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Research Article

Growth Response of Two *Phaseolus mungo* L. Cultivars Induced by Arbuscular Mycorrhizal Fungi and *Trichoderma viride*

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The present investigation aimed to quantify the difference in response of two *Phaseolus mungo* L. cultivars (i.e., UH-1 and IPU-94-1) to *Glomus mosseae* (G), that is, *Funneliformis mosseae*, *Acaulospora laevis* (A), and *Trichoderma viride* (T), in different combinations or alone. All the treatments were inoculated with *Bradyrhizobium japonicum* to ensure nodulation as soil used in the experiment was sterilized. After 120 days of inoculation, plants were analyzed for chlorophyll content, nodulation, mycorrhization, leaf area, and protein content. Results indicate variation in growth response of two cultivars with different treatments. Triple inoculation of plants with G + A + T proved to be the best treatment for growth followed by G + T in both cultivars. Our work allowed the selection of *P. mungo* L. cultivar UH-1 as highly mycorrhizal responsive as compared to IPU-94-1 and *G. mosseae* to be an efficient bioinoculant as compared to *A. laevis* for growth enhancement of *P. mungo*. Further characterization of *P. mungo* genotypes will enhance our knowledge of physiological and genetic mechanism behind increase in plant growth and yield due to AM symbiosis.

1. Introduction

Grain legumes are a vital source of protein-rich edible seeds, comprising the chief source of dietary protein for majority of population in developing countries. India is an agriculture dominated nation where a variety of legumes are grown. *Phaseolus mungo* L. an important legume crop is one of the heavily priced pulses of India. More than two-thirds of the world's total production of *P. mungo* is contributed by India, which is largest in the world [1]. The legume is highly nutritional with about 60% carbohydrates, 24% proteins, and 1.3% fats. In addition to this, the legume has fair amount of minerals like calcium, phosphorus, potassium, and vitamins A, B, and C [2, 3]. Due to high nutritional qualities, this pulse is an ideal crop for achieving developmental goals of reducing poverty and hunger, improving human health and nutrition, and increasing ecosystem flexibility. In agricultural systems, the legumes are important rotation crops which help in supplying nitrogen to the cereal crop and reducing soil pathogens. In India, multiple crops are grown by farmers in a single growing season in order to fetch good profits. As a result of this, withdrawal of nutrients by the plants occurs faster than they are replenished. Soil becomes nutrient depleted which needs to be supplemented with chemical fertilizers but the extensive use of chemical fertilizers and indiscriminate use of pesticides in modern agriculture have resulted in disruption and degradation of agroecosystems. In order to secure environmental quality as well as healthy food demands of future, sustainable productivity of both agricultural and natural soil-plant systems is essential.

A variety of interactions occur among soil microbes and plants in the heterogeneous rhizosphere. Among these, it is predominantly the symbiotic interaction of arbuscular mycorrhizal fungi (AMF) with the roots of plants which determines soil fertility and plant health. The AM fungi amend the root architecture of host plant by enhancing lateral root formation [4]. The extra radical hyphal (ERH) network of fungus increases the volume of soil explored, resulting in increased water and nutrient uptake [5]. The fungi also produce glomalin protein helping in formation of stable soil aggregates, resulting in improvement of soil structure. Role of AMF in mineral nutrition has recently been reviewed by Azcón-Aguilar and Barea [6]. Nearly 80% of the plant phosphorus (P) and nitrogen (N) are now admitted to be provided by mycorrhizal fungi [7]. The effect of AMF on the growth enhancement of some legumes has been confirmed

by Hassan and Abakeer [8] as well as by Ndoye et al. [9]. In addition to AMF, the role of *Trichoderma* species as phytostimulators and biocontrol agents in agricultural and horticultural systems has also been investigated [10, 11]. The exploitation of biocontrol agents requires consideration as they facilitate root colonization by AM fungi [12]. So keeping in mind the concept of sustainability and to enhance the productivity, the present investigation was aimed to find out the effect of different biofertilizers alone and in different combinations on growth enhancement of two cultivars of *P. mungo*.

