SYNERGISTIC EFFECT OF ARBUSCULAR MYCORRHIZAE AND *Trichoderma* sp. ON GROWTH, NUTRIENT UPTAKE AND YIELD OF *Phaseolus mungo* L. CULTIVARS

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ABSTRACT

This study compared the response of two *Phaseolus mungo* L. cultivars, UH-1 and IPU-94-1, to arbuscular mycorrhizal fungi (AMF) and *Trichoderma viride* in combination or alone in pot culture under green house conditions. After 120 days of inoculation, plants were analysed for different growth parameters and it was found that inoculation of *Glomus mosseae*, *Acaulospora laevis* and *T. viride* followed by dual inoculation of *G. mosseae* and *T. viride* in both varieties stimulated growth parameters, phosphatase activity, nutrient uptake and yield. Better plant response to the different treatments was exhibited by variety UH-1 as compared to IPU-94-1 but variety IPU-94-1 responded better than UH-1 for increasing fresh and dry shoot weight, fresh root weight, and acidic phosphatase activity when both the varieties were treated with *A. laevis*. Among the two mycorrhizal fungi used, *G. mosseae* proved to be better bioinoculant compared to *A. laevis* for improving growth in both the cultivars.

Key words: *Trichoderma viride*, AM Fungi, Phosphorus, *Phaseolus mungo*, Phosphatase activity

INTRODUCTION

The functioning of terrestrial ecosystems as well as the above ground biodiversity is influenced by below ground biotic interactions altering the physical environment and soil nutrient cycling (Bardgett and van der Putten 2014). Among the variety of organisms constituting the below ground community, arbuscular mycorrhizal (AM) fungi are the major pervasive obligate symbionts having an important role in nutrient acquisition and growth of plants. In soil-food web, they add to the fungal energy channel acting as a major plant carbon sink. Manipulation of diverse microbial populations, especially arbuscular mycorrhizae and certain rhizobacteria for crop improvement is of great significance due to their remarkable role in sustainability (Yadav et al. 2013). In both associations, the macro-symbiont is benefited by nutrient supply in return for acquisition of photo-assimilates by micro-symbionts (Gourion et al. 2011). Most of unseen soil microbes in the nutrient deprived ecosystems form important ecological relationships with majority of the plant roots for the acquirement of limiting micro and macro nutrients (Marcel et al. 2008).

The ability of legumes to form mutual symbiotic relationship for atmospheric nitrogen fixation with rhizobacteria and AM fungi to meet N and P requirements in natural and agricultural ecosystems has established marvelous impact (Silveira and Cardoso 2004; Aslam et al. 2010; Samac and Graham 2007). Since, nitrogen fixation by rhizobia requires high supply of phosphorus, mycorrhizal infection is vital for legumes as it helps in providing adequate amount of phosphorus to the legumes (Mohammadi et al. 2011). Association of mycorrhizae with the roots of the plants help to explore larger soil volume thus increase in the acquisition of immobile mineral elements such as phosphorus (P), water absorption, water use efficiency and resistance to soil borne pathogens (Beltrano et al. 2003). Besides phosphorus, an uptake of several other immobile mineral elements like copper, zinc is increased and in some circumstances an uptake of potassium calcium magnesium sulphur and iron is also increased (Alizadeh 2012). The use of *Trichoderma* sp. in horticulture and agricultural systems is being investigated due to their role as phyto-stimulators or biocontrol agents, where their compatibility with AM fungi must be verified. The use of biocontrol agents needs consideration as they possibly help in stimulation of mycorrhizal colonization (Tanwar et al. 2013; Tanwar and Aggarwal 2013). On the other hand, microbial population in the rhizosphere directly or indirectly is controlled by

mycorrhizal formation through changes in fungal exudates or through root exudation patterns, termed 'as mycorrhizosphere effect' (Toljander et al. 2006).

