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Growth, nodulation and yield of mash bean (*Vigna mungo* L.) as affected by Rhizobium inoculation and soil applied L-tryptophan

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Abstract

Most of the grain legumes in Pakistan are poorly nodulated either because of low indigenous rhizobial population or due to inefficient strains of native rhizobia. However, improvement in nodulation could be achieved through inoculation with effective rhizobial strains. A pot trial was conducted to evaluate the effect of Rhizobium inoculation along with L-tryptophan (L-TRP), a precursor of indole acetic acid (IAA) on growth, yield and nodulation of mash been. The results indicated that Rhizobium inoculation along with L-tryptophan application (6 mg kg^{-1} soil) significantly improved plant height (44.6%) as compared to control. Different improvements in root length (72.4%), oven dry root weight (Five fold), no. of pods plant^{-1} (86.4%), no. of grains per pod (42.8%), 100-grain weight (18.9%) and nitrogen concentration in grains (two fold), was noted as compared with the control where L-TRP was applied @ 2 mg kg^{-1} soil. There was a significant increase in number of nodules plant^{-1} (one fold), nodule fresh weight plant^{-1} (two fold) and nodule dry weight plant^{-1} (four fold) with Rhizobium inoculation along with L-TRP application @ 2 mg kg^{-1} soil as compared to Rhizobium inoculation alone. The study showed that Rhizobium inoculation along with L-TRP application could be a better approach for sustainable legume production.

Keywords: *Bradyrhizobium japonicum*, L-tryptophan, IAA, nodulation, legumes

Introduction

Significant contributions have been made in Pakistan to basic and practical aspects of increasing nitrogen fixation. Most of the grain legumes in Pakistan do not nodulate properly either because of low indigenous populations or due to inefficient strains of native rhizobia (Azam and Memon, 2001). However, in some previous studies an improvement in nodulation was obtained by using rhizobial inocula (Asad *et al*., 1991). Idris *et al.* (1986) observed that all *Rhizobium* strains under investigation had a positive effect on nodulation of mung bean. Many researchers have reported that Rhizobium inoculation improves the yield of crops as a result of biological nitrogen fixation and some other mechanisms of action like auxin production and phosphorus solublization (Etesami *et al*., 2009).

Plant growth regulators are biologically active substances, which have far reaching effects on physiological processes of plants even at extremely low concentrations and the microorganisms are considered as potential source of these substances (Frankenberger and Arshad, 1995, Arshad and Frankenberger, 1998). Exogenous application of plant growth regulators has shown dramatic effects on growth and development of various crops (Khalid *et al.,* 2001). It is highly likely that the effectiveness of the microorganisms as crop inoculants could be enhanced by the application of plant growth regulators and/or their precursors. L-Tryptophan is a precursor of indole acetic acid (IAA), which has been reported to significantly influence the growth and yield in rice, soybean and wheat (Zahir *et al*., 1998, 2000).

Plant growth regulators are organic substances grouped into auxins, cytokinins, ethylene, gibbrellins and abscisic acid. The primary active auxin in most plants is indole-3-acetic acid (IAA). Auxin, being plant hormone, enhances growth rate, initiate root, promote flowering, fruit setting, and may be involved in nodulation process. Many rhizobial species are able to produce IAA, which improves root growth and as a result, uptake of nutrients by plants (Kevin, 2003; Khalid *et al*., 2004). To produce IAA, bacteria use L-TRP as a precursor (Sarwar and Frankenberger, 1994). So, Rhizobium inoculation along with L-TRP application could help in higher production of auxin resulting in increased root growth and ultimately enhanced crop yield.

Mash bean occupies an important place in Pakistan's agriculture. It is produced for both human consumption and cattle fodder (Zukovsky, 1971). Although its contribution to pulse production in the world is low, in some regions it is a key component of cropping system. This is especially true for the Indo-Pakistan subcontinent, where mash bean provides significant dietary protein as its seed contains 25-32% protein (Allen and Allen, 1981).

Thus, combined application of Rhizobium and L-tryptophan could be used as an effective strategy for

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improving the growth, nodulation and yield of legumes under field conditions, therefore, the present study was conducted to evaluate the effectiveness of co-inoculation of *Bradyrhizobium japonicum* and L-tryptophan to enhance growth, nodulation and yield of mash bean.