2. Materials and Methods

2.1. Mass Multiplication of Bioinoculants. In this study, the two dominant AM species *Glomus mosseae* (i.e., *Funneliformis mosseae*) and *Acaulospora laevis were* used. These species were isolated from the rhizospheric soil of *P. mungo* grown in botanical garden of Botany Department, Kurukshetra University, Kurukshetra. The AM species were propagated in association with maize as host under standard pot culture conditions after preparation of starter inoculum of each species by "Funnel Technique" of Menge and Timmer [13]. *Trichoderma viride* was mass multiplied on a modified wheat bran-saw dust medium [14], while *Bradyrhizobium japonicum* culture (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was multiplied by using nutrient broth medium.

2.2. Preparation of Pot Mixture. The experiment was laid out in a randomized complete block design, with five replicates per treatment. Top soil (0–30 cm) consisting of 20.8% silt, 3.78% clay, with pH 8.05, total N, 0.0485%, and available P, 0.015%, was collected from Kurukshetra University, Kurukshetra. This soil was air-dried and sieved. The soil : sand mixture (3 : 1, v/v) autoclaved for 20 minutes at 121°C and 15 psi was used in the experiment. For AM treatment, 10% (w/w of soil) of the selected AM inoculum having 870–890 AM spores and chopped AM colonized root pieces with an infection level of 94% was added. *Trichoderma viride* inoculum with density 3.4×10^8 cfu gm^{-1} was added per the treatment.

Seeds of two varieties of *P. mungo*, namely, IPU-94-1 and UH-1, were acquired from Haryana Agriculture University Hisar, Haryana, India. Seeds were surface-sterilized with 10% solution of sodium hypochlorite for 1-2 minutes and then rinsed thoroughly with distilled water. Before the seeds were sown, 10 mL of a liquid suspension of bacteria with a density 10^8 cells/mL was applied to each pot to ensure nodulation as the soil used in experiment was sterilized. Nutrient solution [15] which contained half the recommended level of phosphorus without nitrogen was used to fertilize the pots after 15 days and plants were watered once every two days. Pots were treated either with single inoculum, combined inoculum, or no inoculums as summarized in the following:

(1) Uninoculated, that is, autoclaved sterile sand : soil with no inoculum (control).

(2) *G. mosseae* (G).

(3) *A. laevis* (A).

(4) *Trichoderma viride* (T).

(5) G + A.

(6) G + T.

(7) A + T.

(8) G + A + T.

2.3. Plant Harvest and Analysis. After 120 days, plants were harvested and some physiological parameters were analyzed. Chlorophyll content was estimated using the method of Arnon [16]. Leaf proteins were estimated according to Bradford method [17]. The number of nodules per plant and yield in terms of number of pods and weight of pods per plant was recorded. Leaf area was assessed by using leaf area meter (Systronics 21, Ahmedabad, India).

2.4. Identification and Quantification of the Number and Colonization by AM Spores. Isolation of AM spores was done by using "wet sieving and decanting technique" of Gerdemann and Nicolson [18]. Identification of AM spores (*G. mosseae* and *A. laevis*) was done by using the identification manual used by Walker [19], Schenck and Perez [20], Morton and Benny [21], and Mukerji [22]. Quantification of the number of AM spores was done using "Grid Line Intersect Method" of Adholeya and Gaur [23]. "Rapid Clearing and Staining Method" of Phillips and Hayman [24] was used to determine mycorrhizal root colonization. Percent AM colonization of roots was calculated as number of root segments colonized/number of root segments studied × 100.

2.5. Statistical Analysis. Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver. 11.5, Chicago, IL, USA).

3. Results

Inoculation of *P. mungo* plants with mycorrhizal fungi and *Trichoderma* significantly increased chlorophyll content, leaf proteins, leaf area, nodulation, and mycorrhization in both varieties (UH-1 and IPU-94-1) over control (Tables 1(a), 1(b), 2(a), and 2(b)).

