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Acid Phosphatase Enzyme Activity and Various Growth Parameters of Common Bean as Influenced by Applications of Different Biofertilizers with Different Levels of Phosphorus Under Intercropping System

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Abstract: The present study was conducted during *kharif seasons* of 2012 and 2013 at the Krishi Vigyan Kendra (KVK) of Shere-e-Kashmir University of Agricultural Sciences and Technology, Budgam, Jammu and Kashmir. The climate of the experimental site was temperate with mild summers and cold winters, showing wide variations in mean maximum and minimum temperatures.. Common bean variety "Shalimar Rajmash-1" and maize variety "C-15" were used for the present study. The experiment was laid out in a complete randomized design (CRD) with each treatment replicated three times. Different levels of DAP (20 and 40 kg) along with different biofertilizer combinations like Rhizobium (*Rhizobium leguminosarum*), Azotobacter (*Azotobacter vinelandi*), VAM (*Glomus mosseae*) have been used during the research. The results of the experiment revealed that the growth, in terms of morphology and physiology, of all the inoculated plants was much better as compared to control plants. The various growth parameters like plant height, fresh and dry weights and length of the roots of the plants inoculated with *Rhizobium* + VAM @ 20 kg P/ha did best as compared to other treatments as well as control. *Rhizobium* + VAM @ 20 kg P/ha in the present research also showed significant impact on acid phosphatase activity in roots of common bean. Significant increase in N, P and K uptake was recorded when plants were inoculated with *Rhizobium* + VAM @ 20 kg P/ha. Overall the significant increase in growth and development was due to positive interactions among different microorganisms leading to healthy and vigorously growing plants.

Key words: Common bean, phosphorus, biofertilizers, acid phosphatase, nutrient uptake.

Introduction

The most important source of proteins is the legumes which are used for direct human consumption and common bean (*Phaseolus vulgaris*) comprising 50 % of the grain legumes consumed worldwide [1]. The common bean is an important legume crop for human nutrition and a main protein and calorie source in the world [2]. But its yield remains low to moderate because of the scarce nodulation, high inputs of chemical fertilizers and low grade technologies used [3]. Legume and non-legume intercropping system has been widely encouraged in sustainable agriculture because legumes have the great potential to improve the yield significantly and also allow plants to exploit soil N more efficiently [4], which is beneficial for reducing the amount of chemical fertilizer supplies and has positive consequences on the environment. A substantial amount of N is transferred in different communities, including N_2-

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fixed and non-N_2 fixed plants [5]. The common bean is usually considered very poor nitrogen (N) - fixing legume. Inoculating common bean with rhizobium can significantly increase the various growth, yield parameters and also increased N uptake of wheat (*Triticum aestivum* L.cv. Long 17) and faba bean (*Vicia faba* L. cv. Linxia Dacandou) and further improve the various intercropping advantages [6]. However, different environmental factors, such as acid soil conditions, salinity, low soil nitrogen (N) or phosphorus (P) levels are very essential constraints for leguminous crops and particularly for its production in most farms where this crop is mostly grown [7]. In most parts of the world the productivity of this crop is limited by the soil P deficiency, along with N. It has been reported that 40% of crop production in the world's cultivable land is mostly limited by the availability of P and sub-optimal levels of P.

Application of ecofriendly and cost effective microbial bio-fertilizer could be a better solution for overcoming nutritional deficiency related problems, low P low availability, limiting symbiotic nitrogen fixation process, root growth, the process of photosynthesis, translocation of sugars and other functions [8]. Various sudies related to structural and physiological studies have revealed that legumes form tripartite symbiotic associations with nitrogen fixing, nodule forming rhizobia and AM fungi [9]. Biological fertilizers contain one or several specific micro-organisms causing better development of root systems and also accelerate components for better absorption of different nutrients. In both associations, micro-symbionts are benefited by photo assimilates from the host plant. The macro-symbionts fix atmospheric nitrogen by a special process called nitrogen fixation processed in in root nodules of different lehuminous plants [10], and immobile most esential nutrients, especially phosphate, in the case of AM symbiosis [11]. Several studies carried out by different scients also indicated that inoculating both AMF and rhizobium promote the various growth parametrs of different crops and also improve the yield and nutrient uptake of various crops [12]. AMF is considered to be of great importance in plant symbiosis and promoting nutrient uptake, especially P [13]. The mycelium of AM can extend to the area outside the rhizosphere, connect roots with the different soil microhabitats and enlarge the surface area of roots to absorb various essential nutrients [14]. Thus, water and essential nutrients can be easily transported by the huge hyphae network to be finally absorbed by plants [14]. Inoculation of soil with different mycorrhizalstrains significantly increase various plant growth parameters and enhance phosphorus uptake especially under low supply of phosphorus. Under application of low phosphorus, most plant roots are strongly infected and ultimately increase plant growth, but under high dosage of phosphorus, a slight reduction in root occurs [15]. It has been reported that various mycorrhizal fungi improve phosphorus uptake under deficiency conditions which has positive effects on nitrogen fixation. Therefore, it is likely that plants infected with VAM fungi can significantly benefit from rock phosphate application in these soils. Indeed, the well known VAM-dependent plant species, such as *Stylosanthes* and *Leucaena*, have been known to respond favorably to VAM inoculation at low levels of soil phosphorus [16].

