Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 9 (6): 337-343, 2010
ISSN 1682-3974

Growth Response of Sweet Corn (*Zea mays*) to *Glomus mosseae* Inoculation over Different Plant Ages

Ali A.A. Mustafa, Radziah Othman, M.A. Zainal Abidin and V. Ganesan
Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract: A glasshouse study was conducted to investigate the growth response of sweet corn (*Zea mays*) to mycorrhizal fungi inoculation over different plant ages (2, 4, 6, 8 and 10 weeks) and to determine the interaction between the host and mycorrhizal fungus on mycorrhizal development, using soil substrate as inoculum for *Glomus mosseae*. Inoculation had no significant effect on growth parameters in plants harvested at early ages in terms of plant height, total root length, root and shoot dry weights. The significant effect of mycorrhizal inoculation was observed on plants of eight weeks old. Percentage of mycorrhizal colonization and number of spores increased significantly at plants of 2 weeks old (24.1 and 39.2%), respectively while; the highest AMF spores level was recorded at plants of four weeks old. Inoculation with *G. mosseae* enhanced growth of sweet corn by increasing concentration of N, P and K (24.2, 8.4 and 18.2%), respectively. We concluded that the mycorrhizal inoculation need a time (not less than one month in sweet corn) until showed the beneficial effects on host plant to be desired.

Key words: Symbiotic colonization, benefit of AMF, AM fungi, sweet corn, *Glomus mosseae*

INTRODUCTION

More than 90% of terrestrial plants are associated with root-colonizing fungi, establishing a permanent and intimate mutualistic symbiosis, called Arbuscular Mycorrhiza Fungi (AMF). Several types of mycorrhiza exist, defined by plant/fungus combination and the symbiotic structure. The AMF are represented by more than 150 species of the Zygomycota included in the Glomales (Morton and Benny, 1990), by forming the extended, interact hyphal network. In soil AMF are found as spores, hyphae, or infected root pieces. All these are sources of inoculum, with the extraradical mycelium as the main source (Sylvia and Jarstfer, 1992).

The inoculum potential is a product of the abundance and vigor of the propagules in the soil and can be quantified by determining the rate of colonization of a susceptible host under a set of standard conditions (Liu and Luo, 1994). Arbuscular mycorrhizal fungi and plant roots are widespread in the natural environment and can provide range of benefits to the host plant these include improved nutrition, enhanced resistance to the soil –born pests and diseases, improved resistance to drought, tolerance of heavy metals and better soil structure. Many agricultural crops are mycorrhizal and there is widespread of equivocal evidence that crop plants benefits from the AMF (Gosling *et al*., 2006). AMF promote host plant growth by supplying phosphorus, absorbed from the soil solution by the external hyphae. In turn the fungi obtain their carbon from the photosynthetic activity of the host plant (Pearson, 1993). The nutrient exchange between host plant and mycorrhizal fungus is interrelated and depend upon environmental conditions.

The application of AMF is one of the interests for the reclamation and revegetation of degraded lands (Wu *et al*., 2002) an aspect of particular interest in the tropics. Moreover, it is a challenge to develop AMF strategies applicable for sustainable low-input but a reasonably productive and ecologically sound agriculture (Thompson, 1994). It was hypothesized that the effects of AMF on host growth are context dependent and predicted that beneficial effects of AMF on host growth would be most evident when host plants experienced the dual stresses of drought and root damage. Studies exploring compatibility of AMF-plant interactions have demonstrated that pairings of host and AMF may vary in degree or even direction of the effect on host performance (Bennett and Bever, 2007). The external AMF mycelium phase is the fungal phase which is in contact with the soil and thus responsible for nutrient acquisition and transport to the internal mycelium inside the root before any transfer to the plant occurs. In return, triacylglycerides can be transported from the internal to the external mycelium phase to support the glyoxyl cycle

