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IMPACT OF VA-MYCORRHIZA, *RHIZOBIUM* AND PHOSPHORUS ON GROWTH AND YIELD OF *PHASEOLUS VULGARIS* L.

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Field experiments were conducted with the four vesicular arbuscular mycorrhizal fungi namely *Gigaspora albida, Glomus albidum, Sclerocystis sinuosa* and *Scutellospora erythropa* in combination with chemical phosphorus and *Rhizobium* to study their impact on growth and yield of french bean (*Phaseolus vulgaris* L.). Plants inoculated with mycorrhizal fungi plus *Rhizobium* besides better plant growth had maximum number of pods/plant, pod length, seeds/pod, seeds/plant and total seed wt/ plant. The dual inoculation of both the symbionts showed synergistic effects. Phosphorus application in combination with AM-fungi further improved growth and yield of plant. Different species of AM-fungi varied in their respective ability to stimulate plant growth and yield of plant. Inoculation with *Gigaspora* either with *Rhizobium* or phosphorus produced highest yield of pods per plant, while *Scutellospora* was best in the terms of general plant growth. While comparing both the treatments i.e., AM-fungus plus *Rhizobium* and AM-fungus plus phosphorus, the response of dual inoculation using both the symbionts were better. However, both the treatments significantly improved in plant growth and yield as compared to uninoculated control.

Keywords : AM-fungi; P-fertilizer; *Phaseolus; Rhizobium*; VAM.

Introduction

Arbuscular-mycorrhizas (AM) are symbiotic association between zygomycetes and roots of the majority of vascular plants[1]. This mutualistic association benefits plant growth by enhancing soil nutrient uptake[2], altering host physiological and biochemical properties[3]. *Phaseolus vulgaris* L. or French bean - commonly known as Rajmah is mainly cropped in northern parts of Uttar Pradesh for seeds which contain 22-25% protein[4]. Unlike other leguminous crops, it does not nodulate with native *Rhizobia*. Therefore, requirement of fertilizer for this crop is of prime importance[5]. Dual inoculation of AM-fungi with nitrogen fixer i.e., *Rhizobium* or alternatively treatment of AM-fungi with phosphorus fertilizer may bring considerable yield increase owing to their supplementary effects as *Rhizobium*-legume symbiosis begins with two free living organisms and ends with their intimate cellular co-existence[6]. *Rhizobium* species have been used worldwide as legume inoculants to procure nitrogen. This endosymbiotic association reduces the dependency of agricultural crops on nitrogenous fertilizers. Popularising the use of AM-fungi either with *Rhizobium* or with half of recommended phosphorus dose to reduce the dependence on chemical fertilizers and to contribute to pollution free atmosphere is the greatest need of the day. Hence, the experiments were conducted to observe the response of French bean to dual inoculations of different AM-fungi with *Rhizobium* or chemical phosphorus.

Material and Method

On the basis of results obtained from the pot experiments (unpublished data), field experiments were carried out on *Phaseolus vulgaris* L. var PDR 14 obtained from Indian Institute of Pulse Research, Kanpur, during winters of 2003-2004. Soil based inoculum of four AM-fungal genera namely, *Gigaspora albida, Glomus albidum, Sclerocystis sinuosa* and *Scutellospora erythropa* with *Rhizobium* - a bacterial nitrogen-fixing symbiont and phosphorus (KH_2PO_4) were given to the bean plants. The inoculum of VA-Mycorrhizal fungi was raised and maintained on maize crop. It consisted of both, 300-400 spores per 100g soil and chopped, colonized root fragments. Mycorrhizal inoculation was done by placing its culture 3-4 cm beneath the soil. The recommended level of phosphorus for French bean is 15 kg phosphate/ha of which half dose was given as basal dressing. For dual inoculation, seeds were coated with 48 h old *Rhizobium* culture and then sown over a thin layer of mycorrhizal inoculum. French bean seeds @150 kg/ha were sown in 12.0 x 4.50 mt microplot, in 10 rows. In a row, uniform distance of 10 cm was maintained in between the plants by thinning and gap filling 15 days after sowing (DAS). There were ten treatments including phosphorus full dose and control (without any symbiont or phosphatic fertilizer). Observations of plant growth i.e., shoot and root length per plant, fresh and dry weight of shoot, leaves and root per plant were determined regularly after every 15^{th} day from 30^{th} to 120^{th} DAS. Ten plants per treatment were randomly selected and the average data were recorded. Number of pods/plant, pod length, seeds/pod, seeds/plant and total seed wt/plant were also recorded after harvesting the mature crop.