Grain legumes have an important role in ensuring nutritional as well as economic security for developing nations. As compared to cereals, they supplement the farmer's income by fetching higher prices when grown as feed crop in many farming systems. In cropping systems, food legumes act as important rotation crop with cereals, which reduce soil pathogens and increase the fertility of the soil by supplying nitrogen through nitrogen fixing rhizobacteria thus, economically replace the expensive nitrogenous fertilizers partially. *Phaseolus mungo* is one of the palatable food legume having high nutritional qualities. It has its origin in central Asia and India and now, it is found in different tropical areas of Asia, Africa and Madagascar. In Australia and USA, it is cultivated as fodder crop (Jansen 2006). It is easily digestible and highly nutritious with about 1.3% fats, 24% proteins and 60% carbohydrates in seeds (Ali et al. 2002). The seeds are also rich in minerals like calcium, potassium, phosphorus as well as vitamin A, B and C (Sarwar et al. 2004).

In order to increase the yield of pulse legumes, large doses of chemical fertilizers are being used which have various adverse effects such as soil salinization. An alternative for sustainable enhancement of productivity is application of ecofriendly as well as cost effective biofertilizers (Kadian et al. 2013). AMF are an indispensible component of agricultural ecosystems for sustainable enhancement of productivity. Therefore, two dominant AM genera associated with *P. mungo* L. and *Trichoderma viride* were tested for their efficacy to improve growth and nutritional factors of two high yielding cultivars of *P. mungo* which is an economically important pulse crop of India. The study was aimed to determine the mycorrhizal responsiveness of the two high yielding cultivars of *P.mungo*.

MATERIALS AND METHODS

Mass multiplication of bio-inoculants

In this investigation, two dominant AM species *Glomus mosseae* and *Acaulospora laevis* used were isolated from rhizosphere of *P. mungo* L. grown in Botanical Garden, Kurukshetra University, Kurukshetra, India. Soil for AM spore isolation was kept in sterilized polythene bags at 10^0C for further processing in the laboratory. Starter inoculum of each AM fungus was prepared by 'Funnel Technique' of Meng and Timmer (1982) using maize as host plant. Following the standard pot culture conditions, the isolated AM species were propagated in association with maize as a host. A modified wheat bran-saw dust medium was used for multiplication of *T. viride* (Mukhopadhyay et al.1986).

Preparation of pot mixture

Top soil (0 to 30 cm) collected from the Botanical garden, Department of Botany, Kurukshetra University, Kurukshetra, was used for the experiment. This soil was air dried and passed through a 2 mm sieve. The soil consisted of, 3.78% clay, 20.8% silt, total N and P were 0.0489 and 0.015% respectively and pH of soil was 8.05. The experiment was laid out in a randomized complete block design, with five replicates for each treatment. In the sterilized-soil experiment, soil:sand mixture (3:1, v/v) autoclaved at 121 °C and 15 psi for 20 minutes was used. *T .viride* inoculum with approximately 3.4×10^8 cfu g^{-1} was added to each treatment. While, for AM treatment 10% (w/w of soil) of the selected AM inoculum having 870 to 890 AM spores and chopped AM colonized root pieces with an infection level of 94% was added to each pot as a band below the top soil layer in the pot.

Seeds of two varieties of *P. mungo* namely, IPU-94-1 and UH-1 procured from CCS Haryana Agricultural University Hisar, Haryana, India, were surface sterilized with 10% solution of sodium hypochlorite for 1 to 2 minutes and then washed thoroughly with distilled water and were sown in the pots. After 15 days, nutrient solution (Weaver and Fredrick 1982) containing half of the recommended level of phosphorus without nitrogen was added to each pot and the pots were watered regularly.

Treatments used were uninoculated or sterilized sand:soil without inoculum (control), *G. mosseae* (G), *A. laevis* (A), *T. viride* (T), *G. mosseae* and *A. laevis* (G + A), *G. mosseae* and *T. viride* (G + T), *A. laevis* and *T. viride* (A + T) and *G. mosseae*, *A. laevis* and *T. viride* (G + A + T).

