Technical Journal of Engineering and Applied Sciences
Available online at www.tjeas.com
©2011 TJEAS Journal-2011-1-1/20-25
ISSN 0000-0000 ©2011 TJEAS

Phosphorus acquisition by two wheat cultivars supplied with rock phosphate and inoculated with *Glomus intraradices* in an alkaline soil

Seyed MohammadReza Ehteshami

Assistant Professor, Agronomy and Plant Breeding Department, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Corresponding author email: Seyed Mohammad, Reza Ehteshami@yahoo.com

ABSTRACT: The effects of wheat inoculation with *Glomus intraradices* and fertilization with rock phosphate were studied in investigated pot soils in phytotron at University of Western Australia. The experimental treatments were arranged in four randomized complete blocks in a factorial experiment. Two wheat genotype consisting of Brookton and Krichauff; three different rock phosphates, i.e. MonoCalcium phosphate ($CaHPO_4$)(Control), Hydroxy apatite [$Ca_{10}(PO_4)_6(OH)_2$] and North Carolina rock phosphate; and two fungal treatments, i.e. inoculation and uninoculation with *G. intraradices* were studied in this experiment. Wheat fresh weight, dry weight, leaf area, stem height, P and Zn concentration in shoot and root, soil phosphorus content and root colonization percentage evaluated in physiological maturity stage. The greatest positive interaction effect on growth and phosphorus contents of wheat plants was obtained in those treatments which received MonoCalcium phosphate and were inoculated with *G. intraradices*. Plants grown with Hydroxy apatite had higher growth and P concentration than those grown with North Carolina rock phosphate. All of measured parameters, except leaf area, were higher in Krichauf. Inoculation treatments significantly increased all of measured characteristics in compared with uninoculation treatments. The present finding showed that mycorrhizal colonization increased tissue P concentrations in different experimental treatments.

***Keywords*:** Wheat, Phosphorus, Mycorrhiza, Rock Phosphate, Root Colonization Percentage

INTRODUCTION

The availability of phosphorus in the soluble state is of high agronomic value (Singal et al., 1994; Hameeda et al., 2006). Plant mineral nutrition depends mainly on the phosphorus content of soil, which can be assimilated only as soluble phosphate. Hence the use of rock phosphate (RP) as a fertilizer for P-deficient soils has received significant interest in recent years since they are natural, inexpensive and available fertilizers. However their solubilization rarely occurs in nonacidic soils (Caravaca et al., 2004, 2005a). Rock phosphate, although relatively insoluble, is abundantly found and easily mined. When indigenous rock phosphate is readily available, it is obviously very important to know under which conditions it may be profitable as an alternative source of phosphorus either to replace or complement these conventional sources. Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible (Cakmakci et al., 2006). Most of the studies on microbial dissolution of phosphate have been confined to the solubilization of TriCalcium phosphate or Hydroxy apatite, which are solubilized easily when compared with rock phosphate (Omar, 1995). It was reported that many soil fungi and bacteria can solubilize inorganic phosphate (Singal et al., 1994). These microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization or applying low-grade rock phosphate (Hameeda et al., 2006). Addition of an inoculum of phosphate-solubilizing-microorganisms to soil has also been found to improve the efficiency of rock phosphate as a phosphorus source (Omar, 1998). Although some studies have shown an increase in P uptake by plants in response to the addition of free-living fungi, there have been few attempts to quantify the source of the extra P absorbed. Among various soil microorganisms, vesicular arbuscular mycorrhiza (VAM), the most

widespread and agronomically important type of plant symbiont, has a great value in promoting phosphorus, nitrogen, minor elements and water uptake and also increase plant growth and yield of several crops (Allen, 1996; Ibijbijen et al., 1996; Auge, 2001). It was found that seed inoculation with VAM fungi increased P uptake by mycorrhizal compared with non-mycorrhizal controls (Ruiz-Lozano and Azcon 1993). Among microbial groups that could solubilize mineral phosphates and improve plant phosphorus nutrition are AM fungi. Arbuscular mycorrhizal fungal inoculation induced spectacular stimulations of the plant growth and P foliar content (Guissou et al., 2001; Caravaca et al., 2004, 2005a,b; Duponnois et al., 2005). The beneficial effect of phosphate-solubilizing fungi, alone, on the yield and P uptake of wheat was reported by Wahid and Mehana (2000) and Zaidi and Khan (2005). Many of these studies were done in either sterilized soil or in a small volume of non-sterile soil. Little is known also about the participation of rock-phosphate-solubilizing fungi and mycorrhizal inoculation on growth and P nutrition of crop plants in non-sterilized soil, especially under field conditions, where introduced fungi must compete with the indigenous fungal population (Omar, 1998). The fertilizer effectiveness of rock phosphate depends on factors relating to rock phosphate itself (minerology, chemical reactivity and rate of application), soil factors (pH, phosphate and calcium status) and the mycorrhizal status of plants. Plants inoculated with VAM utilize more soluble phosphorus from rock phosphate than non-VAM plants (Manjunath et al., 1989; Antunes and Cardoso 1991). The simplest explanation is that mycorrhiza develop an extrametrical mycelium which increases the root P-absorbing sites, as suggested by Bolan (1991).