Corresponding Author: Radziah Othman, Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia Tel: +601 3552706

for metabolic activity (Lammers *et al*., 2001). However, despite the obvious importance of the external mycelium in nutrient acquisition, few fungal transporters have been characterised. These include a phosphate (Harrison, 1999), an ammonium (Lopez-Pedrosa *et al*., 2006) and a putative zinc transporter (Gonzalez-Guerrero *et al*., 2005). In addition, an aquaporin (water channel proteins) gene, GintAQP1, has been discovered in the external mycelium of *Glomus intraradices* which showed increased expression in parts of the AMF mycelium not experiencing osmotic stress compared to parts that were (Aroca *et al*., 2009). This suggests communication between of the mycelium subject to the differing external conditions, a behavior that is well established in roots (Hodge, 2009).

Corn has a high demand for N and P nutrients (Jokela and Randall, 1989; Olson and Sander, 1988). In order to get a high yield, large amounts of N and P fertilizers are applied (Barry and Miller, 1989). Consequently, N and P fertilization levels are high in a large percentage of corn fields.

Although AMF have a significant positive effect on maize development, there is insufficient information on the stage of growth response to mycorrhizal inoculation, A relatively few number of studies suggest that AMF colonization similarly alters maize root morphology in both low and high P soils, with these alterations primarily involving an increase in lateral root growth during early host development. Therefore, the following research was carried out to investigate the progress of sweet corn to AMF colonization, determine the effects of AMF on the growth of sweet corn and evaluate the biochemical, physiological and morphological changes in the host plant.

MATERIALS AND METHODS

Soil: Mixed soil (Serdang series, sand and peat 3:2:1 v/v) was used in all experiments and soil chemical analysis was carried out before used (Table 1). Soil was sterilized at 121°C for two hours, then packed in open pot culture (20×20 cm). Modified Hoagland solution (NO_3 210, P 31, K 234, Mg 48, Ca 160, S 64, Fe 2.5, Mn 0.5, B 0.5, Cu 0.02, Zn 0.05, Mo 0.01 mg L^{-1}) was added after the first week of sowing in amount of 50 mL per pot.

Plant material: Seeds of sweet corn (*Zea mays*) were obtained from Department of Agriculture, Malaysia. Seed were surface sterilized by gently shaking in a 5% NaClO for 3 min and rinsed four times for 5 min and three times for 20 min in sterilized water. Seeds were pre-germinated on wet sterilized filter paper at 28°C for three days to test the viability. Seeds were sown in pots at 5 seeds per pots. Plants were thinned to three plants per pot. Seedlings were maintained by regular irrigation and arranged in complete randomize design under glass house conditions (32±5°C, 12/12 light/ dark photoperiod, 65% relative humidity).

Fungal material: *Glomus mosseae* Inoculum was obtained from Department of Land management, Faculty of agriculture, Universiti Putra Malaysia (UPM), fungus identification was checked using light microscope. *G. mosseae* was multiplied in open pot culture of sweet corn (Zea *mays*) under glasshouse conditions.

Mycorrhizal inoculation: Soil was inoculated with AMF before seeds were sown at ratio of 120 spores per 1 kg soil using soil as inoculum substrate, while non mycorrhizal (control) plants were without inoculation. Plants were harvested at five different ages (2, 4, 6, 8 and 10 weeks old after inoculations of AMF by uprooting, roots were washed gently under stream of tap water to remove adhering soil particles. Different parameters were taken (estimation of AMF colonization, root density, root length, concentration of minerals in shoot tissues, stem width, plant height and dry weight of shoot and root).