Materials and Methods

Collection of Rhizobium

A pre-selected strain of *Bradyrhizobium japonicum* was obtained from Soil Microbiology and Biochemistry Lab. of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

Inoculum preparation

Broth culture was prepared by using yeast extract manitol (YEM) medium. The broth was inoculated with *Bradyrhizobium japonicum* and incubated at 28$\pm$1 ^{o}C for 72 hours. After incubation, optical density of the inoculated broth culture was determined by spectrophotometer to have (10^7-10^8 CFU mL^{-1}). Surface-disinfected seeds were coated with *Bradyrhizobium japonicum* by using slurry prepared with sterilized peat, broth culture (10^7-10^8 cfu mL^{-1}) and sterilized sugar solution (10%) in the ratio 5:4:1. For the un-inoculated control, sterilized (autoclaved) broth was used in the slurry.

Pot trial

Pot experiment was conducted in the net house. Inoculated seeds were sown in pots filled with 12 kg soil each. The Soil used in pot was analyzed for EC_e (Rhoades, 1982), pH_s (Mclean, 1982), organic matter (Nelson and Sommers, 1982), total N (Tecator, 1981), particle size analysis (Gee and Bauder, 1986), P (Olson and Sommers, 1982), and K as described by US Salinity Laboratory Staff (1954). Recommended NP fertilizers @ 25 and 62 kg ha^{-1} were applied as urea and diammonium phosphate (DAP), respectively, at the time of sowing. After fifteen days of seed germination, different levels (1, 2, 4, 6, and 8 mg kg^{-1} soil) of L-TRP were applied to pots in solution form. For comparison, one treatment was neither treated with inoculum nor with L-tryptophan (L-TRP). Rhizobium alone was also applied to compare the effect of Rhizobium in combination with different levels of L-TRP. Each treatment was replicated six times using completely randomized design (CRD). Three replications were used for recording number of nodules $plant^{-1}$, fresh and oven dry weight of nodules, root length; fresh and oven dry weight of roots at flowering stage. At harvesting, data regarding number of pods $plant^{-1}$, number of grains pod^{-1}, 100-grain weight, fresh and oven dry weight of straw were recorded. Plant samples were analyzed for N-contents. Analysis of variance (ANOVA) technique was used and Duncan's Multiple Range (DMR) test was applied to see the significance of differences among treatments means (Duncan, 1955).

Results

The soil used for the pot trial was free from salinity/ sodicity and alkaline in reaction, calcareous in nature, deficient in organic matter and P, and medium in K. The texture was sandy clay loam (Table 1).

Table 1: Physico-chemical characteristics of soil used for pot trial

Soil Characteristic	Unit	Value
Textural class	-	Sandy clay loam
EC_e	dS m^{-1}	2.7
pH	-	7.4
$CaCO_3$	%	6.2
Organic matter	%	0.43
Total nitrogen	%	0.021
Available phosphorus	mg kg^{-1}	3.0
Extractable potassium	mg kg^{-1}	150

The effect of Rhizobium inoculation along with L-TRP application significantly increased shoot length as compared to control (Table 2). Maximum increase in shoot length (44.6 % over control and 31.1 % over Rhizobium alone) was observed in the treatment where L-TRP was applied @ 6 mg kg^{-1} soil along with Rhizobium inoculation. The results revealed that shoot length increased with increasing concentration of L-tryptophan.

Root length due to different treatments was affected significantly as compared to control. Maximum root length (26.2 cm) was observed where L-tryptophan was applied @ 2 mg kg^{-1} soil along with Rhizobium inoculation; it was 72.4% higher than control and 40.9% higher than single inoculation. The minimum increase in root length (2.6% over control) was recorded when L- tryptophan was applied @ 8 mg kg^{-1} soil along with Rhizobium inoculation (Table 2).

The results showed a significant increase in root fresh weight $plant^{-1}$ over control due to Rhizobium inoculation alone and in combination with L-TRP (Table 2). Maximum root fresh weight (2.1 g $plant^{-1}$) was found with Rhizobium + L-TRP @ 2 mg kg^{-1} soil. It was 91% higher than control and 50% higher than Rhizobium alone.

The effect of Rhizobium inoculation alone and in combination with L-TRP application on root dry weight $plant^{-1}$ indicated that all the treatments except where L-TRP was applied @ 8 mg kg^{-1} soil along with Rhizobium inoculation showed significant improvement over control and Rhizobium inoculation alone. Maximum root dry weight (0.5 g $plant^{-1}$) was recorded with L-tryptophan application @ 2 mg kg^{-1} soil along with Rhizobium inoculation. The increase was four fold higher than control and one and half fold higher than Rhizobium inoculation alone, respectively.

