Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 08, pp.7973-7977, August, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND PHOSPHORUS APPLICATIONS ON PLANT GROWTH AND NODULATION OF *Vigna radiata* (L.)

***Shalu Malik, Sarita Devi and Ashok Kumar**

Department of Botany, C.C.S. University, Meerut-250004, India

ARTICLE INFO

Article History:
Received 08th May, 2014
Received in revised form
16th June, 2014
Accepted 07th July, 2014
Published online 31st August, 2014

Key words:
Increase, Mycorrhiza, Nodulation.

ABSTRACT

This paper reports the effect of mycorrhiza as a biofertilizer and different concentrations 5%, 10% and 15% of phosphorus on seed germination, nodulation and growth of *Vigna radiata* (L.). A significant increase was observed in all parameters over control. At 15% concentration of phosphorus, the *Vigna radiata* (L.) showed favourable effect on seed germination, seedling growth and production of fresh and dry matter as compared to 5 and 10%.

INTRODUCTION

Green gram (*Vigna radiata* L.), commonly known as mung bean, a protein rich pulse crop has a growing demand in Asia, specially in India. It contains light protein, easily digestible, and does not cause the flatulence as many other legumes do (Hussain *et al*., 2012). A group of soil micro-organism that live in extremely close contact with the root are arbuscular mycorrhizal (AM) fungi. A large numbers of plant species form symbiotic associations with mycorrhizal fungi, forming mycorrhizae. This significantly increase nutrients transfer from the soil to the root, as the fugal hyphae take up phosphate and transferring it to fungal structure inside the cortical cell of the root. Arbuscular mycorrhiza has a world-wide symbiotic association that are describes as the result of co-evolution event between fungi and plant where both association have advantages from the reciprocal nutrient exchange (Bonfante and Genrse, 2008). These fungi are known to assist the plant in the uptake of nutrients and to improve plant growth (Douds *et al*., 2005). Legumes need adequate phosphorus supply for satisfactory nodule production and nitrogen fixation. At low levels of phosphate, roots are highly infected with mycorrhizal fungi and levels of expression of the plasma membrane that take up phosphate directly from the soil are low. At high level of phosphate, the mycorrhizal association is suppressed and the expression of direct transports is increased (Smith *et al*., 2010). In addition, much published evidence shows that suppression of disease in plants is due to colonization by AM fungi (Kasiamdari *et al*., 2002). Mycorrhizal fungi can also be stabilize soil aggregates (Piotrowski *et al*., 2004), and some reports show that mycorrhizal plant may be more resistant to stresses such as drought (Neumann and George, 2004), or salinity (Tian *et al*., 2004). Plant phytohormone level can also be affected by micorrhizal fungus. Mycorrhizal fungi act as biofertilizer and have the unique ability to convert nutritionally important elements from unavailable to available form through biological process (Vessey, 2003). The beneficial effect of indigenous AM fungi on the nutrition of agricultural plants depends on both the abundance and type of fungi present in the soil (Prabhu *et al*., 2013).

****Corresponding author: Shalu Malik,***
Department of Botany, C.C.S. University, Meerut-250004, India.

MATERIALS AND METHODS

The present work was conducted during the month of April to June in 2013 to study the effect of mycorrhiza and phosphorus on *Vigna radiata* (L.). The experiments were carried out in the field of Department of Botany, C.C.S. University Meerut. Experiment was designed in five plots of equal size, four plots for treatment and one plot for control. Healthy seeds of *Vigna radiata* (L.) were selected and washed with distilled water. Standard agronomic practices were adopted and no fertilizer was applied to the field.

Five treatments were performed in the present work as followed:

1) Treatment with mycorrhiza alone.
2) Treatment with 5% concentration of P_2O_5 alone.
3) Treatment with 10% concentration of P_2O_5 alone.

4) Treatment with 15% concentration of P_2O_5 alone.
5) Untreated plants considered as control.

AM fungi (*Glomus fasciculatum*) was obtained from Indian Agricultural Research Institute, New Delhi to evaluate the inoculation effect of AM Fungi and phosphorous levels on the growth of *Vigna radiata* (L.). Hundred grams of AM fungal inoculum were mixed with the top 6 cm soil of mycorrhiza treated plot. Three different solutions with different concentrations of P_2O_5 were prepared as 5%, 10% and 15%. Fifty healthy seeds of *Vigna radiata* (L.) were sown in each plot. Control plots were irrigated with tap water and others four plots with three concentrations of P_2O_5 solution and mycorrhiza. The seed germination percentage was calculated after counting the difference between germinated and un-germinated seeds. Nodules were detached from the plant root with the help of forceps from plants. Fresh weight of nodules was measured immediately, followed by drying of nodules at 60ºC for 24 h to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. Leghemoglobin quantity was measured spectrophotometrically as hemochromogen following the method of Hartee (1957). Olsen *et al*. (1954) method was used to measure Phosphorus amount in soil before and after crop sowing.

