INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 1, p. 1-8, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

The symbiotic response of three millet varieties to Arbuscular mycorrhizal fungal (*Glomus* spp.) inoculation in marginal soil: implication in bio-fertilizer

Tanvir Burni*[1], Sayeda Sarah[1], Ikramullah Khan[2], Ghulam Nabi[3], Subhan Ullah[1]

[1]*University of Peshawar, Department of Botany, Peshawar, Pakistan*

[2]*Department of Weed Science, The University of Agriculture, Peshawar, Pakistan*

[3]*Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei, China*

Article published on July 21, 2017

Key words: AM, Millet varieties, Host growth responses, Mycorrhizal dependency, Proximate analysis

Abstract

The effect of three *Glomus* species on growth parameters in terms of shoot length, root length, dry weight proximate composition and quantitative evaluation of mycorrhizal dependency of three millet (*Pennisetum glaucum* L.) varieties i.e. Bajra super 1, PARC-MS 2, PARC-MS 3 were evaluated. Thirty earthen pots were filled with 8kg of soil. Each millet variety was represented by ten replicates i.e. five control and five test pots. The test pots were inoculated with soil containing spores of *Glomus* spp. The *Arbuscular mycorrhizal* (AM) inoculated plants showed significantly better performance than the non-inoculated plants in terms of plant height, number and length of leaves, root length, number of seminal roots and dry weight. Proximate analysis showed enhancement in crude protein, fat, moisture and ash content in mycorrhizal plants, except carbohydrate and crude fibers. Regarding mycorrhizal dependency (M.D), maximum value was noted in PV2 (71.67%) variety while least was observed in PV1 (55.91%). This study clearly indicates the potential of using indigenous AM fungi (*Glomus* species) as bio fertilizer in millet crop in low fertility soils. The use of AM fungi as bio fertilizer will not only reduce demand for chemical fertilizer, but will diminish chemical pollution.

***Corresponding Author:** Tanvir Burni ✉ tanvirburni@yahoo.com

Introduction

Mycorrhizas are universal mutualistic associations between soil fungi and vascular plants and are essential in improving plant fitness and soil quality. It improves the resilience of plant communities against environmental, nutritional and drought stresses (Barea *et al*., 2011).

Mycorrhizae mean fungus roots. In this mutually beneficial partnerships, root of the host plant provide a convenient substrate for the fungus and also supply food in the form of simple carbohydrates. In exchange for this free room and board, the mycorrhizal fungus provides several benefits to the host plant (Wilkinson, 2008). Arbuscular mycorrhiza (AM) is a root endosymbiosis between plants and glomeromycete fungi. It is the most ubiquitous terrestrial plant symbiosis, improving plant growth, plant protection, soil quality, plant uptake of water and mineral nutrients (Mahmood and Rizvi, 2010). AM fungi are known to be of great importance due to their great capability to increase growth, yield and crop quality through efficient nutrient acquisition in infertile soils & therefore lessen the prerequisite for phosphate-based fertilizers (Sawers *et al*., 2008; Roy-Bolduc & Hijri, 2010). In turn, the fungi get carbon from the host plant. AM fungi are able to absorb and transfer all of the 15 major, macro and micro nutrients essential for plant growth (Lester, 2009).

Millet (*Pennisetum glaucum* L.) locally known as "Bajra" is also a coarse grain cereal crop which ranks fifth in Pakistan. It is grown on 34.6 million hector area in the world with the production of 28.8 million tons per annum. In Pakistan, it is grown on 0.3 million hectare area with total production of 0.20 million tons and per unit yield of 563 kg ha^{-1} which is very low than the world average (831 kg ha-1) (FAO, 2005).

Soils of Pakistan like most of the arid and semiarid soils of world are mostly Phosphorus (P) deficient due to their alkaline and calcareous nature, affecting the maize crop adversely (Memon *et al*., 1992; NFDC, 2001).

Throughout the world, scientists are now focused on developing alternative technology to minimize the dependence on chemical fertilizers. Although remarkable research work has been done on various aspects of AM, but the issues of Asian countries including Pakistan such as nutrient deficiency and host growth responses of various crops are least addressed.

Keeping the importance of AM fungi as bio-fertilizers present investigations was carried to find out the feasibility of inoculation of some high yielding tropical millet varieties with indigenous tropical AM fungi.