Root architecture: After harvest, roots were washed gently 3 times under stream of tap water and were kept in the refrigerator at 4°C, till analysis. Root total length, tips number, root volume were analyzed using RHIZO 2000c PC program (Regent Instrument, Qubec city, Canada) an interactive scanner-based image analysis of root samples. Roots were placed in the plexiglass trays (150×250 mm)

Table 1: Chemical properties of mixed soil

Element	P	K	Mg	Ca	S	Cu	Fe	Mn	Mo	Zn
Mg kg^{-2}	0.41	2.7	1.4	3.2	3.8	0.1	72.1	0.8	0.004	0.6

Table 2:Percentages of *Glomus mosseae* colonization and number of spores of sweet corn over different plant growth stage

	Plants age (weeks) at time of sampling				
Parameters	2	4	6	8	10
Colonization (%)	45.6e	59.7d	69.74c	74.b	77.4a
Number of spores	34.5e	56.7d	65.2c	74.5b	82.7a

Means of colonization percentage and number of spore when p = 0.05, means followed with same letters showed no significant different

with 5-10 mm deep water layer depending on root size. Roots were spread on the tray before scanning to minimize overlapping. The instrument makes reading of root length in meters or centimeter.

Chemical analysis of shoot tissues: Shoots were sampled for nutrient analysis. Tissues were dried at 70°C, weighed and digested in a sealed chamber method (Anderson and Henderson, 1986). About 500 mg of powdered tissue was placed into a glass centrifuge tube and 1-2 mL of a 7:3 (v:v) mixture of $HC10_3$ and H_2O was added and the tube was tightly capped. After-2 h or overnight pre-digestion at ambient temperature, 1 mL of H_2O, was added and the tube was again tightly sealed and placed under the fumed on a hot plate for 10-30 min until the acid extract turned to colorless. The digested samples were filtered and diluted to 25 mL with d H_2O. All the minerals except nitrogen were determined with an inductively coupled argon plasma spectrophotometer (Model IRIS Advantage, from thermo elemental, USA). The N content was estimated (Sivasankar and Oaks, 1995) using an Elemental Analyzer (Perkin Elmer Series II 2400, USA).

Estimation of AMF colonization: At each harvest time, a sample of fresh roots were cut into 1 cm length and stored in FAA (formalin-acetic acid-50% ethanol) (5:5:90, v/v/v) solution. Roots were then, washed with tap water and soaked in 10% KOH at 90°C for 1 h. Root samples were washed with tap water three times and then aciditified with 1% HCL for 3 min. Roots were stained in 0.05% trypan blue in lactophenol at 90°C for 15 min and root samples were examined under microscope. The number of root segments colonized were counted and expressed as percentage of total root segments (Kormanik and Mc Graw, 1982).

$$\text{Colonization (\%)} = \frac{\text{Number of colonized sample}}{\text{Total number of segments examined}} \times 100$$

Spore density and percentage of colonization: To assess spores density in the soil, wet sieving decanting method was used, ten grams of soil was collected randomly after harvesting and placed in 200 mL water in a large beaker. Soil mixture was blended for 2 min. then the mixture was poured into arrange sieves (250, 100 and 42 μm). Soil was entrapped to settle at every sieve. The spore suspension was examined under compound microscope.

Experimental design: The study was conducted on Feb. to May 2008 at Faculty of Agriculture, UPM, Serdang, Malaysia. Pots were arranged in a completely randomized experimental design. Three plants were placed in each pot with 5 replicates for each treatment; plants were harvested at different ages, 2, 4, 6, 8 and 10 weeks. Data were analyzed using SAS software program version 8.0.ANOVAs were carried out to determine significance. Means were separated using the LSD test at 5% level of probability.

RESULTS

All mycorrhizal plants showed significant differences ($p>0.05$) in the most measured parameters compared with non- mycorrhizal plants (control). However, the time factor affected all parameters within mycorrhizal plants (Fig. 1-4).

Effect of AM inoculation on shoot and root dry weight: The shoot dry weight of mycorrhizal plants was increased 7.1-27.5% compared with nonmycorrhizal plants (Table 2), the greatest increase in shoot dry weight was recorded in plants of eight weeks old (after 4 week of inoculation) and this explain the suitable duration of AM successful colonization in sweet corn. Inoculation with AMF was increased root dry weight in rate by 9.7%-75.8 compared with noninoculated plants. Four weeks old mycorrhizal plants showed a significant difference in growth compared with other plants.

