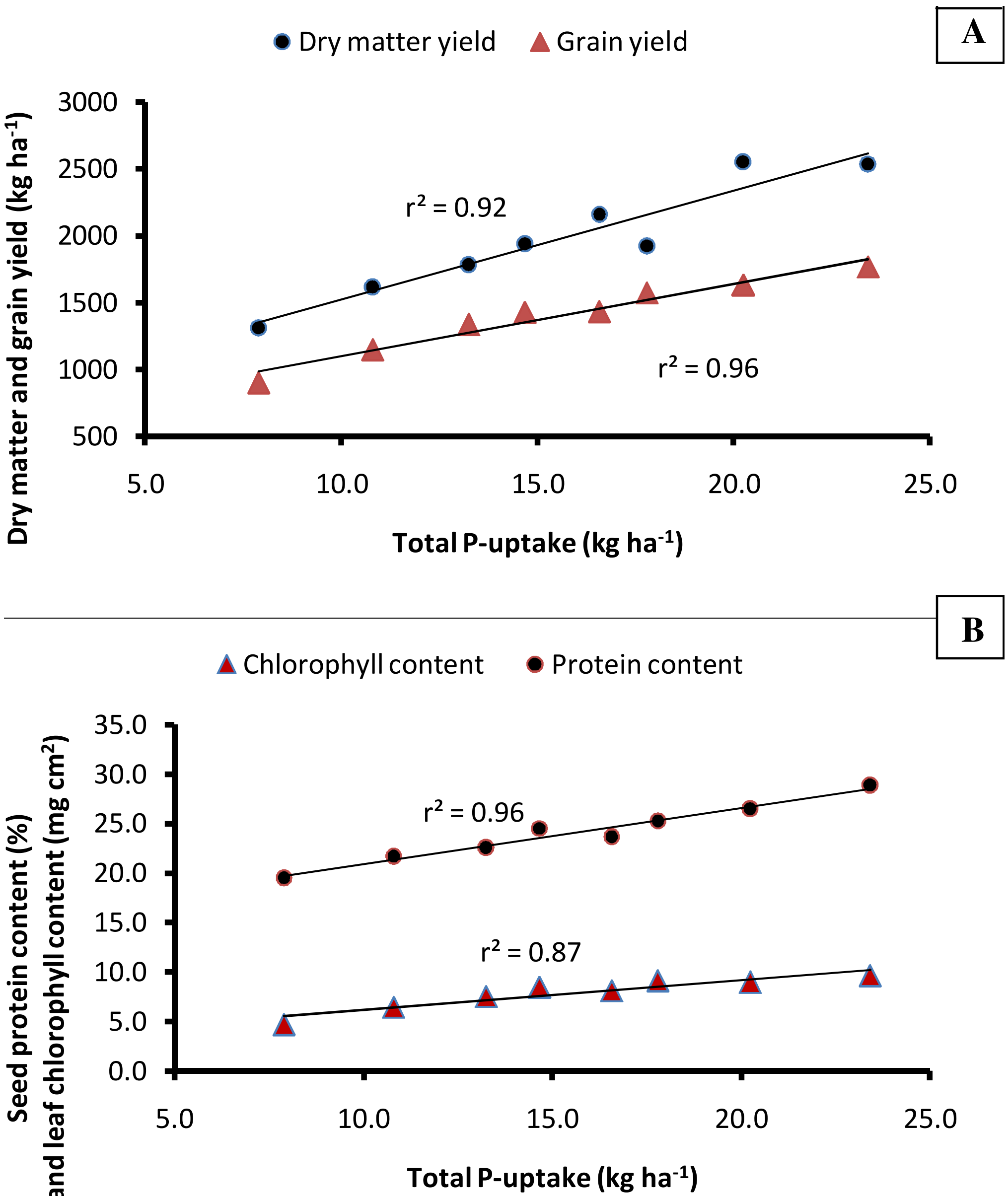

Figure 5. Correlation between (A) total (shoot + seed) common bean P-uptake vs. dry matter yield, total (shoot + seed) common bean P-uptake vs. grain yield and (B) total (shoot + seed) common bean P-uptake vs. chlorophyll content, total (shoot + seed) common bean P-uptake vs. seed protein content.

Phosphorus is a key component of energy generation in plants; thus, it is reasonable to believe that growth alterations occurring during this study resulted from increase in energy availability. It is conceivable that increase in cell division due to P application results from increase in the amount of ATP at growth centers (Chiera et al., 2004) which affect the growth characteristics. In a study with beans subjected to P stress, 50% reductions in the level of ATP in leaves and 60% reductions in total leaf area were observed (Mikulska et al., 1998). Zafar et al. (2011) reported that primary whole plant response to P addition was an enhancement in shoot growth, caused by increases in leaf expansion. Application of P significantly increased most of the growth characteristics of soybean (Abbasi et al., 2008). Yadegari et al. (2010) also reported significant increased in plant growth in response to inoculation with *Rhizobium* strains.