Therefore, the objective of the present study was to study the high and low-level application of phosphorus in combination with various biofertilizers on acid phosphatase enzyme activity and various growth parameters of common bean.

Materials and methods

Experimental site

The present experiment was conducted at the Krishi Vigyan Kendra (KVK) of Shere-e-Kashmir University of Agricultural Sciences and Technology, Budgam, Jammu and Kashmir. The climate of the experimental site is temperate with mild summers and cold winters, showing wide variations in mean maximum and minimum temperatures. The temperature of the site varies from 5°C in winter to a maximum of 34°C.

Details of treatment and crop culture

For smooth and proper preparation of beds, present research field was ploughed thoroughly twice with a tractor. The plots in the research field were properly leveled for even and efficient fertilizer/water distribution. The gross plot size of each plot was 16.5 square meters (sqm) and the net plot size was 9.6 sqm. The research experiment was laid out in a complete randomized design (CRD)with each treatment replicated thrice. The treatment details are presented in Table 2. The varieties of Common bean and maize which were used in the present study were "Shalimar Rajmash-1" and "C-15" respectively. The seeds of the used varieties in the present study were procured from KVK, Budgam, Jammu and Kashmir. The seeds of maize were sown at row to row distance of 75 cm and plant to plant distance of 20 cm. The seeds of common bean were sown in between the maize rows. Sowing of both the seeds was done in the last week of April, 2012 and 2013 and seeds were hand dibbled at a depth of about 2 cm in soil.

Treatments

(T_1) Maize + common bean (control). **(T_2)** Maize + common bean treated with *Rhizobium*. **(T_3)** Maize + common bean both treated with *Azotobacter*. **(T_4)** Maize + common bean both treated with Arbuscular mycorrhizae. **(T_5)** Maize + common bean both supplied with 20 kg Phosphorus/ha. **(T_6)** Maize + common bean both supplied with40 kg Phosphorus/ha. **(T_7)** Maize + common bean treated with *Rhizobium* + Arbuscular mycorrhizae. **(T_8)** Maize + common bean treated with *Azotobacter* + Arbuscular mycorrhizae. **(T_9)** Maize + common bean treated with *Rhizobium* + Arbuscular mycorrhizae + 20 kg Phosphorus/ha. **(T_{10})** Maize + common bean treated with *Azotobacter* + Arbuscular mycorrhizae + 20 kg Phosphorus/ha. **(T_{11})** Maize + common treated with *Rhizobium* + Arbuscular mycorrhizae + 40 kg Phosphorus/ha. **(T_{12})** Maize + common bean treated with *Azotobacter* + Arbuscular mycorrhizae + 40 kg Phosphorus/ha. **(T_{13})** Maize + common bean treated with *Rhizobium* + *Azotobacter* + Arbuscular mycorrhizae.

Application of different biofertilizer and chemical fertilizers

The seeds of both the varieties of maize and common bean were thoroughly surface sterilized by sodium hypochlorite (0.1%) for 2 minutes, thoroughly rinsed with distilled water and completely soaked in distilled water for 6 hours before sowing them in proper plots. Peat based *Rhizobium leguminosarum* inoculum, vesicular arbuscular mycorrhizae (*Glomus mosseae*) and *Azotobacter vinelandi* was procured from the Division of Microbiology, IARI (New Delhi) India. The seeds of both the varieties were moistened in sugar solution (48%) in order to geta uniform coating of *Rhizobium* and *Azotobacter* on the seeds and finally sowing the seeds in field. After inoculation, the seeds were then completely shade. The inoculum of mycorrhizal was applied after seed sowing at the rate of 25 Kg/ha by a special method called planting hole method.

The fertilizers for maize (120 N, 30 K_2O_5 Kg/ ha) and for common bean (30 N, 30 K_2O_5Kg/ha) were applied according to plant population in the research field under intercropping system. Phosphorus was applied as per the treatments. Half doses of both nitrogen and potassium were applied at sowing time as basal dose and the remaining dose of nitrogen was top dressed when true leaves emerged (25 days).