Plant harvest, growth and nutrient analysis

After 120 days, plants were harvested to analyse some morphological and physiological parameters by uprooting them. Root length, shoot length and yield in terms of number of pods and weight of pods per plant was recorded. Root and shoot fresh weights were determined by harvesting at 120 days of sowing and weighing them. For dry weights of roots and shoots, the samples were oven dried at 70°C until constant dry weight was obtained**.** Root and shoot phosphorus content was determined by Vanado-molybdo-phosphoric acid colorimetric method (Jackson 1973). Nitrogen was estimated by Kjeldhal's method while potassium was determined by Flame Photometer model Systronics flame photometer 128 as described by Toth and Prince (1948). Phosphatase activity was assayed using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolysed by the enzyme to p-nitro phenol (Tabatabi and Bremer (1969). Yield in terms of number of pods and weight of pods per plant was recorded.

STATISTICAL ANALYSIS

Data were subjected to two way variance analysis and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.17.0, Chicago, IL, USA).

RESULTS

The plants of both varieties namely IPU-94-1 and UH-1 when inoculated with AM fungi exhibited a significant increase in all the growth parameters as compared to control plants. This positive effect of mycorrhizae on *P. mungo* was modified when both AM fungi were combined with *T. viride.* Variety UH-1 exhibited better response to the different treatments as compared to IPU-94-1 in terms of the growth parameters, phosphatase activity, nutrient uptake and yield. However, this was not found to be true for the plants with single inoculation of *A. laevis* in both varieties which enhanced fresh and dry shoot weight, fresh root weight, and acidic phosphatase activity in IPU-94-1 as compared to UH-1, indicating better responsiveness of variety IPU-94-1 to *A. laevis*. A comparison between the efficiency of two mycorrhizal fungi taken in the study reveals *G. mosseae* to be a better bioinoculant for both varieties as compared to *A. laevis.* Also, yield in terms of number of pods was found to be similar upon treatment with *A. laevis*.

Plant growth

The effect of inoculation with G+A+T followed by double inoculation with G+T significantly resulted in better growth in both varieties. After 120 days of inoculation with G+A+T, variety UH-1 showed significant increase in plant height, root length, fresh and dry weight of shoot as well as root dry and fresh weight as compared to IPU -94-1. In UH-1, all the treatments gave taller plants than IPU-94-1 except dual inoculation of G+A which resulted in plants taller than UH-1 (Table 1).

Phosphorus content and yield

In variety UH-1, the highest shoot phosphorus and root phosphorus was observed in the plants having inoculation of G+A+T, minimum being found in plants with single treatment of *T. viride* (Table 2). For variety IPU-94-1, the highest root phosphorus concentration was recorded with G+A+T while maximum shoot phosphorus concentration was found with dual inoculation of G+ T. Data on yield revealed that bioinoculation of plants resulted in a significant increase in the number andweight of the pods and consequently greater yield as compared to the control. For both varieties, treatment with a mixture of *G. mosseae, A. laevis* and *T. viride* resulted in the higher yield in terms of the number and weight of pods followed by those plants treated with *G. mosseae* and *T. viride*.

Table 1. Interactive effect of AM fungi and *T. viride* on growth response of *P. mungo* var. UH -1 and IPU-94-1 after 120 days