These P solublizers may increase the availability of phosphorus to plant by mineralizing organic phosphorus compounds and by converting inorganic phosphorus into more available form (Baryosef et al., 1999). The ability of PGPR strains to solubilize insoluble P and convert it to plant available form, is an important characteristic under conditions where P is a limiting factor for crop production. Kouas et al. (2009) reported that limited P supply decreased significantly root dry weight of two common bean cultivars and the decrease ranged between 58 and 70% as compared to the control.

Similarly, Elkoca et al. (2010) reported that the bacterial inoculations as single, dual and triple inoculations with *Rhizobium*, N_2-fixing *Bacillus subtilis* (OSU - 142) and P-solubilizing *Bacillus megaterium* (M-3) significantly increased shoot dry weight and chlorophyll content. Bacterial inoculations increased shoot dry weight by 19.7 to 54.3% and chlorophyll content by 34.1 to 59.3% over control. Abbasi et al. (2011) reported that application of PGPR significantly increased wheat plant height, shoot fresh weight and shoot dry weight by 25, 45 and 86%, respectively, while increase in root length, root fresh and dry weight was 27, 102 and 76%, respectively, over the un-inoculated control. Similar findings for improved bean growth parameters were also reported by Valverde et al. (2006); Remans et al. (2007); Suneja et al. (2007); Yadegari et al. (2010) and Stajkovic et al. (2011).

Nodulation

A combined application of P inputs and PGPR increased the number and fresh/dry weight of nodules in plant roots. Averaged across treatments, a combine application of P sources and PGPR increased nodules number between 24 to 158%, nodules fresh weight was increased ranging from 21 to 107% and nodule dry weight between 27 to 168% over the control. This is because P is known to initiate nodule formation, increases the number of nodule primordial and is essential for the development and functioning of formed nodules (Waluyo et al., 2004; Tagoe et al., 2008). Several researchers have reported that the supply of P plays important roles in establishment, growth and function of nodules (Shu-Jie et al., 2007; Abbasi et al., 2008) and is essential for initiation of nodule formation as it increased the number of nodule primordial (Waluyo et al., 2004). The strong positive relationship between number of nodules and plant total P uptake of common bean found in this study (r^2 = 0.87), confirms the importance of P in common bean nodulation.

Results of the study indicated that; (i) response of nodule fresh and dry weight to P supply was higher than the nodules number(ii) relative increase both in nodules number and nodule fresh and dry weight was higher with integration of mineral P fertilizers and PM with inoculation of PGPR compared with alone application of either P inputs. On the basis of these findings, it could be inferred that synergistic application of mineral P fertilizers, PM and PGPR increased the N_2 fixation efficiency of common bean because a positive correlation (r^2 = 0.88) has been reported between nodule number and plant total N-uptake of common bean (Figure 4).

Similar findings were reported by (Sangakkara et al., 1996). Kurdali et al. (2002) reported significant correlations between nodule dry weight and %Ndfa (r^2 = 0.40 and 0.74) and total N_2 fixed (r_2 = 0.45 and 0.81) in fababean and chickpea, respectively and stated that nodule mass could be an acceptable parameter reflecting N_2 fixation efficiency. Valverde et al. (2006) reported that plants inoculated with *Mesorhizobium ciceri* C-2/2, in single or dual inoculation, produced higher nodule fresh weight, nodule number and shoot N content than the control. Remans et al. (2007) studied the effect of four PGPR strains on the symbiotic interaction between *Rhizobium* and common bean (*P. vulgaris*) under deficient versus sufficient phosphorus supply. Their results confirmed that the effect on nodulation of three out of four PGPR strains tested was strongly dependent on P nutrition. Kouas et al. (2009) demonstrated that the nodule biomass decreased significantly under P deficiency to a higher extent 85 to 95%. The P deficiency decreased lesser the nodule number than the nodule mass in the two lines. In addition, nodulation was more sensitive to P limiting than the plant growth. Elkoca et al. (2010) showed significant increase in nodulation by native soil *Rhizobium* population in single inoculations.

Yield and yield components

Results of this work have demonstrated that common bean yield and yield components at this experimental site are P limited. Average across the treatments, the control soil (P_0) yielded 899 kg ha^{-1} grains as compared to the best treatment in the study which was DAP + PM + PGPR with 1931 kg ha^{-1} grain yield. This showed more than 100% increase in grain yield. Combined application of P sources and PGPR further increased seed yield ranging from 27 to 115% over the control.

The positive yield response can be attributed to increase pods per plant and individual seed weight due to P nutrition. Similarly, Chiezey and Odunze, (2009) reported that pod yield and 100-seed weight of soybean which is important determinants of grain yield increased with P application, resulted in substantial increase in seed yield. Addition of P inputs and PGPR resulted in significant increase in growth characteristics and N and P uptake in plants which would eventually affect the yield and yield components of common bean.