In variety UH-1, maximum protein content and chlorophyll content were recorded in the plants dually inoculated with *G. mosseae* + *T. viride* followed by triple inoculation of G + A + T (Table 1(a)). The mycorrhizal colonization of roots and number of AM spores recorded for the treated plants were also different (Table 2(a)). Maximum nodulation, leaf area, and number of spores and percentage colonization of roots were recorded for plants treated with *G. mosseae* + *A. laevis* and *T. viride* pursued by dual inoculation of *G. mosseae* and *T. viride*.

In variety IPU-94-1, the inoculation of plants with *G. mosseae* + *A. laevis* and *T. viride* induced maximum increase in chlorophyll content, mycorrhization (AM spore number and % root colonization), nodulation, and leaf area, while the

Table 1: (a) Interactive effect of AM fungi and *T. viride* on chlorophyll content and nodulation of *Phaseolus mungo* var. UH-1 after 120 days of inoculation. (b) Interactive effect of AM fungi and *T. viride* on chlorophyll content and nodulation of *Phaseolus mungo* var. IPU-94-1 after 120 days of inoculation.

(a)

S. number	Parameters→ Treatments↓	Chlorophyll content (mg g^{-1} FM)			Number of nodules (per plant)
		Chlorophyll a	Chlorophyll b	Total chlorophyll	
(1)	Control	0.967 ± 0.004^{h}	0.308 ± 0.006^{h}	1.227 ± 0.004^{h}	09.00 ± 2.73^{f}
(2)	G	2.091 ± 0.003^{d}	0.727 ± 0.007^{d}	2.818 ± 0.005^{d}	20.20 ± 2.38^{cd}
(3)	A	1.607 ± 0.002^{f}	0.554 ± 0.006^{f}	2.162 ± 0.005^{f}	15.20 ± 2.86^{ef}
(4)	T	1.220 ± 0.003^{g}	0.436 ± 0.006^{g}	1.656 ± 0.008^{g}	12.00 ± 2.54^{f}
(5)	GA	2.435 ± 0.002^{c}	0.755 ± 0.004^{c}	3.091 ± 0.004^{c}	22.60 ± 3.04^{bc}
(6)	GT	2.675 ± 0.002^{a}	1.148 ± 0.003^{a}	3.824 ± 0.005^{a}	25.80 ± 2.38^{ab}
(7)	AT	1.822 ± 0.006^{e}	0.632 ± 0.004^{e}	2.455 ± 0.008^{e}	17.40 ± 2.70^{de}
(8)	GAT	2.564 ± 0.002^{b}	1.104 ± 0.005^{b}	3.667 ± 0.009^{b}	29.20 ± 3.70^{a}
	LSD ($P \leq 0.05$)	0.0061	0.0092	0.0121	4.82
	ANNOVA $F_{(7,16)}$	124.121	125.941	659.041	22.294

(b)

S. number	Parameters→ Treatments↓	Chlorophyll content (mg g^{-1} FM)			Number of nodules (per plant)
		Chlorophyll a	Chlorophyll b	Total chlorophyll	
(1)	Control	0.957 ± 0.003^{g}	0.292 ± 0.006^{h}	1.256 ± 0.014^{h}	03.8 ± 2.863^{e}
(2)	G	1.963 ± 0.004^{c}	0.753 ± 0.005^{c}	2.716 ± 0.010^{d}	15.0 ± 4.123^{cd}
(3)	A	1.475 ± 0.008^{e}	0.575 ± 0.006^{e}	2.050 ± 0.013^{f}	12.0 ± 2.540^{d}
(4)	T	1.082 ± 0.004^{f}	0.429 ± 0.006^{g}	1.511 ± 0.003^{g}	06.8 ± 2.387^{e}
(5)	GA	2.286 ± 0.004^{b}	0.609 ± 0.006^{d}	2.896 ± 0.002^{c}	17.8 ± 2.387^{bc}
(6)	GT	2.552 ± 0.137^{a}	0.895 ± 0.035^{b}	3.449 ± 0.101^{b}	21.2 ± 3.033^{ab}
(7)	AT	1.634 ± 0.004^{d}	0.497 ± 0.004^{f}	2.131 ± 0.008^{e}	16.2 ± 2.387^{c}
(8)	GAT	2.559 ± 0.152^{a}	0.987 ± 0.036^{a}	3.546 ± 0.116^{a}	24.8 ± 2.387^{a}
	LSD ($P \leq 0.05$)	0.0938	0.0243	0.0713	3.6352
	ANNOVA $F_{(7,16)}$	372.350	779.971	1.173	30.924