Material and methods

The study was conducted at the Department of Botany, University of Peshawar. Seeds of the three millet varieties i.e. Bajra super 1 (PV1), PARC-MS 2 (PV2), PARC-MS 3 (PV3) were obtained from National Agricultural Research Centre NARC, Islamabad, Pakistan. The soil used was sandy loam with pH 7.8, electric conductivity 0.675 ds/m^2, Nitrogen 0.032% and Phosphorus 0.8mg/kg with low organic matter 0.6%.

Thirty earthen pots were filled with 8kg of soil. Each millet variety was represented by ten replicates i.e. five control pots and five test pots arranged in RCBD having factorial arrangement with two treatments (controlled and uncontrolled). The test pots were inoculated with soil containing spores of *Glomus* spp. Harvesting of millet plants was done after 80 days. After harvesting different growth parameters were taken including plant height, number & length of leaves, root length and number of seminal roots. In the laboratory dry weight of plants was taken by drying the plants in the oven at $65C^o$ for 72 hours. Mycorrhizal inoculum preparation, placement and application were done by the method given by Gaur and Adholeya (2002). Mycorrhizal dependency was also calculated by the following formula.

Experimental data was statistically analyzed by applying ANOVA test; the means were subjected to LSD test.

While, proximate analysis (moisture content, ash, protein, fats and crude fibers) were determined by standard methods of AOAC (2006).

Result and discussion

Growth Parameters and Mycorrhizal dependency

In the present study, all measured parameters of mycorrhizal plants showed significant differences ($P < 0.05$) as compared to control. It is evident from mean data (Table 1, Fig. 1) that MPV2 responded better followed by MPV1 and then MPV3 in terms of plant height in mycorrhizal plants as compared to control. Our results are in opposite to the work of (Shrestha *et al.*, 2009).

Table 1. Plant height, number & length of leaves, root length, number of seminal roots and dry weight (g.) of mycorrhizal (M) and non mycorrhizal (NM) millet varieties. Each value is a mean of five replicates. Values followed by different letters are significantly different ($p < 0.05$).

Varieties	Treatments	Plant height (cm)	No. of leaves	Leaves length (cm)	Root length (mm)	No. of seminal roots	Dry weight (gm.)
PV1	M	112.8^{a}	11.00^{b}	64.82^{ab}	113.8^{ab}	19.00^{b}	9.394^{a}
	NM	66.10^{b}	10.00^{c}	48.96^{b}	84.28^{b}	11.00^{c}	4.140^{bcd}
PV2	M	120.5^{a}	13.00^{a}	78.50^{a}	143.0^{a}	24.00^{a}	6.850^{ab}
	NM	87.02^{ab}	10.00^{c}	62.72^{ab}	104.0^{ab}	14.00^{c}	1.940^{cd}
PV3	M	77.64 ^{b}	13.00^{a}	57.16^{ab}	140.4^{a}	21.00^{ab}	5.260^{bc}
	NM	54.22^{b}	8.000^{d}	45.32^{b}	69.20^{b}	12.00c	1.540^{d}
LSD at 5%		34.48	0.3801	22.84	55.55	4.723	3.410

Table 2. Effect of AM on proximate analysis of millet varieties.

Treatments	Percent on dry matter basis					
	Moisture	Ash	Crude fiber	Crude Fat	Crude protein	Carbohydrate
MPV_1	9.28	14.51	27.62	2.69	9.01	36.11
$NMPV_1$	9.22	10.66	28.96	2.61	8.95	39.6
MPV_2	10.11	13.80	31.51	3.72	10.11	30.75
$NMPV_2$	7.51	12.57	32.72	3.00	8.10	36.1
MPV_3	12.46	12.79	28.08	2.77	9.03	34.87
$NMPV_3$	9.77	12.51	30.36	2.69	8.62	36.05

It has been suggested that in VAM inoculated plants the mitotic activity of stem cells may enhance, resulting in taller plants (Tarafdar & Marschner, 1995). This may be because of extra-radical mycelium (being smaller in diameter than roots) better penetrates beyond the depletion zone for better acquisition of nutrients (Sylvia *et al.*, 1993; Mengel and Kirkby, 2001).

The result regarding length and number of leaves of millet (Table 1, Fig. 2, 3) showed that mycorrhizal plants shows better performance than non-mycorrhizal plants. The data shows that increase in number of leaves in mycorrhizal plants range from 11.11 - 62.5% in millet varieties.