		Plant Height (cm)	Shoot weight (g)		Root Length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
CULTIVAR UH-1	**Control**	30.78±2.184^{k}	2.35±0.037^{l}	0.92±0.185^{n}	07.5±0.36^{j}	1.106±0.005^{k}	0.280±.0.003^{k}
	G	58.42±1.407^{e}	4.22±0.025^{e}	3.63±0.035^{g}	18.9±0.33^{c}	3.708±0.372^{f}	3.054±0.358^{e}
	A	47.44±1.499^{g}	3.66±0.022^{h}	2.00±0.031^{l}	15.0±0.33^{e}	2.826±0.060^{h}	1.646±0.009^{h}
	T	36.88±1.340^{i}	3.00±0.036^{j}	1.40±0.031^{m}	13.2±0.34^{g}	1.956±0.027^{j}	0.842±0.004^{i}
	GA	63.68±1.768^{c}	5.12±0.023^{c}	4.32±0.030^{d}	19.0±0.31^{c}	4.430±0.015^{d}	3.694±0.020^{c}
	GT	74.30±1.392^{b}	5.59±0.023^{b}	4.80±1.811^{b}	19.8±0.29^{b}	5.024±0.222^{b}	4.172±0.003^{b}
	AT	51.54±1.725^{f}	4.05±0.175^{f}	2.51±0.031^{j}	16.2±0.32^{d}	3.150±0.171^{g}	2.388±0.002^{f}
	GAT	94.46±1.368^{a}	5.97±0.294^{a}	5.11±0.031^{a}	20.4±0.27^{a}	5.500±0.079^{a}	4.780±0.025^{a}
CULTIVAR IPU-94-1	**Control**	33.06 ±1.021^{j}	1.90±0.02^{m}	0.63±0.03^{o}	05.12±0.31^{k}	0.91±0.023^{l}	0.13±0.047^{k}
	G	46.84 ±1.339^{g}	4.02±0.17^{f}	3.44±0.03^{h}	15.04±0.24^{e}	3.23±0.130^{g}	2.04±0.279^{g}
	A	37.36±1.228^{i}	3.41±0.02^{i}	2.22±0.03^{k}	12.24±0.32^{h}	1.81±0.041^{j}	0.95±0.013^{i}
	T	33.62±1.639^{j}	2.59±0.02^{k}	0.96±0.28^{n}	09.78± 0.23^{i}	1.00±0.027^{kl}	0.51±0.159^{j}
	GA	72.14±0.896^{c}	4.72±0.03^{d}	3.88±0.03^{f}	14.38±0.37^{f}	3.61±0.223^{f}	2.54±0.201^{f}
	GT	73.60±1.820^{bc}	5.03±0.15^{c}	4.13±0.02^{e}	14.80±0.27^{e}	4.20±0.053^{e}	3.04±0.213^{e}
	AT	44.14±1.393^{h}	3.84±0.16^{g}	2.64±0.02^{i}	13.20±0.29^{g}	2.31±0.097^{i}	1.51±0.155^{h}
	GAT	74.36±1.567^{b}	5.54±0.04^{b}	4.59±0.02^{c}	16.14±0.20^{d}	4.82±0.229^{c}	3.32±0.203^{d}
	Cultivar (C)	247.617	201.748	186.889	2918.703	535.583	591
	Treatments(T)	1592.890	1179.943	2884.571	1669.419	112.388	799
	C ×T	87.896	3.347	30.559	22.452	9.383	19.684

G†: *Glomus mosseae*, A: *Acaulospora laevis*, T: *Trichoderma viride*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different

Table 2. Interactive effect of AM fungi and *T. viride* on phosphorus uptake, phosphatase activity and yield of *P. mungo* var. UH-1 and IPU-94-1 after 120 days