Effect of AM inoculation on root length: Total root length of mycorrhizal plants was showed significant effect at plants of 4 weeks age and the great root length was recorded in plants of 6 weeks of age, same results were obtained when the difference measured between mycorrhizal and nonmycorrhizal plants, although the greater difference was at plants of 10 weeks age (Fig. 3).

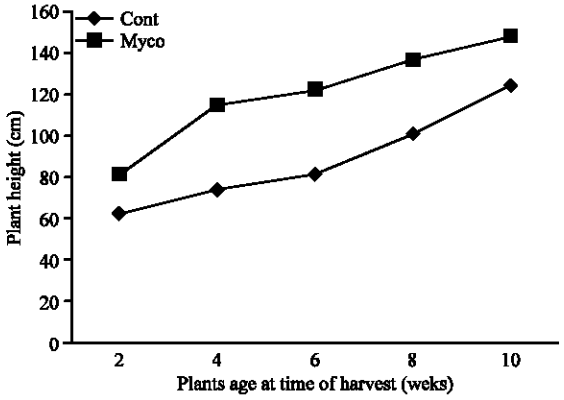

Fig. 1: Effect of mycorrhizal inoculation on the height of sweet corn

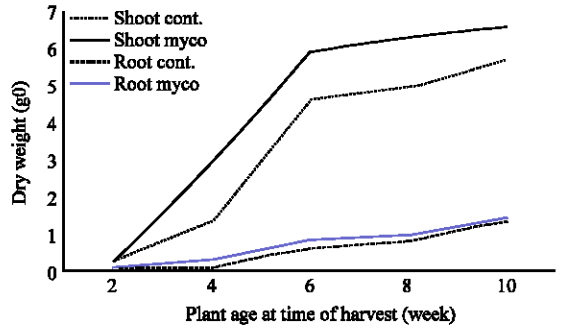

Fig. 2: Shoot and root dry weight (g) p< 0.05%. *cont.= non-mycorrhizal plants, Myco = mycorrhizal plants

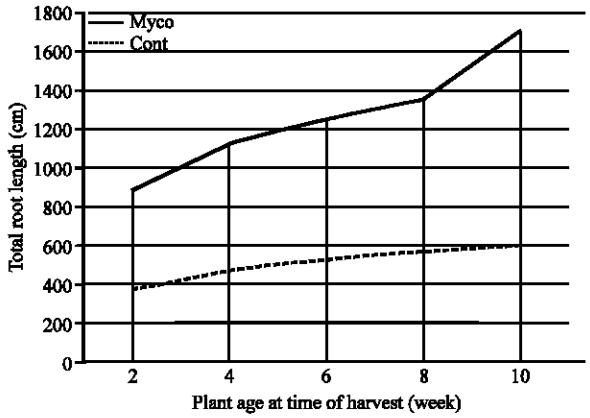

Fig. 3: Effect of mycorrhizal inoculation on total root length

Effect of AM inoculation on stem height and width: The effect of mycorrhizal inoculation on plant height was measured. Inoculation of *G. mosseae* increased plant height significantly (Fig. 2). Plants height of 4 and 6 weeks were highly increased due to AM inoculation compared with other non mycorrhizal plants. However, plants of two weeks of age not showed significant different between mycorrhizal and non-mycorrhizal plants

Similarly stem width was also affected with AMF inoculation although, there is no significant different in plants of 2 weeks old. The greater stem width was recorded with plants of 6 and 8 weeks age as shown in Fig. 3.