These results are also in agreement with the findings of Wu & Xia, (2006) in *Citrus tangerine* and Wu *et al.* (2008) in *Poncirus trifoliate* who reported that VAM inoculation resulted in greater number of leaves per plant as compared to the control plants.

The positive effect may be attributed to the enhancement of P nutrition (Henrike *et al.*, 2007) and water uptake by hyphae (Faber *et al.*, 1991).

Ghazi & Zak, (2003) investigated that improved plant growth in terms of leaf length, leaf water turgidity and stomatal activities might be due to enhanced uptake of water and nutrients like Zinc (Zn) and Copper (Cu).

Our present results (Table 1, Fig. 4) evidently showed that in millet varieties, the maximum root length (143.0mm) was recorded in MPV2, while least (113.8mm) was observed in MPV1. Our findings are in agreement with the work of Nzanza *et al.* (2011) and Ayoob *et al.* (2011).

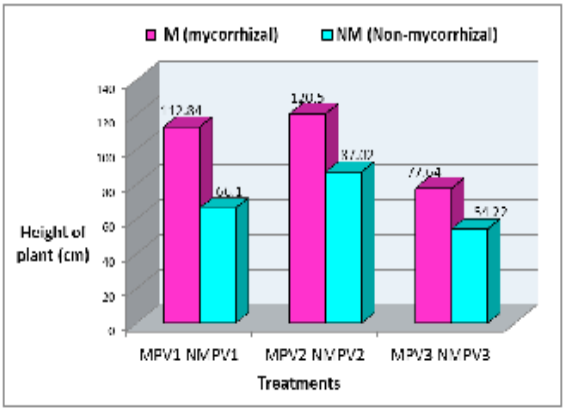

Fig. 1. Effect of mycorrhiza on plant height (cm) of *Pennisetum glaucum* L.

The possible explanation may be that mycorrhizal inoculation stimulates rooting and growth (Kumar *et al.*, 2007) and also reduces soil compaction which results in root development (Miransari, 2007).

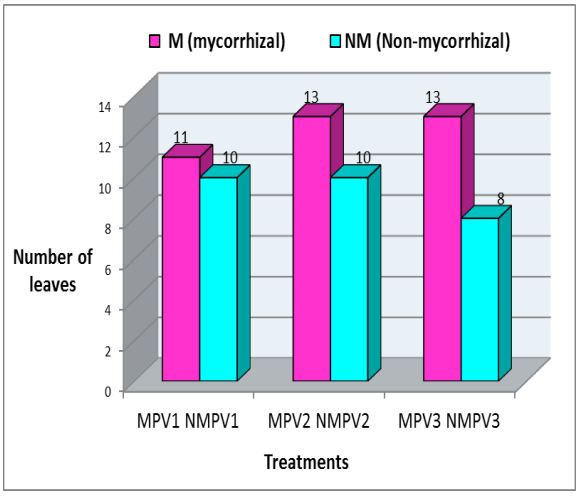

Fig. 2. Effect of mycorrhiza on number of leaves of *Pennisetum glaucum* L.

Regarding number of seminal roots in, millet varieties mycorrhizal plants showed greater number than control plants. The number of seminal roots in millet varieties was increased by 75%, 72% and 71.42% in MPV3, MPV1 and MPV2 respectively.

The results (Table 1, Fig. 5)are in consistent with the fact that mycorrhizal inoculation changed the root morphology (Subramanian *et al.*, 2008) and bring about stimulation for development of the root system, generally by increasing the formation of lateral roots (Berta *et al.*, 2002).

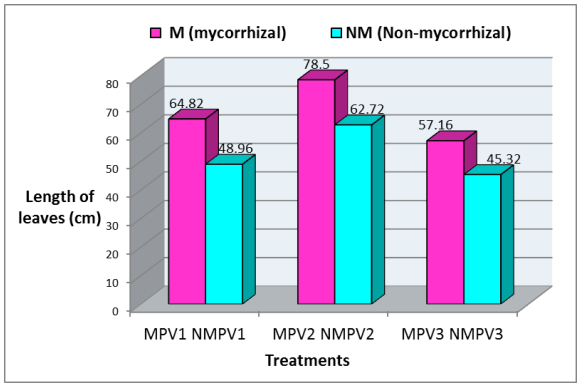

Fig. 3. Effect of mycorrhiza on length of leaves (cm) of *Pennisetum glaucum* L.