Harvest of plant and various growth analysis

Common bean plants were harvested at the time of flowering by simply uprooting them from the soil and different morphological and physiological features were measured. For determining root, shoot fresh and dry weight, roots and shoots were weighed by using simple measuring balance and then, oven dried at 70 °C and then again weighed. Phosphatase enzyme activity was assayed by using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol[17].

Statistical analysis

All the statistical data were simply loaded with Microsoft excel and then subjected to the analysis of variance using online OPSTA, CCS Haryana Agricultural University, Hisar. The significance of data obtained was judged from the critical difference at the 5 % level of significance.

Results

The results of the present research revealed that both the application of phosphorus and biofertilizers at various levels had a significant effect on fresh and dry weight of leaves and stem of common bean under intercropping with maize. Maximum fresh weight of both leaves and stem were observed in treatment, receiving dual inoculation of *Rhizobium* + VAM + 20 kg P/ha (T_9) followed by treatment, receiving *Azotobacter* + VAM + 20 kg P/ha (T_{10}) and then treatment inoculated with triple inoculation of *Rhizobium* + VAM + *Azotobacter* (T_{13}) [Table 1]. Other remaining treatments receiving both inoculation of *Rhizobium* + VAM and *Azotobacter* + VAM excluding any application of phosphorus also showed positive impact on fresh weight of leaves and stem. Minimum fresh weight of leaves and stem were recorded in the control (T_1). Also similar results were observed for both dry weight of leaves and stem.

Applying phosphorus along with different biofertilizers significantly affected the shoot and root length in common bean (Table 2). The highest root and shoot length were recorded for those treatments which were inoculated with *Rhizobium* + VAM + 20 kg P/ha) followed by those treatments which were inoculated with *Azotobacter* + VAM + 20 kg P/ha and also treatments which were inoculated with *Rhizobium* + VAM + *Azotobacter*. The minimum shoot and root length were recorded in control treatments (T_1). Among all the treatments, T_9 recorded maximum fresh and dry weight of roots followed by T_{10} and T_{13} respectively. The minimum fresh and dry weight of root per plant were observed in control plants (T_1) followed by T_3 [Table 2].

The data obtained on acid phosphatase enzyme activity in roots of common bean as influenced by combining application of phosphorus along with different biofertilizers revealed significant differences among the various treatments (Table 2). Significantly highest acid phosphatase enzyme activity was recorded in control plants (T_1) followed by single inoculation treatment T_2. The

Table 1. Impact of phosphorus and biofertilizers on fresh and dry weight of leaves and stem of common bean under intercropping of common bean + maize

Treatments	Fresh weight of leaves (g)	Dry weight of leaves (g)	Fresh weight of stem (g)	Dry weight of stem (g)
T_1 (Control)	1.97±0.06	0.29±0.00	3.52±0.02	1.05±0.00
T_2 (*Rhizobium*)	2.36±0.03	0.35±0.01	4.40±0.03	1.32±0.01
T_3 (*Azotobacter*)	2.22±0.01	0.33±0.00	4.18±0.02	1.25±0.00
T_4 (VAM)	2.34±0.00	0.34±0.04	4.59±0.03	1.38±0.01
T_5 (20 kg P)	2.37±0.00	0.35±0.00	4.72±0.02	1.42±0.00
T_6 (40 kg P)	2.31±0.01	0.34±0.03	4.41±0.03	1.32±0.00
T_7 (Rhz.+VAM)	2.55±0.02	0.38±0.01	4.90±0.01	1.47±0.06
T_8 (Az.+VAM)	2.45±0.03	0.36±0.02	4.75±0.02	1.42±0.09
T_9 (Rhz.+ VAM+20 kg P)	2.90±0.01	0.43±0.00	5.00±0.02	1.50±0.01
T_{10} (Az.+ VAM+20 kg P)	2.84±0.01	0.42±0.03	4.91±0.01	1.47±0.06
T_{11} (Rhz.+VAM+40 kg P)	2.67±0.06	0.39±0.03	4.67±0.04	1.40±0.02
T_{12} (Az.+VAM+40 kg P)	2.74±0.09	0.41±0.00	4.83±0.02	1.45±0.03
T_{13} (Rhz.+Az.+VAM)	2.80±0.09	0.43±0.07	4.88±0.02	1.46±0.09
C.D. @ 5 %	0.006	0.007	0.021	0.007

Rhz. = *Rhizobium*
Az. = *Azotobacter*
VAM = *Vesicular arbuscular mycorrhizae*
P = Phosphorus
C.D. = Critical difference