Table 2: Effect of Rhizobium inoculation and soil applied L-tryptophan on growth parameters and nodulation of mash bean

(Average of three replicates)

Treatment	Shoot length (cm)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Number of nodules plant^{-1}	Nodule fresh weight g plant^{-1}	Nodule dry weight g plant^{-1}
Control (un-inoculated without L-TRP)	30.0 d	15.2 d	1.1 e	0.1 d	--	--	--
Rhizobium alone	33.1bc	18.6 c	1.4 d	0.2 cd	31.0 e	0.6 b	0.1 c
Rhizobium+L-TRP @ 1 mg kg^{-1} soil	34.4 b	22.8 b	1.9 b	0.4 ab	88.3b	1.1 a	0.3 b
Rhizobium+L-TRP @ 2 mg kg^{-1} soil	35.4 b	26.2 a	2.1 a	0.5 a	104.0 a	1.3 a	0.5 a
Rhizobium+L-TRP @ 4 mg kg^{-1} soil	41.5 ab	22.0 b	1.8 b	0.3 b	40.7 c	1.0 a	0.3 b
Rhizobium+L-TRP @ 6 mg kg^{-1} soil	43.4 a	19.5 c	1.6 c	0.3 b	36.0 d	0.7 ab	0.1 c
Rhizobium+L-TRP @ 8 mg kg^{-1} soil	32.4bc	15.6 d	1.2 dc	0.2 cd	16.3 f	0.4 c	0.1 c

Means sharing the same letter do not differ significantly at p=0.05

Table 3: Effect of Rhizobium inoculation and soil applied L-tryptophan on yield parameters and nitrogen concentration in straw and grains of mash bean

(Average of three replicates)

Treatment	Number of pods plant^{-1}	Number of grains plant^{-1}	100- grain weight (g)	N-conc. in straw (%)	N- conc. in grains (%)
Control (un-inoculated without L-TRP)	11.8 de	4.2 e	5.3 c	1.5 d	2.1 e
Rhizobium alone	14.2 c	4.9 d	5.6 bc	2.4 bc	3.2 bc
Rhizobium+L-TRP @ 1 mg kg^{-1} soil	17.8 b	5.7 ab	6.2 a	2.5 bc	4.3 a
Rhizobium+L-TRP @ 2 mg kg^{-1} soil	22.0 a	6.0 a	6.3 a	2.6 b	4.4 a
Rhizobium+L-TRP @ 4 mg kg^{-1} soil	17.0 bc	5.4 bc	6.0 ab	2.6 b	4.1 ab
Rhizobium+L-TRP @ 6 mg kg^{-1} soil	14.3 c	5.0 cd	5.8 abc	3.2 a	3.8 b
Rhizobium+L-TRP @ 8 mg kg^{-1} soil	12.0 d	4.7 d	5.6 bc	2.3 c	3.0 d

Means sharing the same letters do not differ significantly at p=0.05

The results (Table 2) revealed that the number of nodules plant^{-1} was significantly improved by Rhizobium inoculation alone and in combination with L-TRP over control. The maximum number of nodules plant^{-1} (104) was obtained by the application of L-tryptophan @ 2 mg kg^{-1} soil along with Rhizobium inoculation. The increase was 2 fold higher than Rhizobium inoculation alone (Table-2). There were significant increases in nodule fresh weight plant^{-1} (two fold) and nodule dry weight plant^{-1} (four fold) with Rhizobium inoculation along with L-tryptophan application @ 2 mg kg^{-1} soil as compared to only inoculation.

A significant increase in number of pods plant^{-1} was observed due to Rhizobium inoculation alone and in combination with L-TRP application as compared to control. The application of L-Tryptophan (TRP) @ 2 mg kg^{-1} soil along with Rhizobium inoculation yielded maximum number of pods plant^{-1} (86.4 % over control and 55% over Rhizobium inoculation alone). Rhizobium inoculation along with L-TRP application @ 2 mg kg^{-1} soil increased number of pods plant^{-1} and number of grains pod^{-1} up to maximum extent in this experiment. Maximum number of grains pod^{-1} (6) was observed with Rhizobium + L-TRP @ 2 mg kg^{-1} soil (Table 3). The increase in number of grains pod^{-1} was 42.8 % higher over control and 22.4 % over Rhizobium inoculation alone. The minimum increase in number of grains pod^{-1} (11.9% over control) was recorded when L-TRP was applied @ 8 mg kg^{-1}soil along with Rhizobium inoculation.