The present results (Table 1, Fig. 6) clearly showed that AMF increased the dry weight of mycorrhizal plants than non-mycorrhizal plants. Our results are supported by the work of Sharif *et al.*, (2011) who reported that millet crop inoculated with AMF enhanced root and shoot dry matter by 21% and 20% respectively.

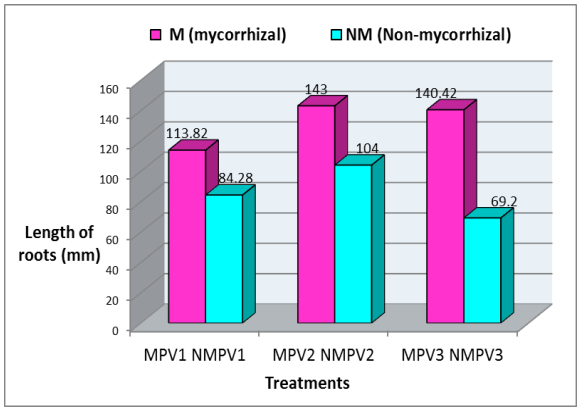

Fig. 4. Effect of mycorrhiza on length of roots (mm) of *Pennisetum glaucum* L.

Mycorrhizal dependency

In present research work three varieties of millet were investigated for mycorrhizal dependency (MD) (Fig. 7). The degree of mycorrhizal dependency was found a maximum in PV2 (71.67%) and least in PV1 (55.91%).

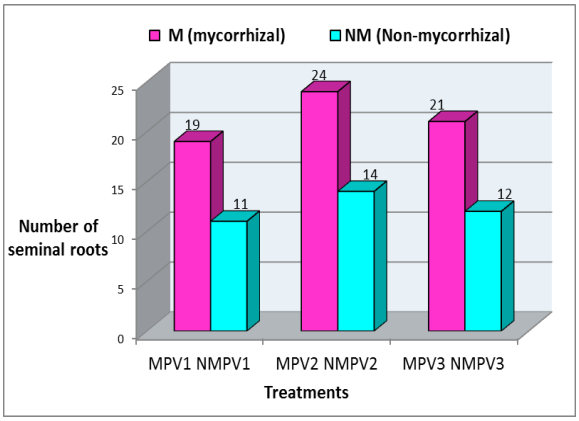

Fig. 5. Effect of mycorrhiza on number of seminal roots of *Pennisetum glaucum* L.

The results of our investigation have showed that cereals were more dependent on mycorrhiza association for better growth in nutrient deficient soil. Root branching determines plant dependence on symbiosis (Smith & Read, 1997; Barakh and Heggo, 1998). The results showed that all varieties were found to be differs in their mycorrhizal dependency as also shown by Xavier & Germida, (1998) and Sawers *et al.*, (2008). They found that mycorrhizal dependency is not the same in plant species and even in their cultivars also. This difference in general is related to root geometry, soil type, soil phosphorus, plant growth rates and mycorrhizal species (Plenchette *et al.*, 1983; Hatrick *et al.*, 1993).

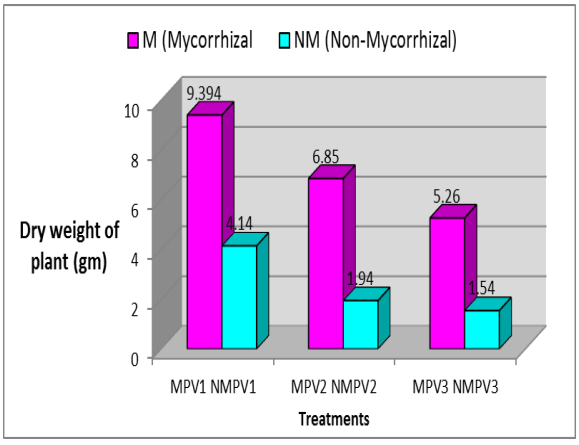

Fig. 6. Effect of mycorrhiza on dry weight of plant (gm) of *Pennisetum glaucum* L.