Effect of AM inoculation on nutrients concentration in shoot system: Mycorrhizal inoculation increased tissue phosphorus content significantly; however the rate of enhancement was not stable 5.8, 19.9, 5.7, 3.5 and 7.1% respectively, tissues from plants of 4 weeks age were

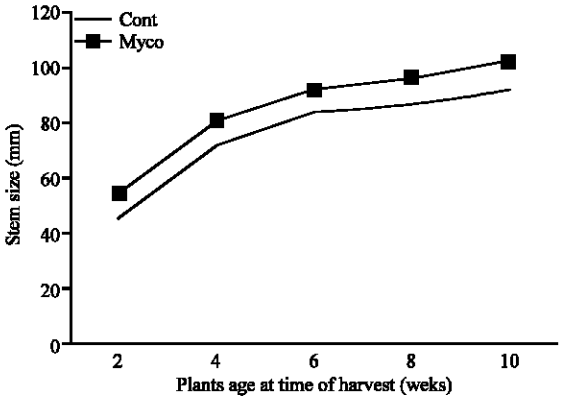

Fig. 4: Effect of mycorrhizal colonization on stem width of sweet corn

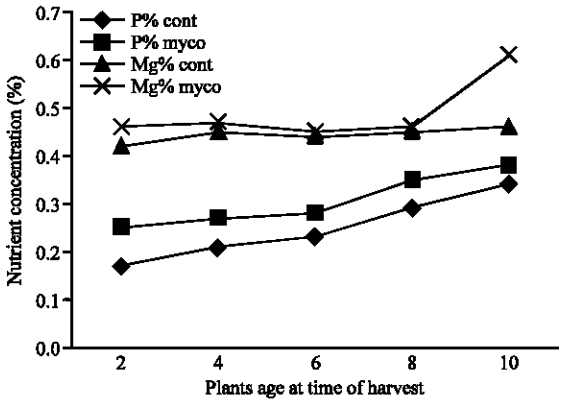

Fig. 5: Concentration of phosphorus and magnesium in shoot tissue of mycorrhizal and non-mycorrhizal plants at different ages

significantly affected due to mycorrhizal inoculation in terms of phosphorus enhancement rate while the low enhancement was observed in tissue from plant of 8 weeks age.

Mycorrhizal plants of 10 weeks age showed highly calcium content compared with others whereas no significant differences, which is contrast of Mg results no significant differences between mycorrhizal and non-mycorrhizal plants (Fig. 5).

Inoculation with *G. mosseae* increased potassium concentration in average by 18.2% compered with nonmycorrhizal plants while the highest K concentration was recorded with plants of 8 weeks age (Fig. 6).

Nitrogen content was affected with mycorrhizal inoculation especially plant of 10 weeks age the rate of increase was 33.8% compared with lowest rate 12.9% in plants of 2 weeks (Fig. 6).

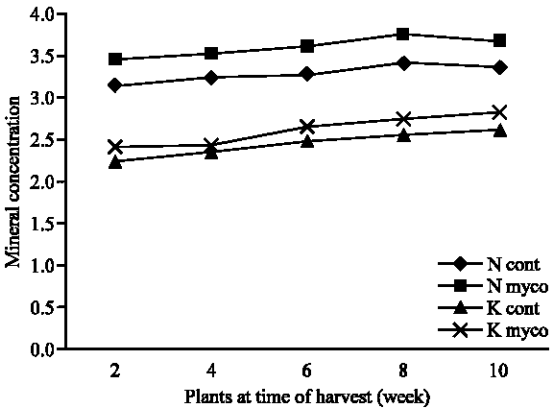

Fig. 6: Percentage of potassium and nitrogen in shoot tissue of mycorrhizal and non-mycorrhizal plants at different ages

Degree of AM colonization and spore density: Results showed that the number of the spores was increased according to the plants growth stage which explain the relationship between plant age and spores production, although the different in the number of spores between different plant ages was (39.2, 13, 12.5 and 9.9%) respectively (Table 2) which explains that although the spores density were enhanced with increase in time but the rate of spore density is decrease with time.

There were differences in percentage of mycorrhizal colonization between different plant age were (24.1, 14.4, 6.3 and 3.9%), respectively (Table 2). Thus, mycorrhizal colonization after four weeks was not affected by plant age. There was a relationship between AMF colonization and spores production although there were differences in rate of increase.

