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Nitrogen content and uptake in leaves and stem of common bean (Phaseolus vulgaris L.) as influenced by application of various 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" was 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 nutrient content and uptake in leaves and stem of all the inoculated common bean plants was higher as compared to control plants. Rhizobium + VAM @ 20 kg P/ha in the present research showed significant impact on nitrogen content in leaves and stem of common bean as compared to all treatments. Significant increase in nitrogen uptake was recorded when plants were inoculated with Rhizobium + VAM @ 20 kg P/ha. Overall the significant increase in nitrogen content and uptake of common bean was due to positive interactions among different microorganisms leading to healthy and vigorously growing plants.

Key words: Common bean, phosphorus, biofertilizers, nitrogen content and uptake

I. INTRODUCTION

Among food legumes, Rajmash or common bean (*Phaseolus vulgaris* L.), family Fabaceae, is regarded as „grain of hope‟ as it is an important component of subsistence agriculture. It accounts for about half of the total pulses production. Since it is a short duration (85-110 days) crop, it can very well form a component of intercropping and sequence cropping in many of the agro climatic zones. Pulses act as natural resource of nitrogen as they have inbuilt capacity to fix biological nitrogen. Most of the pulse crops fix approximately 50-70 kg nitrogen per

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hectare. The root nodules of pulses are rich source of nitrogen and the root residues left in the field enhance the fertility of the soil. Intensive agriculture in irrigated agro-ecosystem particularly in Punjab and Haryana has declined the fertility and productivity of soil. Inclusion of pulses in cereals-based cropping system is of utmost importance to revive the soil health in terms of chemical, physical and biological properties.

the growth and yield of crops is governed by nutrients to the crops (Kumar *et al*., 2012). Even though pulse crops have unique properties of nodulation through *Rhizobium* bacteria that is biological nitrogen fixation (BNF). These bacteria through BNF meet about 80-90 per-cent of their total N-requirements and also make it available to non-legumes grown in intercrops. Likewise phosphate-solubilizing bacteria (biological phosphate fixation (BPF) have the capability to solubilize the residual or fixed soil P, increase the availability of phosphorus in the soil, produce growth promoting substances (Kumar *et al*., 2009) and thereby increase the overall P-use efficiency of the crops. Thus, application of bio fertilizers may help in sustainable crop production. However the determination of appropriate fertility requirements whether inorganic or bio fertilizers (either alone or in combination) for the maximum gain in terms of economic yield or other growth and development traits in the need under intercropping systems.

II.MATERIALS AND METHODS

Treatments Details and Crop Culture: The detailed treatments are presented in Table 2. Common bean variety "Shalimar Rajmash-1" and maize variety "C-15" were used for the present study. The maize seeds were sown at row to row distance of 75 cm and plant to plant distance of 20 cm. The common bean seeds were sown in between the maize rows. Sowing 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.

Biofertilizers and chemical fertilizers application: The seeds were surface sterilized by sodium hypochlorite (0.1%) for 2 minutes, thoroughly rinsed with distilled water and soaked in distilled water for 6 hours before sowing in 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. For *Rhizobium* and *Azotobacter* inoculation, the seeds were moistened in sugar solution (48%) before the application of inoculums to get a thin uniform coating of inoculum on the seeds, immediately before sowing the seeds in field. The seed were then shade dried after inoculation.The mycorrhizal inoculum was applied after seed sowing at the rate of 25 Kg/ha by planting holes method.

III.TREATMENTS

(T_1) Maize + common bean (control).

(T2) 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.

IV.MINERAL ANALYSIS

Estimation of nitrogen

Nitrogen content in different plant parts (leaves, stem) of common bean and maize was estimated as per Kjeldhal method (Jackson, 1973). The dried material was ground to powder in wiley grinding mill. 1g of sample was placed in a Kjeldahl digestion tube to which 5g of digestion mixture (5 g K_2SO4 + 10 g CuSO4+1 g $FeS0_{4)}$ and 20 ml conc. sulphuric acid were added. The samples were digested for two hours. After adding 25 ml NaOH (40%), the samples were then distilled and the ammonia liberated was collected in boric acid (4%) and titrated with 0.1N hydrochloric acid. A blank was prepared and treated in the same manner except that the tube was free of sample.

Nitrogen percentage was calculated according to the formula: N (%) = Titration value x 1.4 x 10/ sample wt.

Estimation of Phosphorus and Potassium

Phosphorus was estimated by vanadomolybdo phosphoric acid, yellow colour method and estimation of potassium was carried out as per Jackson (1973). Plant samples were dried, powdered and sieved. 5 g of sample was taken in a shaking bottle to estimate available potassium. 50 ml neutral 1N ammonium acetate solution was added and shaken in a reciprocating shaker for 5 minutes. The sample was then filtered through Whatman No. 42 filter paper. 5 ml of the filtrate was pipetted into a volumetric flask and made up to 50 ml with 1N ammonium acetate and potassium was estimated using a flame photometer (Flame photometer Model 128 Systronics) using potassium filter.

Uptake of Nitrogen, Phosphorus and Potassium

Uptake of nitrogen, phosphorus and potassium in different plant parts of common bean was worked out by multiplying nutrient contents by respective dry matter.