Proximate analysis

Dried powders of the plants were analyzed for moisture, ash contents, crude protein, crude fiber, fats, and carbohydrate on dry matter basis and the results are given in (Table 2). The present results evidently showed that mycorrhizal plants enhanced the amount of crude protein, crude fat, moistures and ash contents as compared to non-mycorrhizal plants (Fig. 8).

As evident from the result that amount of crude protein enhanced in mycorrhizal plants as compared to non-mycorrhizal plants.

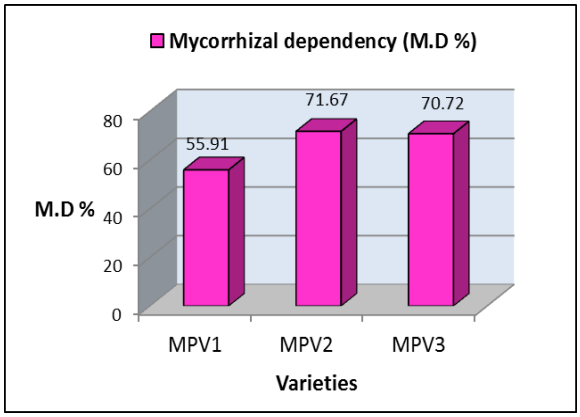

Fig. 7. Mycorrhizal dependency index (MD) of millet varieties.

The data revealed that increase of crude protein was 24.81% in MPV2, as compared to control. Our results are supported with the findings of Khalafallah & Abo-Ghalia, (2008) who reported that AM inoculation increased crude proteins contents of wheat plant by 6% than non-mycorrhizal. Our results are also in agreement with the finding of other workers (Wu *et al.*, 2006b; Manoharan *et al.*, 2008) who observed that the crude protein content is higher in AM than non-AM plants. Similarly, highest rate of increase of crude fat was 24% in MPV2. Our results agreed with the work of Cooper & Losel, (1978), according to them infected roots contained more total lipid than uninfected roots. Moreover, Omomowo *et al.* (2009) found that inoculation with *Glomus mosseae* has higher fat content of cowpea than un-inoculated control. An increase of 36.11% ash content was recorded in MPV1 of inoculated maize variety. According to Mehrvarz & Chaichi, (2008) inoculated plants of Barely (*Hordeum vulgare* L.) exhibited higher level of total ash (8.05%) than non-mycorrhizal (7.84%).

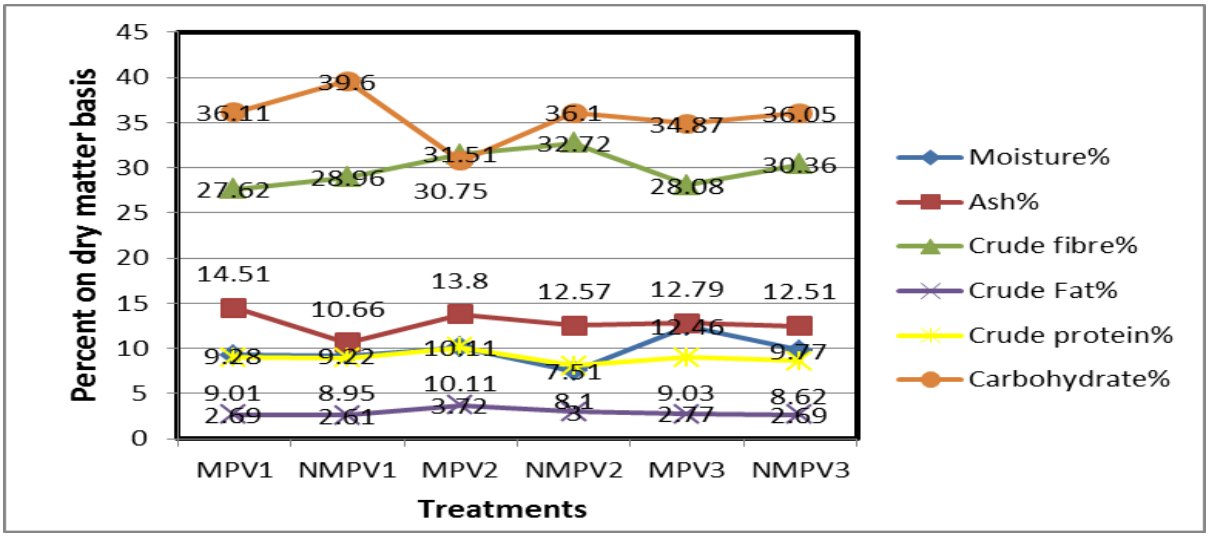

Fig. 8. Proximate analysis of Millet varieties.