Table 2. Impact of phosphorus and biofertilizers on shoot length, root length, fresh, dry weight and acid phosphatse activity of roots of common bean under intercropping of common bean + maize

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight of roots (g) per plant	Dry weight of roots (g) per plant	Acid phosphatase activity (mg p-N P g^{-1} h^{-1})
T_1 (Control)	26.60±0.12	6.89±0.06	4.13±0.09	0.94±0.00	9.95±0.04
T_2 (*Rhizobium*)	33.54±0.18	8.89±0.08	4.56±0.06	1.15±0.10	8.93±0.06
T_3 (*Azotobacter*)	33.46±0.23	8.63±0.05	4.34±0.06	0.99±0.03	8.68±0.09
T_4 (VAM)	33.51±0.13	8.71±0.06	4.50±0.01	1.03±0.03	6.81±0.03
T_5 (20 kg P)	33.57±0.04	9.26±0.09	4.57±0.06	1.04±0.03	4.58±0.08
T_6 (40 kg P)	33.35±0.14	8.96±0.04	4.38±0.09	1.00±0.06	3.57±0.15
T_7 (Rhz.+VAM)	34.31±0.17	9.70±0.07	4.78±0.02	1.11±0.04	6.62±0.12
T_8 (Az.+VAM)	33.70±0.09	9.41±0.05	4.63±0.01	1.09±0.03	5.80±0.13
T_9 (Rhz.+VAM+20 kg P)	35.77±0.17	9.96±0.09	4.97±0.01	1.14±0.06	2.54±0.12
T_{10} (Az.+VAM+20 kg P)	35.58±0.20	9.81±0.08	4.85±0.01	1.11±0.03	2.56±0.07
T_{11} (Rhz.+VAM+40 kg P)	31.66±0.88	9.03±0.06	4.79±0.06	1.09±0.00	2.90±0.08
T_{12} (Az.+VAM+40 kg P)	33.58±0.09	9.05±0.08	4.82±0.03	1.10±0.03	2.95±0.09
T_{13} (Rhz. +Az. +VAM)	34.37±0.19	9.17±0.02	4.84±0.01	1.11±0.04	3.68±0.07
C.D. @ 5 %	0.644	0.135	0.017	0.100	0.113

Rhz. = *Rhizobium*
Az. = *Azotobacter*
VAM = *Vesicular arbuscular mycorrhizae*
P = Phosphorus
C.D. = Critical difference

lowest acid phosphatase enzyme activity was recorded in those treatments receiving dual inoculation of *Rhizobium* + VAM @ 20 kg P/ha followed by also those treatments receiving dual inoculation of *Azotobacter* + *Arbuscular mycorrhizae* + and those treatments inoculated dual inoculation of *Rhizobium* + *Azotobacter* + *Arbuscular mycorrhizae*.

Discussion

Application of phosphorus at different doses along with single, dual or multiple uses of different biofertilizers had significant impact on various growth parameters of common bean. The data obtained during the whole time research revealed that maximum fresh and dry weight of leaves of common bean were observed with treatment combination of *Rhizobium* + VAM + 20 Kg P/ ha. Similarly, both fresh and dry weights of stem of common bean were maximum in treatment combination of *Rhizobium* + VAM + 20 Kg P/ha which was significantly higher than other single inoculation treatments of either biofertilizer or phosphorus. The present results are in agreement with the findings of Salamone *et al.* [18] who reported that combine inoculation of *Rhizobium* + VAM in presence of nitrogen and phosphorus increased dry weight of shoot as compared to single inoculation. Phosphorus is the main component of energy production in different plants. Actually the increase in cell division due to the phosphorus application which results from accelerating the amount of adenosine triphosphate (ATP) at various growth centers, which ultimately affect the growth characteristics [19]. The studies have revealed that common beans subjected to those soils which are under phosphorus stress show reduction in ATP in different plant parts, mostly leaves and 60% reduction in other plant parameters like total leaf area [20].

Zaffer *et al.*[21] observed that plants respond to phosphorus application by the enhancement in the growth of shoot and increases leaf area in leaf. However, it is very essential to note that by applying excessive phosphorus to the soil may not result in any significant improvement in various growth parameters of different plants as excessive phosphorus in the soil gets fixed and ultimately become unavailable to the plants for usage. Yadegari et al.[22] reported a significant increase in plant growth in response to *Rhizobium* inoculation. Similarly, Elkoca et al. observed that by applying different bacterial inoculations as single, dual and triple along with *Rhizobium* or other nitrogen fixing bacteria (NFB), or phosphate solubilizing bacteria (PSB) significantly increased dry weight of shoot and chlorophyll content in the leaves. Abbassi et al.[24] recoded that by applying different plant growth promoting rhiobacteria (PGPR) increased different growth parameters of the plant like height, shoot fresh weight and shoot dry weight over un-inoculated control.