		Phosphorus content (%)		**Phosphatase activity ($IUg^{1}FW$)**		**Yield (per pot)**	
		Root	Shoot	Acidic	Alkaline	No. of pods	Weight (g)
CULTIVAR UH-1	**Control**	0.863 ± 0.003^{n}	0.689 ± 0.008^{m}	0.066 ± 0.009^{g}	0.089 ± 0.005^{g}	04.00 ± 2.73^{f}	1.146 ± 0.308^{hi}
	G	1.503 ± 0.006^{f}	1.318 ± 0.006^{f}	0.113 ± 0.005^{cde}	0.143 ± 0.006^{cdef}	10.00 ± 3.39^{cd}	3.354 ± 0.352^{e}
	A	1.087 ± 0.007^{j}	0.977 ± 0.005^{i}	0.106 ± 0.004^{e}	0.140 ± 0.005^{cdef}	05.20 ± 2.86^{ef}	2.752 ± 0.330^{f}
	T	0.936 ± 0.008^{l}	0.848 ± 0.007^{k}	0.080 ± 0.008^{f}	0.094 ± 0.009^{g}	04.60 ± 1.94^{ef}	1.874 ± 0.288^{g}
	GA	1.631 ± 0.018^{d}	1.402 ± 0.006^{d}	0.116 ± 0.006^{bcd}	0.147 ± 0.009^{bcd}	14.40 ± 2.88^{b}	6.172 ± 0.299^{c}
	GT	1.781 ± 0.008^{b}	1.544 ± 0.006^{b}	0.124 ± 0.008^{ab}	0.161 ± 0.006^{a}	15.80 ± 2.86^{ab}	6.898 ± 0.269^{b}
	AT	1.221 ± 0.005^{h}	1.158 ± 0.005^{g}	0.111 ± 0.003^{cde}	0.132 ± 0.006^{ef}	07.60 ± 2.88^{def}	2.902 ± 0.330^{f}
	GAT	1.972 ± 0.007^{a}	1.605 ± 0.007^{a}	0.131 ± 0.007^{a}	0.151 ± 0.007^{abc}	18.60 ± 2.96^{a}	7.858 ± 0.388^{a}
CULTIVAR IPU-94-1	**Control**	0.734 ± 0.007^{o}	0.587 ± 0.006^{n}	0.064 ± 0.007^{g}	0.087 ± 0.025^{g}	4.2 ± 2.387^{f}	0.884 ± 0.255^{i}
	G	1.361 ± 0.007^{g}	1.167 ± 0.006^{g}	0.109 ± 0.006^{de}	0.141 ± 0.009^{cdef}	7.4 ± 2.701^{def}	2.462 ± 0.407^{f}
	A	1.002 ± 0.006^{k}	0.895 ± 0.005^{j}	0.105 ± 0.005^{e}	0.137 ± 0.004^{def}	5.2 ± 2.863^{ef}	1.528 ± 0.358^{gh}
	T	0.870 ± 0.006^{m}	0.730 ± 0.007^{l}	0.079 ± 0.005^{f}	0.092 ± 0.008^{g}	4.4 ± 3.209^{f}	1.312 ± 0.347^{hi}
	GA	1.525 ± 0.006^{e}	1.319 ± 0.006^{f}	$0.113\pm0.005b^{cde}$	0.145 ± 0.004^{cde}	9.0 ± 2.549^{de}	2.714 ± 0.436^{f}
	GT	1.691 ± 0.010^{c}	1.531 ± 0.006^{c}	0.120 ± 0.006^{bc}	0.160 ± 0.008^{ab}	9.0 ± 4.527^{de}	3.442 ± 0.472^{e}
	AT	1.134 ± 0.007^{i}	1.083 ± 0.010^{h}	0.117 ± 0.005^{bcd}	0.130 ± 0.005^{f}	5.6 ± 2.701^{ef}	1.862 ± 0.324^{g}
	GAT	1.777 ± 0.008^{b}	1.390 ± 0.007^{e}	0.129 ± 0.008^{a}	0.152 ± 0.004^{abc}	13.4 ± 3.847^{bc}	4.290 ± 0.332^{d}
	Cultivar (C)	3611.272	4345.266	0.758	0.749	16.667	537.797
	Treatments(T)	2251.106	21625.609	104.968	104.968	20.688	269.108
	C ×T	61.793	53.445	0.671	0.671	2.077	42.167

G†: *Glomus mosseae*, A: *Acaulospora laevis*, T: *Trichoderma viride*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different

Table 3. Interactive effects of AM fungi and *T. viride* on nutrient uptake of *P. mung*o var. UH-1 and IPU-94-1 after 120 days