DISCUSSION

Significant findings of this study were; sweet corn was appeared high response to mycorrhizal inoculation at all ages, especially plants of four and six weeks ages then; the response was decreased in plants of old age. One of the major factors that determine potential benefits from mycorrhizal inoculation is crop species (Miller *et al*., 1986). Some plants species and even cultivars of the same species are more dependent on mycorrhizal infection than others (Graham and Syversten, 1985).

McAllister (1994) found that inoculation with *G. mosseae* was significantly increases root dry weight of lettuce, while root dry weight not affected with inoculation with *Trichoderma koningii*, similar results were found by Tahat *et al*. (2008) when they investigated the response of tomato (*Lycopersicum esculentum* Mill) to *G. mosseae*, they found that *G. mosseae* was increased shoot dry weight 2.82 g this totally agree with our results most likely as enhanced in root length also, because the experiments were conducted under similar conditions.

Augmentation of soil with different beneficial microorganisms including AMF species that results in plant growth promotion has been demonstrated by several workers over four decades (Xavier and Germida, 2001). The primary benefit derived by plants involved in mycorrhizal symbioses is generally perceived to be enhanced nutrient uptake, achieved by the fungus expanding the zone of nutrient uptake farther away from the rhizosphere and/or more efficiently taking up and transporting nutrients.

Significant differences in plant height between mycorrhizal and nonmycorrhizal plants, similar were observed at plants of four weeks age, these results in the same line with Tarafdar and Marschner (1995) who found a significant different in plant height at mycorrhizal wheat plants of four week ages, this can be explained that corn and wheat are classified under one family (Graminceae).

Shoot concentration of P was significantly higher at plants of ten weeks old while, there were slight differences at plants of other ages. While, Mg concentration in shoot was significantly different when compared mycorrhizal inoculated and non-inoculated plants over all plants growth age (Fig. 5).

Mycorrhizal plants showed significant differences in most measured parameters than non- mycorrhizal plants (control), however the time factor was affected on all parameters within mycorrhizal plants. There was a relationship between plant ages and AMF colonization ratio even the highest colonization ratio was produced in the plants of four weeks ages. Plant age was influenced the successful interaction between the plant and AM fungi, maximum infection occurred at 6 weeks old plants. In terms of the relationship between spores density and roots colonization our results showed there were a positive relationship between them, this agreed with Smith and Read (2008) who stated that the density of the spores in soil can be determined but, although this sometimes shows a correlation to the extent of root colonization, this is certainly not always the case, the relationships complex.

Our research on the responsiveness of sweet corn (*Zea mays* L.) to AMF (*G. mosseae*) inoculation concluded that there is a positive relationship between

host response to AMF and growth stage of the host. Therefore, mycorrhizal colonization needs a time to show the desired benefits on plant growth.

ACKNOWLEDGMENTS

We wish to thank Sudan University of Science and Technology and Universiti Putra Malaysia for financing this study. Our thanks extend to Dr. M. M. Tahat at Faculty of Agriculture, UPM for his valuable efforts in reviewing the article.

REFERENCES

Anderson, D.L. and L.J. Henderson, 1986. Sealed chamber digestion for nutrient analyses. Agron. J., 78: 937-939.

Aroca, R., A. Bago, M. Sutka, J.A. Paz, C. Cano, G. Amodeo and J.M. Ruiz-Lozano, 2009. Expression analysis of the first *Arbuscular mycorrhizal* fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. Mol. Plant-Microbe Interactions, 22: 1169-1178.

Barry, D.A.J. and M.H. Miller, 1989. Phosphorus nutritional requirement of maize seedlings for maximum yield. Agron. J., 81: 95-99.

Bennett, A.E. and J.D. Bever, 2007. Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology, 88: 210-218.

Gonzalez-Guerrero, M., C. Azcon-Aguilar, M. Mooney, A. Valderas, C.W. Mac-Diarmid, D.J. Eide and N. Ferrol, 2005. Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet. Biol., 42: 130-140.