The effect of rock phosphate application and VAM inoculation on many plant species has been the subject of numerous studies (Wang et al., 2006) in which rock phosphate was applied at one rate or more but to confirmed that root colonization by VAM was not inhibited even at high rates of rock phosphate application. These studies also showed that VAM was able to improve growth and facilitated plant recovery of P from applied rock phosphate. However, because of the lack of interval assessment during the time course of the experiments and in many cases the very high rates of rock phosphate application (Babana and Antoun, 2006) may have precluded a realistic assessment of the rock phosphate-VAM interactions.

The objective of our study was to evaluate growth responses of two wheat cultivars and mineral nutrient acquisition over time to rock phosphate application and inoculation with *Glomus intraradices* in a highly alkaline soil.

MATERIALS AND METHODS

This experiment was carried out in controlled environment (phytotron) at university of Western Australia. The soil had the following characteristics: Colwell P, 5 mg kg^{-1}; Nitrogen, 3 mg $kg^{-1;}$ Potassium, 14.5 mg kg^{-1}; Conductivity, 0.078 ds/m; pH: 8-9. The experimental treatments were arranged in four randomized complete blocks in a factorial experiment, having 12 treatments in a block. Two wheat genotype consisting of Brookton and Krichauff; three different rock phosphate, i.e. MonoCalcium phosphate ($CaHPO_4$)(Control), Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] and North Carolina; and two fungal treatments, i.e. inoculation and uninoculation with *G. intraradices* will apply in this experiment. The inoculum of *G. intraradices* was prepared by Dr. Mark Tibbett in Soil Biology Laboratory of the University of Western Australia. This inoculum is consisted of spores that was mixed with sand. Soil thoroughly was mixed and sterilized by steam (80-90 C) for two hours, twice. Then, sterilised soil was passed through a 2 mm sieve.

In inoculation treatments, the sterilised soil mixed with inoculum (9:1) and place in 1 Kg pots, while in non-inoculation treatments, the sterilised soil mixed with autoclaved inoculum (9:1). Nutrients were added to the soil/sand mixture as the following chemical compounds (in solution form) per soil kg: KH_2PO_4, 45.363g/l; K_2SO_4, 14.522g/l; $CaCl_2.2H_2O$, 49.005g/l; $MgSO_4.7H_2O$, 10.25g/l; $CuSO_4.5H_2O$, 0.42g/l; $MnSO_4.H_2O$, 3.0g/l; H_3BO_3, 0.133g/l; $Na,MoO_4.2H_2O$, 0.033g/l; $ZnSO_4.7H_2O$, 0.88g/l; NH_4NO_3, 18.6g/l.

Five uniform-size seeds of wheat (*Triticum aestivum* L.) would been surface-disinfected with 0.5% (v-v) sodium hypochlorite solution for 45 min and then washed in distilled water. Pre-germinated seeds sown in 5cm deep in pots. The pots kept in a phytotron set at 20/15 C and 65% relative humidity with ambient light intensity. Seedlings thined to three plants pot^{-1}, 15 days after germination. The plants are watered on a daily basis with distilled water to 60% field capacity.

The whole plants harvested before heading. The maximum of P uptake by plant, mainly occurs during this time of plant growth and mycorrhizal colonization approaches plateau value at this stage according to previous studies under the same conditions (Zhu and Smith, 2001). To estimate shoot dry weight, the shoots severed at the soil level and dry at 75° C until a constant weight obtained.