		Nitrogen content (%)		Potassium content (%)	
		Root	Shoot	Root	Shoot
CULTIVAR UH-1	**Control**	0.362 ± 0.0031^{l}	0.921 ± 0.0031^{h}	0.812 ± 0.0238^{j}	0.521 ± 0.0034^{n}
	G	0.570 ± 0.0023^{d}	1.325 ± 0.0039^{f}	1.279 ± 0.0027^{c}	1.022 ± 0.0025^{g}
	A	0.425 ± 0.0028^{j}	1.042 ± 0.0025^{i}	1.023 ± 0.0023^{g}	0.899 ± 0.0054^{j}
	T	0.391 ± 0.0020^{k}	0.984 ± 0.0023^{g}	0.974 ± 0.0323^{h}	0.751 ± 0.0027^{k}
	GA	0.590 ± 0.0025^{c}	1.421 ± 0.0031^{d}	1.293 ± 0.0027^{c}	1.124 ± 0.0031^{c}
	GT	0.612 ± 0.0025^{b}	1.629 ± 0.0023^{a}	1.498 ± 0.0031^{a}	1.354 ± 0.0031^{a}
	AT	0.477 ± 0.0025^{g}	1.163 ± 0.0025^{g}	1.124 ± 0.0035^{e}	0.955 ± 0.0031^{h}
	GAT	0.705 ± 0.0031^{a}	1.600 ± 0.0036^{b}	1.456 ± 0.0031^{b}	1.291 ± 0.0030^{b}
CULTIVAR IPU-94-1	**Control**	0.224 ± 0.0027^{o}	0.762 ± 0.0028^{j}	0.714 ± 0.0039^{l}	0.401 ± 0.0029^{o}
	G	0.462 ± 0.0031^{i}	1.148 ± 0.0027^{h}	1.066 ± 0.0031^{f}	0.929 ± 0.0028^{i}
	A	0.354 ± 0.0034^{m}	0.983 ± 0.0030^{g}	0.931 ± 0.0031^{i}	0.631 ± 0.0025^{m}
	T	0.301 ± 0.0023^{n}	0.899 ± 0.0033^{i}	0.780 ± 0.0028^{k}	0.450 ± 0.0024^{o}
	GA	0.472 ± 0.0027^{h}	1.331 ± 0.0023^{e}	1.194 ± 0.0277^{d}	1.042 ± 0.0031^{f}
	GT	0.564 ± 0.0035^{e}	1.502 ± 0.0039^{c}	1.204 ± 0.0031^{d}	1.100 ± 0.0074^{e}
	AT	0.391 ± 0.0028^{e}	1.002 ± 0.0028^{j}	0.970 ± 0.0025^{h}	0.713 ± 0.0025^{l}
	GAT	0.518 ± 0.0028^{f}	1.419 ± 0.0023^{d}	1.288 ± 0.0028^{c}	1.114 ± 0.0025^{d}
	Cultivar (C)	28339.084	37207.542	3423.705	58204.823
	Treatments(T)	17044.850	79828.797	3136.114	60982.693
	C ×T	584.844	603.795	77.883	1443

G†: *Glomus mosseae*, A: *Acaulospora laevis*, T: *Trichoderma viride*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different

The average number and weight of pods were high in UH-1 compared to IPU-94-1 variety.

Phosphatase activity

Significantly higher acidic and alkaline phosphatase activities were observed for varieties UH-1 and IPU-94-1 when inoculated with G+A+T and G+T (Table 2). Significant differences have been encountered between two varieties with regard to acidic and alkaline phosphatase activities. The highest value for acidic phosphatase activity was detected in UH-1 with treatment of G+A+T while alkaline phosphatase activity was found to be highest for treatment with G+T. Acidic and alkaline phosphatase activities were significantly lower in plants inoculated with *T. viride* as single bioinoculant.