G: *Glomus mosseae*; A: *Acaulospora laevis*; T: *Trichoderma viride*; ±: each value is a mean of five replicates; FW: fresh weight; FM: fresh matter; ±: standard deviation; AM: arbuscular mycorrhizae; values in columns followed by same letter are not significantly different ($P \leq 0.05$), least significant difference test.

dual combination of *G. mosseae* + *T. viride* increased protein content in leaves. Among the different dual inoculation treatments, the combination G + T performed best for all the parameters studied. Among the different treatments used, *Trichoderma* was found to be least effective (Tables 1(b) and 2(b)). The combined effect of the bioinoculants resulted in highest mycorrhization in the plants than single strain. *G. mosseae* alone or in combination with *T. viride* proved to be a compatible strain for growth enhancement of pulse legume as compared to *A. laevis*.

4. Discussion

The ability of AMF to improve growth of plants by increasing nutrient uptake and water absorption makes them especially important for sustainable farming systems. In the present study, a positive interaction was observed between the AM fungi and *T. viride* for growth improvement because of stimulatory role of the latter in mycorrhization. Our results confirm the findings of Shuab et al. [25] and Bagheri et al. [26], who also observed an improvement in growth of the plants treated with microbes. High concentration of photosynthetic pigments in the leaves of mycorrhizal plants is attributable to increased uptake of magnesium and phosphorus, increased transpiration, stomatal conductance, and carbon assimilation [27, 28]. High concentration of photosynthetic pigments results from higher nutritional uptake by plants and thus increased energy [29]. Another reason could be AM mediated increase in number and size of chloroplasts as reported by Arumugam et al. [28] and Krishna and Bagyaraj [30]. Mycorrhizal infection increases nitrogen and phosphorus content in the plants [6, 31] which contributes to increased protein synthesis. The higher shoot protein content observed in AMF treated plants may be due to stimulation of protein synthesis in host after infection [32]. Similar observations have been made by Mathur and Vyas [33], Sandhya et al. [34], and Lenin et al. [35]. The plants treated with AM fungi had higher nodulation than non-AM plants. It may be due to the ability of AMF to supply adequate amount of P required for nitrogen fixation by *Rhizobia*. Role of AM fungi in improving

Table 2: (a) Interactive effect of AM fungi and *T. viride* on protein content, leaf area, and mycorrhization in *Phaseolus mungo* var. UH-1 after 120 days of inoculation. (b) Interactive effect of AM fungi and *T. viride* on protein content, leaf area, and mycorrhization of *Phaseolus mungo* var. IPU-94-1 after 120 days of inoculation.

(a)