Maximum shoot and root length were observed with treatment combination of *Rhizobium* + VAM + 20 Kg P/ha. Similarly, higher fresh and dry weights of roots were also found in T_9. The application of phosphorus along with PGPR resulted in a significant increase in growth characteristics, i.e., shoot length, root length, root fresh weight and root dry weight of common bean. The results are in accordance with Abbasi et al.[25] who reported that phosphorus application significantly increased the most of the growth characteristics in soybean. Artusson et al.[26] reported that arbuscular mycorrhizal (AM) fungi and *rhizobacteria* could interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition (Nitrogen and phosphorus bioavailability) and inhibition of fungal plant pathogens. Abu and Aly[27] showed the positive effect of phosphate solubilizing microorganisms on the most plant growth parameters of tomato. The effect of phosphate solubilizing bacteria on growth may be due to the activity of phosphate solubilization caused by the strain and increased further mineral availability uptake.

Stamfordet al.[28] observed that applying nitrogen biofertilizer showed positive impact on nitrogen uptake and nitrogen content in cowpea and same results were obtained by others for soybean[29].The combined applications of different nitrogen and phosphorus biofertilizers on various field plants were more effective for nitrogen, phosophorus uptake compared to their single application[30]. Hence, we concluded that by applying inorganic phosphorus fertilizer along with nitrogen and phosphorus biofertilizers resulted in better nitrogen uptake by the various essential field crops than that obtained by the single usage of inorganic phosphorus fertilizer or either of the biofertilizers. The present data revealed that the APase enzyme activities in roots of common bean enhanced with the deficiency of soil phosphorus. The maximum activity of the enzyme acid phosphate was noticed under control condition and minimum activity of the enzyme acid phosphate was noticed in theT_9 (*Rhizobium* + VAM + 20 kg P/ha).This may be due to the availability of phosphorus mobilizing fungi and *Rhizobium* which show a synergistic effect to make the unavailable phosphorus in the soil easily available to the plant. Further the addition of inorganic phosphorus at optimum levels, relieves the plant from phosphorus limitations. The sufficient supply of phosphorus in the soil for the overall plant growth and development adversely affects acid phosphatase activity. Mandri et al.[31] concluded that deficiency of inorganic phosphorus in the soil increases the activity of enzyme phosphatases in the nodules of *Phaseolus vulgaris*. This could be an adaptation mechanism developed by leguminous plant to overcome the deficiency of phosphorus under stress condition. Increased activity of enzyme phosphatase under phosphorus deficiency appears to be correlated with an increase in the expression of those genes coding for phosphatases[32]. However, various biochemical and molecular studies suggest that the secretion of enzyme phosphatase is an essential part of the mechanism of plant response to low availability of soil P. In general, in agricultural cultivable soils, the solubilization of inorganic phosphate is closely associated to the activity of soil microorganisms including *Rhizobium*, *Azotobacter*, and VAM[33]. Indeed,these beneficial microorganisms can secrete those substances in their culture media during their growth, which can easily allowing inorganic phosphate solubilization [34]. It was found that plants with higher mycorrhizal root colonization had maximum phosphatase activity (alkaline and acidic). These enzymes enhance mineralization of bound P into a soluble form and make it easily available to the plants. This important elemental P is then absorbed by the plants through the AM colonized roots and thus absorbs maximum phosphorus from the soil. Further,the actual physiological role of root acid phosphatases is not clear.The induction of a higher acid phosphatase activity under P deficiency, as observed in bean roots may enhance the P utilization from phytate in the plant and contribute to improve its adaptation to low P soils.

Conclusion

Integrated application of different levels of phosphorus in combination with *Rhizobium* and VAM significantly increased the various growth and physiological parameters of common bean under intercropping system. Also application of different biofertilizers along with different levels of phosphorus plays an essential role in becoming the inorganic phosphorus easily accessible to the plants. Low levels of soil available phosphorus accentuated the enzymatic activity of acid phosphatases in roots of common bean. The decrease in acid phosphatases activity in roots of common bean by applying different biofertilizers along with phosphorus could be an adaptation mechanism developed by common bean bean plants to overcome phosphorus deficiency stress. Therefore, integrated application of phosphorus with *Rhizobium* and VAM can be highly recommended in common bean - maize inter-cropping for enhancing growth and physiological parameters of common bean.

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