While the crude fiber and carbohydrate content showed a relative decrease of -7.50% (MPV3) and -14.81% (MPV2), respectively in mycorrhizal plants. Similar, decrease in crude fiber contents were also reported by Adewole & Ilesanmi, (2011), while Manoharan *et al.* (2008) reported contradictory results to our findings.

The reason behind decrease in carbohydrate content is believed that carbohydrates are transferred from host to the fungal partner (Johnson *et al.*, 1997). However, our results are contradictory to Khalafallah and Abo-Ghalia, (2008) who reported that mycorrrhizal plants shows maximum amount of carbohydrates content of wheat plant than non-mycorrhizal under well watered conditions.

References

Adewole MB, Ilesanmi AO. 2011. Effects of soil amendments on the nutritional quality of okra (*Abelmoschus esculentus* L.). Journal of Plant Nutrition and Soil Science **11,** 45-55.

AOAC. 2006. Official Method of Analysis. Association of Analytical Chemistry, Washington DC.

Ayoob M, Aziz I, Jite PK. 2011. Interaction effects of arbuscular mycorrhizal fungi and different phosphate levels on growth performance of *Catharanthus roseus* Linn. Notulae Scientia Biologicae **3,** 75-79.

Barakh FN, Heggo AM. 1998. Moisture Stress. *Bradyrhizobia*, vesicular arbuscular mycorrhiza and P-Fertilizers effect of soyabean growth, nutrient content and phosphate activity under calcareous soil. Annals of Agriculture Sciences. Cairo **43,** 261-475.

Barea JM, Palenzuela J, Cornejo P, Sanchez-Castro I, Navarro-Fernandez C, Lopez-García A, Estrada B, Azcon R, Ferrol N, Azcon-Aguilar C. 2011. Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain, Journal of Arid Environment **75,** 1292-1301.

Berta G, Fusconi A, Hooker HA. 2002. Arbuscular mycorrhizal modifications to plant root systems: scale, mechanisms and consequences. In Mycorrhizal Technology in Agriculture: from Genes to Bioproducts (Gianinazzi, S., Schuepp, H., Barea, J.M. and Haselwandter, K., Eds.). Basel-Boston-Berlin: Birkhäuser Verlag, 71–85.

Cooper KM, Losel DM. 1978. Lipid physiology of vesicular arbuscular mycorrhiza, I. compostion of lipids in roots of onion, clover and ryegrass infected with *Glomus mosseae*. New Phytologist **80,** 143.

Faber BA, Zasoske RJ, Munns DN, Shaokel K. 1991. A method for measuring hyphal nutrition and water uptake in mycorrhizal plants. Canadian Journal of Botany **69,** 87-94.

FAO. 2005. The analysis of adaptation in a plant breeding programme. J. Agric. Research **14,** 742- 754.

Gaur A, Adholeya A. 2002. Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. Biology and Fertility of Soils **35,** 214-218.

Ghazi AK, Zak BM. 2003. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza **14,** 263-269.

Hatrick BG, Wilson WT, Cox TS. 1993. Mycorrhizal dependency of modern wheat cultivars and ancestors: A synthesis. Canadian Journal of Botany **71,** 512-518.

Henrike P, Dietmar S, Christian B, Paul M, Eckhard G. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of *Pelargonium* plants. Mycorrhiza **17,** 469-474.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism, parasitism and continuum. New Phytologist **135,** 575-586.

Khalafallah AA, Abo-Ghalia HH. 2008. Effect of arbuscular mycorrhizal fungi on the metabolic products and activity of antioxidant system in wheat plants to short term water stress, followed by recovery at different growth stages. Journal of Applied Science Research **4,** 559-569.

Lester D. 2009. Buying and applying mycorrhizal fungi. Max. Yield. USA., 126-131 p. http://www.maximumyield.com/article_shdb.php?articleID=483).

Mahmood I, Rizvi I. 2010. Mycorrhiza and Organic Farming. Asian Journal of Plant Sciences **9,** 241-248.