V.RESULTS

Nitrogen content and uptake in leaves and stem

The results revealed that the application of biofertilizers and phosphorus under different levels significantly influenced N and its uptake in leaves of common bean. The highest nitrogen content (2.63%) was recorded inT_9 followed by T_{10} and T_7 (2.51 and 2.35%). The lowest nitrogen content (1.19 %) was recorded in control plants T_1 followed by T_2 (1.6%). The results further indicated that application of phosphorus and biofertilizers also had significant effect on nitrogen content in stem. Significantly highest nitrogen (0.87%) was recorded in T_9 (*Rhizobium* + VAM + 20 Kg P/ha) followed by T_{10} (*Azotobacter* + VAM + 20 Kg P/ha) (0.82%).Among dual inoculation combination without any phosphorus application, treatment *Rhizobium*+VAM showed highest nitrogen concentration (0.68%) over other dual and single inoculation treatments without any phosphorus application. While lowest nitrogen concentration (0.34%) was recorded incontrol plants (T_1) followed byT_3 (0.50 %).Similarly higher nitrogen uptake (1.31 g/plant)by stem of common bean was recorded in T_9 (*Rhizobium* + VAM + 20 Kg P/ha) followed by T_{10} and T_{13} (1.20 and 1.12 g/plant). Lowest uptake of nitrogen (0.35 g/plant) was recorded in control plants (Table 1).

Table 1:- Impact of phosphorus and biofertilizers on nitrogen content and uptake in leaves,and stem of common bean under intercropping of common bean + maize.

Treatments	Leaves		Stem	
	Nitrogen content (%)	Nitrogen uptake (g/plant)	Nitrogen content (%)	Nitrogen uptake (g/ plant)
T_1 (Control)	1.19±0.02	0.72±0.06	0.34±0.01	0.35±0.01
T_2 (*Rhizobium*)	2.08±0.01	0.34±0.03	0.56±0.03	0.73±0.02
T_3 (*Azotobacter*)	1.61±0.01	0.53±0.03	0.50±0.02	0.62±0.01
T_4 (VAM)	1.84±0.04	0.63±0.01	0.54±0.02	0.74±0.03
T_5 (20 kg P)	2.22±0.02	0.77±0.03	0.57±0.03	0.80±0.02
T_6 (40 kg P)	1.92±0.01	0.65±0.09	0.50±0.01	0.66±0.06
T_7 (Rhz.+ VAM)	2.35±0.05	0.89±0.06	0.68±0.04	0.99±0.00

T_8 (Az.+VAM)	2.24±0.03	0.80±0.02	0.58±0.03	0.82±0.03
T_9 (Rhz.+ VAM+20kg P)	2.63±0.05	1.13±0.06	0.87±0.06	1.31±0.02
T_{10} (Az.+ VAM+20 kg P)	2.51±0.09	1.06±0.01	0.82±0.05	1.20±0.03
T_{11} (Rhz.+VAM+40 kg P)	1.72±0.09	0.67±0.09	0.71±0.03	0.99±0.05
T_{12} (Az.+VAM+40 kg P)	1.64±0.02	0.67±0.06	0.74±0.04	1.07±0.04
T_{13} (Rhz.+Az.+VAM)	2.39±0.02	1.02 ±0.01	0.77±0.05	1.12±0.01
C.D. @ 5%	0.05	0.04	0.03	0.07

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

VI.DISCUSSION

Application of phosphorus and biofertilizer showed significant effect on nitrogen contents and its uptake in leaves and stem. Higher nitrogen contents and nitrogen uptake were observed with the treatment combination of *Rhizobium* + VAM + 20 Kg P /ha in both the crop plants.Nitrogen is a vital element in plant growth which is usually absorbed as nitrate or ammonium, taking part in proteins, enzymes and chlorophyll structures (Malakooti and Tabatabayee, 2005). A cost effective way to provide sufficient nitrogen to plants is the biological nitrogen fixation. The results of the present findings are in complete agreement with the findings of Rahman *et al*.(2010) who reported that dual inoculation of *Rhizobium* +VAM + 25 Kg P/ha recorded the highest amount of nitrogen uptake over the other treatment combination. Further they also reported that plants receiving both the inoculants along with phosphorus fertilizer (*Rhizobium* + VAM + P) registered maximum uptake rather than plants which were dual inoculated without P fertilizers. Zarrin*et al*.(2009) revealed that dual inoculation of *Rhizobium* along with elemental phosphorus significantly increase nitrogen, phosphorus and potassium take in shoots of soybean as compared to control. This increase may be due to supply of phosphorus that seems important for *Rhizobium* to fix relatively more nitrogen from soil, which resulted in increased plant growth and nitrogen uptake by root and then to shoots. Phosphorus plays a vital role in physiological and development process in plant life and favorable effect of this important nutrient might have accelerated the growth process that increases nitrogen uptake in plants.Similar results were reported by Basir, 2005 and Sarawg*et al*. (1999).

A positive effect of nitrogen biofertilizer on nitrogen uptake and nitrogen content has been reported for soybean (Patra *et al*. 2012).The combined applications of nitrogen and phosphorus biofertilizers were more effective (Moinuddin *et al*.2014) for nitogen uptake compared to their single application.Hence, the application of inorganic phosphorus fertilizer together with nitrogen and phosphorud biofertilizers resulted in better nitrogen uptake by the crop than that achieved by the single use of inorganic phosphorus fertilizer or either of the biofertilizers.

VII.CONCLUSION

Integrated application of phosphorus in combination with *Rhizobium* and VAM significantly increased the nutrient content and uptake of common bean under intercropping system. Also application of different biofertilizers along with different levels of phosphorus plays a significant rule in becoming the inorganic phosphorus easily available to the plants. Therefore, integrated application of phosphorus with *Rhizobium* and VAM can be highly recommended in common bean intercropping for enhancing various nitrogen content and uptake of common bean crop.

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