The plant tissue's dried samples digested in diacid mixture in sealed chamber method (Anderson and Henderson, 1986). The digested samples diluted to 50ml with distilled H_2O. The phosphorus content was estimated

using standard micro-kjeldahl and vanadomolybdo phosphoric acid yellow color methods, respectively (Subramanian and charest, 1997). Soil P extracted from 2g dried soil with 40 ml 0.5M $NaHCO_3$ at pH, 8.5 (Olsen et al., 1954). The sampled roots located in a solution of 50% ethanol and 50% distilled water. A portion of the root sample was thawed (Phillips and Hayman, 1970), and after clearing the roots with KOH and staining the roots in 0.05% trypan blue, percentage of root segments colonized by *G.intraradices* estimated by the line-intersect method (Kormanik and McGraw, 1982).

The statistical analysis of data carried out by conducting ANOVA. Means comparison was made using least significant difference (LSD). All the data presented as actual (non-transformed) means. Percentage values arcsin-transformed before statistical analysis.

RESULTS

Table 1 shows that fresh and dry weight of the wheat shoots differed significantly between inoculation and rock phosphate treatments ($p \leq 0.05$). Mycorrhizal colonisation had positive effect on biomass production of two cultivars, and the extent of the growth varied between rock phosphate treatments. Differences in shoot's fresh and dry weight weren't significant between cultivars. Plants produced significantly more dry matter, when grown in the presence of AM and MonoCalcium phosphate (Table 1). Rock phosphate fertilization caused a significant increase in shoot's dry weight of wheat plants, when arranged over all inoculation treatments and compared with uninoculation plants. When all treatments involving rock phosphate are arranged and compared with noninoculated controls, inoculation of sterilized pot soils with fungi increased fresh and dry weight of shoot, but this difference wasn't significant (except the plants treated with MonoCalcium phosphate).

The highest increase in stem height (38.925cm) was recorded by Krichauff+MonoCalcium phosphate+inoculation treatment. However, Hydroxy apatite performed better than North Carolina, but was less than MonoCalcium phosphate in stimulating stem height. Stem height decreased in the treatment receiving Hydroxy apatite and North Carolina in compared with MonoCalcium phosphate treatment (Table 1). The total leaf area of two cultivars was significantly increased by inoculation with AM together with MonoCalcium phosphate, compared to the uninoculated treatment (Table 1). Total leaf area in plants under different treatments ranged from 18.646 (Krichauff + North Carolina + uninoculation) to 88.427 (Brookton + MonoCalcium phosphate + inoculation). The lowest leaf area was recorded in plants grown in uninoculated and North Carolina treatments. Conversely, inoculation with AM enhanced the wheat leaf area regardless of other treatments, but this response was more pronounced in MonoCalcium phosphate treatment.

The highest soil available P was associated with plants grown under AM+MonoCalcium phosphate treatment. This value was significantly ($P \leq 0.05$) higher than that of plants grown under all other treatments in two cultivars. Our study showed that inoculated wheat plants by AM were much more efficient in taking up soil P than non-inoculated plants. All inoculation treatments improved available P content of soil in all of rock phosphate treatments. However, this difference wasn't significant (except the plants treated with MonoCalcium phosphate) (table 2).

Table 1.Means of fresh weight, dry weight, stem height, leaf area and soil P of wheat in different treatments cultivarrock phosphate*inoculation

	Parameters				
Treatment	Fresh Weight (g per plant)	Stem Height (cm)	Dry Weight (g per plant)	Leaf Area (cm^2)	Soil P (mg/kg soil)
K + M + In	6.37 a	38.925 a	1.476 a	78.384 b	14.75 a
B + M + In	6.37 a	35.041 b	1.31 b	88.427 a	14.75 a
K + M + Un	5.045 b	34.4 b	1.102 c	57.154 c	12.25 b
B + M + Un	5.184 b	30.383 c	1.135 c	58.647 c	12 b
K + H + In	1.331 c	20.783 de	0.283 d	22.62 d	8.75 c
B + H + In	1.286 c	19.108 defg	0.248 d	25.679 d	8.25 cd
K + H + Un	1.078 cd	20.166 de	0.237 d	18.521 d	8 cde
B + H + Un	1.046 cd	17.25 fg	0.203 d	22.348 d	7.5 cdef
K + N + In	1.242 cd	21.258 d	0.271 d	22.7 d	6.25 ef
B + N + In	1.056 cd	18.258 efg	0.205 d	21.051 d	6.5 def
K + N + Un	1.02 cd	19.617 def	0.222 d	18.646 d	5.75 f
B + N + Un	0.949 d	16.491 g	0.19 d	19.59 d	6.25 ef

K Krichauff B Brookton; M: MonoCalcium phosphate; H: Hydroxy apatite; N: North Carolina; In: Inoculation; Un Uninoculation
Means with different superscript letters in a column are significantly different at $P \leq 0.05$ according to Duncan test

The percentage root length colonised by arbuscular mycorrhizal fungi was significantly different between different rock phosphates, varying from 17% for Krichauff and North Carolina to 37.225% for Krichauff and

MonoCalcium phosphate ($P \leq 0.05$, Table 2). The percentage of root colonization was significantly higher in the treatments containing MonoCalcium phosphate and AM than the treatments containing Hydroxy apatite and North Carolina. The highest increase in the percentage of root colonization in different rock phosphates was recorded in Krichauff in compared with Brookton (except for North Carolina).