The percent mycorrhizal colonization of roots after clearing and staining[7] was also determined by using the formula used by Chaurasia *et al.* [8].

$$\%mycorrhizal root colonization = \frac{\text{No. of VAM colonized root bits}}{\text{Total no. of root bits examined}} x100$$

Results and Discussion

The effect of VA-Mycorrhizal inoculation along with the *Rhizobium* and phosphatic fertilizer on plant growth, dry biomass production, yield of plants and mycorrhizal colonization are given in table 1 and 2. In general, all treated plants exhibited improved growth and biomass production over control plants but there were variations among treatments of different AMF species. The responses of an AM-fungus plus *Rhizobium* were better than that of AM-fungus plus half dose of phosphorus. *Scutellospora* plus *Rhizobium* was found to be highly effective in terms of vegetative plant growth. The longest root (23.54 cm) and shoot (18.34 cm) as well as the highest dry content in root (210 mg), shoot (3254 mg) and leaves (9520 mg) were recorded for it (Table1).

Yield attributes pattern was studied in terms of pods/plants, pods length, pod wt/plant, seeds/pod, seeds/plant and seed wt/plant (Table 2). The maximum values of these parameters were recorded where AM-fungus was co-inoculated with the *Rhizobium*. On an average, the values of these parameters were recorded maximum in plants dual inoculated with *Gigaspora* + *Rhizobium* as compared to those plants treated with other AMF species either with *Rhizobium* or with half dose of phosphorus. But both the treatments i.e., AM-fungus with half dose of phosphorus and AM-fungus with *Rhizobium* gave much better responses in terms of all the yield attributes as compared to uninoculated control and treatment of plants with only half dose of phosphorus.

The root colonization by AM-fungi was also more in plants inoculated with AM fungus + *Rhizobium* as compared to AM fungus + 1/2 dose of phosphorus or uninoculated control or plants basal dressed only with 1/2 dose of phosphorus. *Scutellospora* alongwith *Rhizobium* showed maximum (73%) average root colonization of French bean plants.

French bean plants showed comparatively maximum growth, yield and mycorrhizal colonization in dual inoculated plants as compared to plants inoculated with AM fungi + half dose of phosphorus. But both the treatments were better in growth than uninoculated control. The mutualistic double symbiosis is accounted for better colonization and plant growth due to interchange of carbon, phosphate and nitrogen in between host, fungus and bacterium. These results bring out a synergistic or additive interaction between AM-fungi and *Rhizobium* with consequential effect on plant growth and yield of French bean. This is in agreement with the earlier findings in other legumes[9] and non-leguminous plants[10] that VA mycorrhizal fungi can have important effect on plant growth. Earlier experimental evidences also showed that when initial soil phosphorus concentration was very low, even small addition of phosphorus tremendously increased biomass[11]. In spite of this, when heavy dose of phosphorus was applied, it reduced root volume and in consequence it decreased the root surface area colonized by AM[12].

The requirement of phosphorus is high in legumes[13] and therefore, leguminous plants as compared to cereals respond more to mycorrhizal colonization which indirectly enhances the biological nitrogen fixation through increased phosphorus availability specially in soil with low phosphorus content[14]. French bean also responds well to application of chemical phosphorus[15]. Attempt on plant growth improvement through combined application of AM-fungi with half dose of phosphorus showed significant response in terms of root and shoot length and plant dry weight[10]. Phosphorus is important in root development and translocation of photosynthates and being the constituent of nucleic acid, phytin and phospholipids, its application increases growth and yield attributing parameters[16]. The results of our study also clearly indicate that french bean plants are benefited when they are raised in the presence of various AM-fungal species. Further, maximum benefits of this fungus-host symbiosis can be harnessed when the soil is amended with half dose of phosphorus, thus minimising application of phosphorus fertilizer through AM-fungal inoculations and resulting in the reduction of cultivation cost. Similar results were also obtained in banana plantlets[17].

In our results, the efficacy of *Scutellospora* was highest in terms of plant growth than any other of the four AM-fungal species used. It might have resulted due to its better ability to colonize roots extensively as evident from the highest infection percentage (Table1). The results are in agreement with the findings of Rajeswari *et al.*[18]. The distinguishable performance of various AM strains on plant growth could be attributed to the variations in the capability and competence of strains to form mycorrhizae rapidly and extensively in the rhizosphere[19].