The results indicated that the effect of Rhizobium inoculation and soil applied L-TRP on 100-grain weight plant^{-1} was significantly better than that of Rhizobium alone. Maximum 100-grain weight plant^{-1} (6.3 g) was obtained with L-TRP application @ 2 mg kg^{-1} soil along with Rhizobium inoculation. The increase was 18.9 % over control and 12.5 % as compared to Rhizobium inoculation alone (Table 3).

Rhizobium inoculation alone and in combination with L-TRP enhanced the nitrogen concentration in straw and grains (Table 3). Rhizobium inoculation along with L-TRP application @ 6 mg kg^{-1} produced maximum increase in the nitrogen concentration (3.2%) in straw over the un-inoculated control. Maximum N-uptake in grain (two fold) was recorded with Rhizobium + L-TRP @ 2 mg kg^{-1} soil as compared to control.

Discussion

Rhizobium inoculation significantly improved plant growth as compared to control. But when inoculation was supplemented with L-TRP, growth was further increased as compared to Rhizobium inoculation alone as well as control. The study revealed that inoculation could be made more effective with the application of L-tryptophan which is a physiological precursor of auxin; a growth regulator, in the presence of indigenous microbes and can improve root growth resulting in more uptake of nutrients by plant. L-Tryptophan has been reported to significantly influence the growth and yield in rice, soybean and wheat (Zahir *et al*., 1998, 2000).

In the present study, integrated use of L-TRP and Rhizobium was found more effective in increasing the growth and yield parameters, and nodulation in mash bean, compared with untreated control. Moreover, it also significantly increased nitrogen concentration in grain and straw as compared to un-inoculated control. The combined application of Rhizobium and L-TRP in our experiment showed more promising results and increased almost all the growth parameters like shoot length, root length, root weight, number of nodule, and nodule weight. Rhizobium inoculation in combination with L-TRP @ 6 mg kg^{-1} soil significantly increased shoot length as compared to untreated control. This might be due to higher production of auxin in the plant due to L-TRP which might be resulted in enhanced cell growth as well as root growth and consequently more nutrients uptake. Other researchers have reported similar kind of results that the use of L-tryptophan along with microbial inoculants enhanced plant growth (Arshad *et al*., 1994; Arshad and Frankenberger, 1998; Zahir *et al*., 1997, 2000, 2005). Similarly, Khalid *et al*. (2004) found that wheat inoculated with PGPR and L-tryptophan, produced higher root yield and plant growth of wheat. The results also go parallel to those of Qureshi *et al*., (2009) who found an increase in number of nodules $plant^{-1}$ due to Rhizobium inoculation along with L-TRP. In present study, a significant increase in number of pods $plant^{-1}$ was observed due to Rhizobium inoculation alone and in combination with L-TRP application as compared to control. Hussain (1995) also reported an increase in number of pods $plant^{-1}$ in lentil while investigating the effect of Rhizobium inoculation and L-TRP application.

The results regarding the 100-grain weight $plant^{-1}$ indicate that Rhizobium inoculation and soil applied L-TRP produced significant improvement over control and Rhizobium alone. An increase in grain yield was reported by Hussain (1995) while working on lentil to study the combined effect of Rhizobium inoculation and L-TRP application.

The accumulation of nitrogen in grains was enhanced due to Rhizobium inoculation alone and in combination with L-TRP. Hussain (1995) studied N-uptake in lentil with Rhizobium inoculation alone or in combination with L-TRP and reported an increase in nitrogen contents in grains. The results were also in line with the findings of Qureshi *et al*. (2009) while working on chickpea to assess the co-inoculation potential of *Mesorhizobium ciceri* and *Azotobacter chroococcum* on the yield of chickpea at two levels of nitrogenous fertilizer. Similarly, Hussain *et al.* (1989) and Shahzad (1993) also found an increase in N-concentration and its uptake by the application of L-tryptophan on *Albizia lebbeck* and maize, respectively.

It is concluded from the study that Rhizobium inoculation may be used as an effective strategy to improve the growth, nodulation and yield under field conditions. But the Rhizobium inoculation supplemented with L-TRP is even more effective for improving the growth, yield, and nodulation of mash bean and could be preferred over the sole inoculation of Rhizobium.

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