Mycorrhizal colonization significantly increased P concentration and Zn content in wheat roots and shoots ($P \leq 0.05$). There were considerable variations in P accumulation and Zn content in shoots, both with and without mycorrhizal colonization (Table 2). Our results shows that shoot P concentration and Zn content differed considerably between cultivars, and it appeared that Brookton is generally more effective in P adsorption than other cultivar. Table 2 reveals that mycorrhizal colonisation increased shoot P and Zn uptake in two cultivars, and there were also considerable variations between rockphosphates. The differences in root P and Zn uptake between mycorrhizal and non-mycorrhizal wasn't significant.

Table2. Means of shoot's P and Zn, root's P and Zn, root colonization percentage of wheat in different treatments cultivarrock phosphateinoculation

	Parameters				
Treatment	Shoot P (%)	Shoot Zn (mg/kg)	Root P (%)	Root Zn (mg/kg)	Root Colonization Percentage (%)
K + M + In	0.151 a	135.55 a	0.117 a	161.13 a	37.225 a
B + M + In	0.149 a	133.675 a	0.104 ab	160.03 ab	34.7 a
K + M + Un	0.14 b	133.45 a	0.101 abc	149.6 abc	0 d
B + M + Un	0.131 c	131.15 a	0.099 abc	148.73 abc	0 d
K + H + In	0.109 ef	109.95 b	0.095 bc	129.6 bc	29.575 ab
B + H + In	0.115 de	110.225 b	0.097 bc	130.55 abc	23 bc
K + H + Un	0.098 gh	39.7 c	0.084 c	34.68 d	0 d
B + H + Un	0.103 fg	44.225 c	0.091 bc	39.35 d	0 d
K + N + In	0.107 f	104.05 b	0.095 bc	118.58 c	17 c
B + N + In	0.119 d	115.075 b	0.097 bc	135.75 abc	19.575 c
K + N + Un	0.093 h	38.725 c	0.082 c	34.03 d	0 d
B + N + Un	0.099 gh	41.85 c	0.088 bc	37.45 d	0 d

KKrichauff; B: Brookton; M: MonoCalcium phosphate; H: Hydroxy apatite; N: North Carolina; In: Inoculation; Un Uninoculation
Means with different superscript letters in a column are significantly different at $P \leq 0.05$ according to Duncan test

As our results indicated, the inoculation of plants with *G.intraradices* considerably increased shoot P and Zn uptake and enhanced plant growth in all of rock phosphate treatments. The effects of experimental treatments on P and Zn contents of wheat plants grown in pots were more pronounced in compared with noninoculated pots. The addition of MonoCalcium phosphate to inoculated had the highest of P and Zn content in compared with other treatments. Our results indicated that mycorrhizal colonization increased tissue P concentrations in two cultivars (Table 2). As observed in this study, increase in tissue P concentrations can be considered as an improvement in plant P nutrition.

DISCUSSION

Experiment results revealed that root colonization was the greatest in plots inoculated with *G.intraradices* and fertilized with MonoCalcium phosphate. Conversely, the maximum of plant dry mass, fresh weight, leaf area, soil P, stem height and P contents were observed in soil inoculated with a mixed inoculum and fertilized with MonoCalcium phosphate, too. These results indicated that the relation existed between root colonization percentage and other parameters. These results may be explained with the respect to the metabolic activity of hyphae and their levels of colonization (Beever and Burns, 1981). These results agree with those of Islam *et al*. (1980). They reported a higher percentage of root infection in field plots than in pot trials, but inoculation with mycorrhiza had no effect on growth and P uptake of cowpea plant in field plots. Powell (1982) studied the effect of some VAM fungi that were active in stimulating growth of clover in sterilized pot soils on growth of plants under nonsterilized pots and field conditions. He found that plant growth was checked in field soil and nonsterile pot soil compared with sterilized pot soil and explained these results on the basis of the inability of fungi to persist in field soils owing to competition from the indigenous mycorrhiza and probably other soil micro-organisms. Accordingly, mycorrhizal fungus used in the present study is good competitor.