Gosling, P., A. Hodge, G. Goodlass and G.D. Bending, 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ., 113: 17-35.

Graham, J.H. and J.P. Syvertsen, 1985. Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytologist, 101: 667-676.

Harrison, M.J., 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Ann. Rev. Plant Physiol. Plant Mol. Biol., 50: 361-389.

Hodge, A., 2009. Root decisions. Plant Cell Environ., 32: 628-640.

Jokela, W.E. and G.W. Randall, 1989. Corn yield and residual soil nitrate as affected by time and rate of nitrogen application. J. Environ. Qual., 81: 720-726.

Kormanik, P.P. and A.C. McGraw, 1982. Quantification of Vesicular-arbuscular Mycorrhizae in Plant Roots. In: Methods and Principles of Mycorrhizal Research, Sheed, N.C. (Ed.). American Phytopathological Society, St. Paul, pp: 37-45.

Lammers, P.J., J. Jun, J. Abubaker, R. Arreola and A. Gopalan *et al*., 2001. The glyoxylate cycle in an *Arbuscular mycorrhizal* fungus. Carbon flux and gene expression. Plant Physiol., 127: 1287-1298.

Liu, R.J. and X.S. Luo, 1994. A new method to quantify the inoculum potential of arbuscular mycorrhizal fungi. New Phytologist, 128: 89-92.

Lopez-Pedrosa, A., M. Gonzalez-Guerrero, A. Valderas, C. Azcon-Aguilar and N. Ferrol, 2006. GintAMT1 encodes a functional high affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. Fungal Genet. Biol., 43: 102-110.

McAllister, C.B., I. Garica-Romera, A. Godeas and J.A. Ocambo, 1994. *In vitro* interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*. Soil Boil. Biochem., 26: 1369-1374.

Miller, J., J. Rajapakse and R. Garber, 1986. Vesicular-arbuscular in vegetable crops. Hortscience, 21: 974-984.

Morton, J.B. and G.L. Benny, 1990. Revised classification of arbuscular mycorrhizal fungi (zygomycetes): a new order glomales, two new suborders, glomineae and gigasporineae and gigasporaceae, with an amendation of glomaceae. Mycotaxon, 37: 471-491.

Olson, R.A. and D.A. Sander, 1988. Corn Production. In: Corn and Corn Improvement, Sprague, G.F. and J.W. Dudley, (Eds.). American Society of Agronomy, Madison, Wisconsin, pp: 639–678.

Pearson, J.N. and I. Jakobsen, 1993. Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. New Phytologist, 124: 481-488.

Sivasankar, S. and A. Oaks, 1995. Regulation of nitrate reeducates during early seedling growth: A role for asparagines and glutamine. Plant Physiol., 107: 1225-1231.

Smith, S.E. and D.J. Read, 2008. Mycorrhizal Symbiosis. 3rd Edn., Academic Press, London, ISBN-10: 0123705266.

Sylvia, D.M. and A.G. Jarstfer, 1992. Sheared root inocula of vesicular-arbuscular mycorrhizal fungi. Applied Environ. Microbial., 58: 229-232.

Tahat, M.M., S. Kamaruzaman, O. Radziah, J. Kadir and H.N. Masdek, 2008. Response of (*Lycopersicum esculentum* Mill.) to different arbuscular mycorrhizal fungi species. Asian J. Plant Sci., 7: 479-484.

Tarafdar, J.C. and H. Marschner, 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.

Thompson, J.P., 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. Soil Biol. Biochem., 26: 1133-1143.

Wu, T.H., W.Y. Hao, X.G. Lin and Y.Q. Shi, 2002. Screening of arbuscular mycorrhizal fungi for the revegetation of eroded red soils in subtropical China. Plant Soil, 239: 225-235.

Xavier, L.J.C. and J.J. Germida, 2001. Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. Soil Biol. Biochem., 33: 45-48.