A significant and positive correlation was found between P-uptake and grain yield (r^2 = 0.87). In addition, application of P sources and PGPR ensured increase in nodules number and mass which increased symbiotic activity culminating in more N_2 fixation in the plant and likely increased seed and dry matter yield. A significant correlation existed between nodulation and seed and biomass yield. The greater increase in seed yield than in total biomass yield in the present study implies that, P fertilization and PGPR inoculation stimulated seed development more than vegetative growth. Results also demonstrate that increase in yield and yield components due to integrated P supply is higher than that of sole application of either P sources.

In addition to the higher seed and pod yields, harvest index was increased overall between 10 to 22% with integration of P sources and PGPR application. Shelling percentage showed no significant results to application of different P inputs and PGPR application. These results are in accordance with the previous studies (Abbasi et al., 2008; Chiezey and Odunze, 2009) indicating that P supply is important in attaining high yields in beans. Nevertheless, the results of this study were in contrast with the findings of other workers who reported that yield components and grain yield in beans were not significantly influenced by P application (Agboola and Obigbesan, 1997; Olofintoye, 2007).

It has been observed that the common bean crop response to P is dependent on P available in the soil (Mallarino and Rueben, 2005). Haq and Mallarino (2005) stated that these responses were frequent when soils having very low or low P (<16 mg P kg^{-1}). The positive response of common bean to P nutrition in this study was obviously due to the low available P of the experimental site that is, 5.14 mg kg^{-1} soil. Elkoca et al. (2010) reported significant increases of the seed yield under different inoculation treatments and increase ranged between 6.6 (*Rhizobium* + OSU - 142 + M-3) and 12.2% (OSU - 142 alone) over the control. Abbasi et al. (2011) confirmed that PGPR increased number of tillers per plant, 1000-grain weight and grain yield by 23, 48 and 59% application over the control. Valverde et al. (2006) reported that the co-inoculation treatment ranked the highest in seed yield (52% greater than the uninoculated control treatment).

Biochemical characteristics

Nutrient accumulation in plant

Higher N uptake due to P supply is ascribed due to; (i) Increase in root mass due to P nutrition resulted in absorption of higher concentration of mineral nutrients from soil including N and (ii) significant increase in number and mass of root nodules due to P and PGPR increased N_2 fixation that lead to increase in N uptake, as significant correlation existed between number of nodules and protein content in this study (r^2 = 0.93). Chaudhary et al. (2008) reported a 40% increase in N concentration in soybean due to P application. Similar results were also shown by Premaratne and Oertli (1994) and Fatima et al. (2007).

Accumulation of P both in plant leaves and seed showed similar response that recorded for N accumulation. The increase P content and uptake by plant (shoot + seeds) due to P nutrition compared to the control is associated with greater and extensive root system developed by common bean, leading to a greater soil volume exploited. A larger root system provides greater root-soil contact and thereby higher uptake of soluble P in the vicinity of the roots and such larger contact is especially important for uptake of low mobility nutrients, such as P (Vesterager et al., 2006).

Higher P content in common bean seed would improve the nutritive value and quality of common bean. An increase in the grain P content, particularly in protein-rich crop, may also be associated with the more vigorous seedling and higher grain yield of the crop. Our results are in accordance with the previous studies indicating increase P accumulation in plants due to P application and PGPR inoculation (Heckman and Kamprath, 1995; Olivera et al., 2004).

Seed protein content

Seed protein is the component which contributes significantly to the economic value of common bean. Therefore, influence of P fertilization and PGPR inoculation on common bean seed protein content was measured and it showed positive response.

Seed protein was increased by 11 and 48% with P fertilization and PGPR inoculation. Integrated use of both P inputs and PGPR application lead to the highest protein content of 29% (in DAP+PM+PGPR) compared to 20% in the control. With regard to PGPR application, significant changes occurred in protein content and highest protein content 26 and 29% was recorded in TSP + PM + PGPR and DAP + PM + PGPR, respectively. These observations demonstrated that combine application of P sources and PGPR application exhibited positive changes in seed composition by increasing protein content of common bean. Zafar et al. (2011) showed that integrated use of P resources significantly increased the grain protein content of maize.

Conclusions

Use of P as fertilizer is common practice among farming community throughout the world but application of P to legumes is limited especially in the developing countries. The results of present investigation, reveal that integrated application of mineral P fertilizer, PM and PGPR in combination significantly increased growth and yield characteristics of common bean. Average across treatments, application of two mineral P fertilizers along with PM, increase common bean yield by 75, 59% while, yield increase due to PGPR inoculation was 96 and 81% over the control, indicating efficiency of P and PGPR towards common bean yield. Similar response was observed for growth parameters and root nodulation and N and P accumulation in plant shoot and grain. Increase concentration of N and P in common bean grain would affect the nutritional quality of common bean grain. Integrated application of P alone or in combination with PM and PGPR, significantly increased the protein content demonstrating importance of P nutrition because, protein content which is the component of grain contribute significantly to the economic value of common bean. Integrated application of P with growth promoting rhizobacteria is highly recommended in common bean for improving yield, enhancing nodulation or N_2 fixation and increasing nutrient uptake as well as grain protein content.

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