From the results obtained from this study, it appears that inoculation of the plants with VAM fungi caused infection of roots rapidly, but Hydroxy apatite and North Carolina rock phosphates application cause a comparatively poor response in all of measured parameters of plant growth. Plant growth and utilization of these rock phosphates were increased by inoculation with VAM fungi. As, our data agree with the findings of , Islam *et al*.

(1980) who reported that inoculation of the plants with AM fungi caused very rapid infection of roots, but rock phosphate application reduced the degree of infection without affecting plant growth. Plant growth and utilization of rock phosphate were increased by inoculation with VAM fungi. In autoclaved pot soil experiments, soil inoculation with *P. bilaii* increased plant dry matter yield by 16% and total plant P uptake by 14%, but addition of rock phosphate did not affect wheat dry mass production nor plant P uptake (Asea et al., 1988). They also found that AM inoculation alone did not have any effect on the plant P specific activity. Double inoculation with VAM and *P. bilaii* was not more effective than addition of *P.bilaii* alone, but was superior to the addition of VAM alone.

In accordance with results of the present study, Asea *et al*. (1988) found that the highest total P uptake was achieved with inoculation treatments. Growth of clover was stimulated by some VA-mycorrhizal species, when grown in sterilized pot soil (Powell, 1982). The promotive effect of rock phosphate solubilizing fungi and mycorrhiza on wheat growth in pots was demonstrated by Kucey (1987), and then Talukdar and Germida (1994). Omar (1995) reported that sterilized pot soil inoculation with AM fungi dramatically increased growth and the percentage of root infection of maize plants, but not P content. These results could be attributed to the ability of these microorganisms to solubilize organic and inorganic phosphorus already present in soil. In agreement with these findings, Barea et al. (1975) observed that in low phosphate soils, plant growth and phosphate uptake were greater after inoculation with both Endogone and phosphate dissolving bacteria than after inoculation with either organism separately.

On the other hand, we suggest that pot size (i.e. soil volume) used in experiments may also partly affect the expression of mycorrhizal responsiveness, particularly for plants with large root systems such as spring wheat. Mycorrhizal responsiveness is likely to be greater, when root length density is lower (i.e. in larger pots), because in this situation the exploration of the soil volume by mycorrhizal hyphae would become more critical for plant acquisition of P and other immobile nutrients. Pot size used in previous researches varied from just over 0.5 kg (Hetrick et al., 1993) to around 6 kg (Khalil et al., 1994), and the large variations in pot size will certainly reduce the comparability of different data sets.

CONCLUSION

In summary, this study revealed that rock phosphate can be used as a crude phosphatic fertilizer by direct application to soil. Double application of rock phosphate and rock-phosphate-solubilizing fungi improved the P contents and growth of plants. Mixed inoculation of soil with mycorrhizal fungi and rock phosphate enhanced growth of wheat plants and plants took more P from the soil. The combined effect was explained as the net result of P solubilization and transfer from soil. These results have an agronomic importance for wheat cultivation and crop yield in Iran. This is because wheat requires a greater amount of phosphorus fertilizers, especially when growth in alkaline soil, because a proportion of these fertilizers become unavailable for plants. This may assist in solving problems encountered with food shortage and crop production economy.

REFERENCES

Allen MF. 1996. The ecology of arbuscular mycorrhizas; A look back into the 20th century and a peek into the 21st. Mycorrhizal Res 100: 769-782.

Anderson DL, Henderson LJ. 1986. Sealed chamber digestion for nutrient analyses. Agron J 78: 937-939.

Antunes V, Cardoso E. 1991. Growth and nutrient status of citrus plants as influenced by mycorrhiza and phosphorus application. Plant and Soil 131: 11-19.

Asea PEA, Kucey RMN, Stewart JWB. 1988. Inorganic phosphate solubilization by two Penicillium species in solution culture and in soil. Soil Biol and Biochem 20: 459-464.

Auge RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

Babana AH, Antoun H. 2006. Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali. Plant and Soil 287: 51–58.

Barea JM, Azcon R, Hayman DS. 1975. Possible synergistic interactions between Endogone and phosphate-solubilizing bacteria in low-phosphate soil. In Endomycorrhiza, (eds) Mosse B, Tinker PT. London, Academic Press.

Beever RE, Burns DJW. 1981. Phosphate uptake, storage and utilization by fungi. Adv Bot Res 8: 127-219.

Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134: 189-207.

Cakmakci R, Donme F, Aydin A, Sahin F. 2006. Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38: 1482-1487.

Caravaca F, Alguacil MM, Azcon R, Parlade J, Torres P, Roldan A. 2005a. Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate and *Aspergillus niger*. Microb Ecol 49: 73–82.

Caravaca F, Alguacil MM, Torres P, Roldan A. 2005b. Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. Soil Biol Biochem 37: 227–233.

Duponnois R, Colombet A, Hien V, Thioulouse J. 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. Soil Biol Biochem 37: 1460–1468.

Guissou T, Ba AM, Guinko S, Plenchette C, Duponnois R. 2001. Mobilisation des phosphates naturels de Kodjari par des jujubiers (*Ziziphus mauritiana* Lam.) mycorhize´s dans un sol acidifie´ avec de la tourbe. Fruits 56: 261–269.

Hameeda B, Harini G, Rupela OP, Wani SP, reddyn G. 2006. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Research, In Press.
Hetrick BAD, Wilson GWT, Cox TS. 1993. Mycorrhizal dependence of modern wheat cultivars and ancestors: A synthesis. Can J Bot 71: 512–518.
Ibijbijen J, Urquiaga S, Ismaili M, Alves BJR, Boodey RM. 1996. Effect of arbuscular mycorrhizas on uptake of nitrogen by *Brachiaria arrecta* and *Sorghum vulgare* from soils labelled for several years with 15^N. New Phytol 183: 487-494.
Islam R, Ayanaba A, Sanders FE. 1980. Response of cowpea (*Vigna unguiculata*) to inoculation with VA-mycorrhizal fungi and to rock phosphate fertilization in some unsterilized Nigerian soils. Plant and Soil 54: 107-117.
Khalil S, Loynachan TE, Tabtabai MA. 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. Agron J 86: 949–958.
Kormanik PP, McGraw AC. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In Methods and Principles of Mycorrhizal Research (N C Schenck, Ed.). pp. 37-45. American Phytopathological Society, st Paul.
Kucey RMN. 1987. Increased phosphorus uptake by wheat and field beans inoculated with a phosphorus-solubilizing *Penicillium bilaii* strain and vesicular arbuscular mycorrhizal fungi. Appl Environ Microbiol 53: 2699-2703.
Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Circular 939: 1-19.
Omar SA. 1995. Growth effects of the vesicular-arbuscular mycorrhizal fungus *Glomus constrictum* on maize plants in pot trials. Folia Microbiol 40: 503-507.
Omar SA. 1998. The role of rock-phosphate-solubilizing fungi and vesicular-arbuscular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J Microbiol Biotech 14: 211-218.
Phillips JM, Hayman DS. 1970. Improved procedure for clearing roots, and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans British Mycol Soc 55: 158-161.
Powell CL. 1982. Selection of efficient VA mycorrhizal fungi. Plant and Soil 68: 3-9.
Ruiz-Lozano JM, Azcon R. 1993. Specificity and functional compatibility of VA mycorrhizal endophytes in association with Bradyrhizobium strains in *Cicer arietinum*. Symbiosis 15: 217-226.
Singal R, Gupta R, Saxena RK. 1994. Rock phosphate solubilization under alkaline condition by *Aspergollus japonicus* and *A. foetidus*. Folia Microbiol 39: 33-36.
Subramanian KC, Charest C. 1997. Nutritional, growth and reproductive responses of maize (*Zea mays* L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling. Mycorrhiza 7: 25-32.
Talukdar NC, Germida JJ. 1994. Growth and yield of lentil and wheat inoculated with three *Glomus* isolates from Saskatchewan soils. Mycorrhiza 5: 145-152.
Wahid OA, Mehana TA. 2000. Impact of phosphate solubilizing fungi on the yield and phosphorus uptake by wheat and faba bean plants. Microbiol Res 155: 221-227.
Wang F, Lin X, Yin R, Wu L. 2006. Effects of arbuscular mycorrhizal inoculation on the growth of *Elsholtzia splendens* and *Zea mays* L. and the activities of phosphatase and urease in a multi-metal-contaminated soil under unsterilized conditions. Appl Soil Ecology 31: 110-119.
Zaidi A, Khan MS. 2005. Interactive effect of rhizotrophic microorganisms on growth, yield and phosphorus uptake of wheat. J Plant Nut 28: 2079-2092.
Zhu YG, Smith SE. 2001. seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi. Plant and Soil 231: 105-112.