S. number	Parameters→ Treatments↓	Protein content (mg g^{-1} FW)	Leaf area (cm^2)	AM spore number/10 g of soil	AM root colonization (%)
(1)	C	0.221 ± 0.017^{f}	07.52 ± 1.733^{h}	07.0 ± 2.23^{f}	00.6 ± 0.29^{g}
(2)	G	0.461 ± 0.017^{c}	25.55 ± 2.037^{d}	87.0 ± 3.16^{b}	74.6 ± 4.15^{c}
(3)	A	0.382 ± 0.019^{e}	18.65 ± 2.095^{f}	67.6 ± 3.64^{d}	55.6 ± 4.39^{e}
(4)	T	0.359 ± 0.022^{e}	14.50 ± 1.818^{g}	57.0 ± 3.16^{e}	42.6 ± 3.36^{f}
(5)	GA	0.475 ± 0.019^{c}	29.27 ± 2.104^{c}	86.2 ± 3.34^{b}	80.4 ± 4.92^{b}
(6)	GT	0.584 ± 0.028^{a}	35.27 ± 2.182^{b}	90.2 ± 4.43^{ab}	84.0 ± 4.52^{ab}
(7)	AT	0.422 ± 0.018^{d}	22.38 ± 1.863^{e}	74.6 ± 3.64^{c}	64.8 ± 3.70^{d}
(8)	GAT	0.519 ± 0.020^{b}	40.24 ± 2.231^{a}	94.8 ± 3.49^{a}	89.4 ± 3.84^{a}
	LSD ($P \leq 0.05$)	0.0358	3.5949	6.03635	5.2888
	ANNOVA $F_{(7,16)}$	89.472	102.206	172.752	149.816

(b)

S. number	Parameters→ Treatments↓	Protein content (mg g^{-1} FW)	Leaf area (cm^2)	AM spore number/10 g of soil	AM root colonization (%)
(1)	C	0.187 ± 0.003^{h}	05.88 ± 1.891^{g}	00.00 ± 0.00^{g}	00.00 ± 0.00^{f}
(2)	G	0.435 ± 0.003^{d}	35.50 ± 2.266^{a}	81.40 ± 4.92^{c}	72.40 ± 3.64^{b}
(3)	A	0.365 ± 0.003^{f}	15.22 ± 1.983^{e}	57.80 ± 3.96^{e}	45.80 ± 3.70^{d}
(4)	T	0.360 ± 0.003^{g}	10.60 ± 1.617^{f}	49.20 ± 3.49^{f}	30.80 ± 5.31^{e}
(5)	GA	0.456 ± 0.002^{c}	21.04 ± 1.939^{d}	88.60 ± 4.15^{b}	77.00 ± 3.67^{ab}
(6)	GT	0.506 ± 0.002^{a}	25.90 ± 2.003^{c}	92.00 ± 3.80^{b}	78.00 ± 5.91^{a}
(7)	AT	0.402 ± 0.002^{e}	19.24 ± 1.553^{d}	66.20 ± 4.20^{d}	58.60 ± 3.64^{c}
(8)	GAT	0.485 ± 0.002^{b}	30.27 ± 2.248^{b}	96.80 ± 2.16^{a}	82.00 ± 3.53^{a}
	LSD ($P \leq 0.05$)	0.0037	2.5158	4.698	5.183
	ANNOVA $F_{(7,16)}$	6.155	129.458	380.026	253.739

G: *Glomus mosseae*; A: *Acaulospora laevis*; T: *Trichoderma viride*; ±: each value is a mean of five replicates; FW: fresh weight; FM: fresh matter; ±: standard deviation; AM: arbuscular mycorrhizae; values in columns followed by same letter are not significantly different ($P \leq 0.05$), least significant difference test.

nodulation has been reviewed by Azcón-Aguilar and Barea [36]. Inoculation with biofertilizers caused leaf enlargement and improved total plant morphology. Our results are in agreement with the findings of Amerian and Stewart [37] and Lenin et al. [35].

5. Conclusion

The use of microbes improved physiological and morphological status of the plants; thus they could be used to economically replace the expensive chemical fertilizers. A combination of *G. mosseae*, *A. laevis*, and *T. viride* can be used for sustainable growth improvement of *P. mungo*. However, the field trails for testing the efficacy of bioinoculants are still to be performed so as to understand the plant establishment and development under these conditions. Results indicate variation in growth of the two cultivars with different treatments of bioinoculants. Our work allowed the selection of *P. mungo* L. cultivar UH-1 as highly mycorrhizal responsive as compared to IPU-94-1.

Competing Interests

The authors declare that they have no competing interests.

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