Manoharan PT, Pandi M, Shanmugaiah V, Gomathinayagam S, Balasubramanian N. 2008. Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. African Journal of Biotechnology **7,** 3431-3436.

Mehrvarz S, Chaichi MR. 2008. Effect of Phosphate Solubilizing Microorganisms and Phosphorus Chemical Fertilizer on Forage and Grain Quality of Barely (*Hordeum vulgare* L.). American-Eurasian Journal of Agriculture and Environmental Science **3,** 855-860.

Memon KS, Rashid A, Puno HK. 1992. Phosphorus deficiency diagnosis and P soil test calibration in Pakistan. Trop. Soil Bulletin **92,** 125-147.

Mengel K, Kirkby EA. 2001. Principles of plant nutrition, 5th Edn. Kluwer, Dordrecht.

Miransari M, Bahrami HA, Rejali F, Malakouti MJ. 2007. Using arbuscular mycorrhiza to alleviate the stress of soil compaction on Wheat growth. Soil Biology and Biochemistry **40,** 1197-1206.

NFDC. 2001. Balanced fertilization through phosphate promotion. Project terminal report NFDC, Islamabad, Pakistan, 2001.

Nzanza B, Marais D, Soundy P. 2011. Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi. African Journal of Microbiology Research **5,** 425-431.

Omomowo IO, Ola IO, Akintokun AK, Bankole MO, Babalola OA. 2009. Direct and Residual Influence of Inoculation with *Glomus mosseae* and *Bradyrhizobium japonicum* on Proximate and Nutrient Element Content of Cowpea Seeds. American.-Eurasian Journal of Sustainable Agriculture **3,** 435-441.

Plenchette C, Fortin JA, Furlan V. 1983. Growth response of several plant species to mycorrhiza in a soil of moderate P fertility. In: Mycorrhizal dependency under field conditions. Plant Soil **70,** 199-209.

Roy-Bolduc A, Hijri M. 2011. The use of mycorrhizae to enhance phosphorus uptake: a way out the phosphorus crisis. Journal of Bio fertilizer and Bio pesticides **2,** 104.

Sawers RJH, Yang SY, Gutjahr C, Paszkowski U. 2008. The molecular components of nutrient exchange in arbuscular mycorrhizal interactions. In: Siddiqui, Z. A., Akhtar, M. S. and Futai, K. (Eds.). Mycorrhizae: Sustainable Agriculture and Foresrty. Springer, Dordrecht, the Netherlands. 37-60 p.

Sharif M, Ahmad E, Sarir MS, Muhammad D, Shafi M, Bakht J. 2011. Response of different crops to arbuscular mycorrhiza fungal inoculation in phosphorus-deficient soil. Communication in Soil Science and Plant Analysis **42,** 2299-2309.

Shrestha G, Vaidya GS, Rajbhandari BP. 2009. Effects of arbuscular mycorrhiza in the productivity of maize and fingermillet relay cropping system Nepal. Journal of Science and Technology **10,** 51-55.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. San Diego, CA: Academic Press; 1997.

Subramanian K, Bharathi C, Jegan A. 2008. Response of maize to mycorrhizal colonization at varying levels of zinc and phosphorus. Biology and Fertility of Soil **45,** 133-144.

Sylvia DM, Hammond LC, Bennett JM, Haas JH, Linda SB. 1993. Field response of maize to a VAM fungus and water management. Agronomy Journal **85,** 193–198.

Tarafdar JC, Marschner H. 1995. Dual inoculation with *Aspergillus fumigates* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Na-phytate. Plant Soil **173,** 97-102.

Wilkinson KM. 2008. Beneficial microorganisms. In: R. K. Dumroese, T. D. Luna, Eds. nursery managements. Volume 1. Nursery manual for native plants: A guide for tribal, nurseries. Washington: USDA Forest service. Agriculture **730,** 246-261.

Wu OS, Xia RX. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal of Plant Physiology **163,** 417-425.

Wu QS, Xia RX, Zou YN. 2006b. Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. Journal of Plant Physiology **163,** 1101-1110.

Wu OS, Xia RX, Zou YN. 2008. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. European Journal of Soil Biology **44,** 122-128.

Xavier LJC, Germida JJ. 1998. Response of spring wheat cultivars to *Glomus clarum* NT4 in P-deficient soil containing arbuscular mycorrhizal fungi. Canadian Journal of Soil Science **78,** 481-484.