A greater availability of phosphorus alongwith AM-fungi inoculation in legumes results in increased plant growth[20]. Dual inoculation with *Rhizobium* and mycorrhizae also induced significant increase in plant growth of Chickpea[21]-a nodulating legume. This endosymbiotic association reduces the dependency of agricultural crops on nitrogenous fertilizers. The high cost of fertilizers, release of pollutants during fertilizer production, leaching of nutrients into ground water etc. have emphasized the need

Table 1. Effect of AM-fungi with *Rhizobium* and Phosphorus on the growth of french bean (105 DAS).

Treatments	Root length (cm)	Shoot length (cm)	Dry wt. of Roots (mg)	Dry wt. of shoots (mg)	Dry wt. of leaves (mg)	Mycorrhizal colonization (%)
Control	17.8	15.78	114	2580	5720	10
P full dose (KH_2PO_4)	18.96[a]	16.66[a]	132[a]	2790[a]	6790[a]	33
S. erythropa + *Rhizobium*	23.54[a]	18.34[a]	210[a]	3254[a]	9520[a]	73
S. erythropa + 1/2 Phosphorus	22.6[a]	17.82[a]	202[a]	3224[a]	9220[a]	62
S. sinuosa + *Rhizobium*	23.14[a]	18.24[a]	208[a]	3218[a]	9490[a]	65
S. sinuosa + 1/2 Phosphorus	21.8[a]	18.08[a]	182[a]	3208[a]	7800[a]	57
G. albida + *Rhizobium*	21.6[a]	18.38[a]	202[a]	3218[a]	8830[a]	68
G. albida + 1/2 Phosphorus	20.92[a]	18.26[a]	204[a]	3194[a]	8370[a]	60
G. albidum + *Rhizobium*	20.94[a]	18.26[a]	186[a]	3194[a]	8310[a]	70
G. albidum + 1/2 Phosphorus	20.7[a]	18.2[a]	168[a]	2954[a]	7940[a]	60

a = Significant at 1% level ; b= Non-significant

Table 2. Effect of AM-fungi with *Rhizobium* and Phosphorus on the yield of French bean.

Treatments	Pod No./Pt.	Average length of Pods / Pt. (cm)	Avg. Dry Pod wt.* (g)	Average No. of seeds/Pod	Total seed No./Pt.	Wt. of Total seed/Pt.# (g)
Control	17	9.7	9.47	4.5	58.7	15.535
P full dose (KH_2PO_4)	18[b]	10.37[b]	9.9[a]	4.7[b]	60.4[b]	17.955[b]
S. erythropa + *Rhizobium*	24[a]	10.96[a]	11.13[a]	5.6[a]	84.4[a]	25.035[a]
S. erythropa + 1/2 Phosphorus	22[a]	10.8[a]	10.09[a]	5.2[b]	77.3[a]	21.335[a]
S. sinuosa + *Rhizobium*	22[a]	11.24[a]	10.77[a]	5.4[a]	78.3[a]	24[a]
S. sinuosa + 1/2 Phosphorus	21[a]	11.18[a]	10.49[a]	5.3[b]	77.1[b]	23.565[a]
G. albida + *Rhizobium*	24[a]	11.25[a]	10.78[a]	5.2[b]	85.9[a]	26.465[a]
G. albida + 1/2 Phosphorus	22[a]	10.68[b]	10.832[a]	4.9[b]	82.5[a]	24.415[a]
G. albidum + *Rhizobium*	24[a]	11.2[a]	11.03[a]	5.4[a]	84.9[a]	25.77[a]
G. albidum + 1/2 Phosphorus	23[a]	10.97[a]	10.098[a]	5.2[b]	81.2[a]	25.005[a]

*120 DAS #150 DAS **a**=Significant at 1% level ; **b**= Non-significant at 1% level of bacterization to increase productivity in legumes[22]. A large number of *Rhizobium* species nodulate bean plants supporting that bean is a promiscuous host and a diversity of bean-rhizobia-interactions exists. Large range of dinitrogen fixing capabilities have been documented among bean cultivars; commercial beans having the lowest values among legume crops[23]. It is evident from the present study that the role of *Rhizobium* is to improve nitrogen fixation while AM-fungi inoculation improves growth through enhancing nutrient uptake particularly phosphorus in the non-nodulating *P. vulgaris*.

From the present study it is concluded that French bean plant are benefited when they are raised in the presence of AM-fungus with the half dose of phosphorus, thus resulting in a reduction of the P-fertilizer application. Co-inoculation of efficient AM-fungi and *Rhizobium* can greatly assist nitrogen- fixation and ultimately increase yield of plants. This dual inoculation gave better response than plants treated with AM fungi plus half dose of phosphorus.

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