Nitrogen and potassium content

The maximum nitrogen concentration has been found with G+A+T and G+T treatments in both varieties. In variety UH-1, treatment G+A+T gave highest root N concentration while G+T was best for shoot N content as well as root and shoot potassium content. In variety IPU 94-1, G+A+T increased shoot and root N concentrations to the maximum while, root and shoot potassium concentrations were found to be highest with G+A+T followed by the dual inoculation treatment of G+T (Table 3).

DISCUSSION

Symbiosis is a biological phenomenon which involves dynamic changes in the metabolism, genome, signaling network, as well as a multidirectional comprehension of these interactions is needed while studying symbiotic organisms (Kawaguchi and Minamisawa 2010). Our results revealed that inoculation of plants with AM fungi caused an improvement in growth of both the varieties compared to uninoculated plants which supported the findings of Lone et al. (2015). The variety UH -1 gave better growth response with different treatments as compared to variety IPU-94-1. This difference in the response of the two cultivars could be attributed to better root architecture and high AM spore number and percent root colonisation in variety UH-1 as compared to the variety IPU-94-1 (Sharma et al. 2016) which might have resulted in increased uptake of nutrient elements and thus yield. The mycorrhizal infection is well known to enhance plant growth by increasing nutrient uptake through increased surface area for absorption, mobilization of sparingly available nutrient sources or by excretion of chelating compounds or ecto-enzymes. A major contribution of mycorrhizal fungi to plant nutrition is increase in the uptake of phosphorous (Al Amri et al. 2013; Sheng et al. 2013). An increase in phosphorus content in AM treated plants in our experiment is in accordance with the findings of Yuan (2015). With the help of gel electrophoresis (Abdel Fattah 2001) and ultracytochemistry (Gianinazzi et al. 1979), alkaline phosphatase (ALP) has been found to be an active enzyme for mycorrhizal symbiosis. It is evident that alkaline phosphatase (ALP) has its role in acquisition of P by mycorrhizal plants as its presence in vacuoles of mature arbuscules is confirmed by ultracytochemical studies (Abdel Fattah 2001). Moreover, Mycorrhizal-Specific phosphatase (MSPase) of fungal origin has been found in mycorrhizal root extracts (Daei 2009). AMF colonisation has been known to enhance the activity of acid and alkaline phosphatase which catalyses the hydrolysis of complex forms of phosphate (Gianinazzi et al.1979). Our results confirmed the findings of Abdel Fattah et al. (2014), where increase in phosphatase activity and plant phosphorus has been observed in the plants inoculated with AM fungi.

The role of mycorrhizal symbiosis for improvement of plant nutrition as well as the molecular basis of nutrient relocation are at present well studied for phosphorus (Plassard and Dell 2010), nitrogen (Jin et al. 2012) and potassium (Garcia and Zimmermann 2014). As evident from the results, treatment of plants with bioinoculants increased nitrogen and potassium content besides phosphorus. In legumes, AMF have been reported to sustain nitrogen fixation by supplying the plant with P and other immobile nutrients like copper and zinc vital for N fixation (Mohammadi et al. 2011; Alizadeh 2012) even

though, specific symbiont combination is an important factor. Thus, in the present experiment, an improvement in plant growth and yield of mycorrhizal plants may be attributed to the increased activity of acid and alkaline phosphatase as well as higher nutrient content.

CONCLUSION

In both varieties of *P. mungo*, inoculation with *G. mosseae*, *A. laevis* and *T. viride* followed by dual inoculation of *G. mosseae* and *T. viride* stimulated growth parameters, phosphatase activity, nutrient uptake and yield. Although mycorrhizal treatment increased growth and yield of *P. mungo* as compared to *T. viride* and control the two cultivars showed variation in their response as the variety UH-1 was more responsive than IPU-94-1. *G. mosseae* alone and in different combinations proved to be a better bioinoculant to enhance yield as compared to *A. laevis*.

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