RESULTS AND DISCUSSION

Seed germination

The seed germination was measured at the different time-intervels 7 day after sowing (DAS), 10 DAS and 15 DAS. Seed germination in the mycorrhiza treated seeds was higher than those in control. 15% concentration of phosphorus improves the germination percentage in the *Vigna radiata* L. as compare to 5% and 10% concentrations (Table 1). However, maximum germination was recorded in the seeds treated with mycorrhiza. Through biological processes symbiotic microorganism can change prime nutrients from an unavailable form to an available form, extend the root system and improve seed germination (Chen 2006; Rajendran and Devaraj, 2004). AM association also revealed a significant increase in percent seed germination with wheat plants (Minaxi *et al*., 2013)

Table1. Seed germination percentage of *Vigna radiata* (L.) with mycorrhiza and different concentrations of P.

Treatment	Germination % 7 DAS	Germination % 10 DAS	Germination % 15 DAS
Control	80	82	85
Phosphorus (5%)	82	88	90
Phosphorus (10%)	84	90	92
Phosphorus (15%)	88	92	94
Mycorrhiza	90	96	98

Growth studies

One of the main objectives of our study was to explore the beneficial effects of mycorrhiza that can be used as biological fertilizers. In general root and shoot length of plant were found significantly higher in mycorrhiza treated plants. Photosynthesis requires intensive phosphorylation process (Louw-Gaume *et al*., 2010). Mycorrhizal infection has known to enhance plant growth by increasing nutrient uptake, the uptake of nitrogen, phosphorus and potassium and also increase the rates of photosynthesis. Photosynthesis and transpiration rates of mycorrhizal *Satsuma mandarian* trees are higher than non mycorrhizal trees (Prabhu *et al*., 2013). Due to increased rate of photosynthesis growth enhanced. Inoculation with arbuscular-mycorrhizal fungi improved the phosphorous uptake in *Coleus aromaticus* (Earanna *et al*., 2001). 15% concentration of phosphorus showed remarkable increase in growth parameters as compare to 5% and 10% (Table 2). It is concluded from the results that P adsorption capacities of plants influenced the supply of P differently to the plants. The maize plant height was greater, even at low level of soil solution of P even though the P adsorption parameters of the soil were low (Rashid and Iqbal, 2012).

Table 2. Root length and shoot length of *Vigna radiata* (L.) with mycorrhiza and different concentrations of P.

Treatment	Root length (cm)	Shoot length (cm)
Control	8.7	31.7
Phosphorus (5%)	8.96	34.06
Phosphorus (10%)	11.21	40.34
Phosphorus (15%)	11.72	41.94
Mycorrhiza	12.88	44.98

Plant biomass

In general root and shoot length and fresh and dry weight of plant were found significantly higher in mycorrhiza treated plants over the phosphorus treated plants and control (Table 3).

Table 3. Root and shoot fresh and dry weight of *Vigna radiata* (L.) with mycorrhiza and different concentrations of P.

Treatment	Root		Shoot	
	Fresh wt. (gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt. (gm)
Control	0.542	0.168	5.66	1.57
Phosphorus (5%)	0.2976	0.1240	8.92	2.80
Phosphorus (10%)	0.7092	0.2214	9.51	3.12
Phosphorus (15%)	0.9040	0.2742	10.21	3.53
Mycorrhiza	1.0376	0.2902	12.85	4.29

15% concentration of phosphorus showed remarkable increase in growth parameters as compare to 5% and 10%. Inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation (Jia *et al*., 2004). In low P condition roots dry mass reduced and simultaneously the root volume did not increase significantly. Lower root density in P-deficient plants must be related to thinner cell walls, which could transport more mineral to root cells (Wahl and Ryser, 2000). Growth and total biomass increase in the presence of sufficient availability of P conditions (Louw-Gaume *et al*., 2010). Different concentrations of phosphorus accumulates higher rates of P in shoots than roots of plants, when grown in the presence of sufficient P condition, though early rapid growth led to similar P amount per weight unit in comparison with P-deficient plants (Basirat *et al*., 2011).

Nodulation

The number of the nodules was higher in mycorrhizal treatment compare to the phosphorus application. Mycorrhizal infections of roots of legumes have been reported to stimulate both nodulation and N_2 fixation, especially in soils low in available (Redecker *et al*., 1997). Mycorrhiza treated plant showed greater increase values of nodules fresh and dry weight of a plant^{-1} compared to non mycorrhiza treated plant (Table 4). In legumes, AM not only stimulated plant growth but also enhance nodulation. Nodule number and biomass have been shown to increase remarkably due to inoculation of micro symbionts (Zhao *et al*., 1997). 15% concentration of phosphorus showed increase in nodulation as compare to 5% and 10%. Phosphorus appears essential for both nodulation and N_2 fixation (Pereira *et al*., 1989). Phosphorus influences nodule development through its basic functions in plants as energy source. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such function which directly or indirectly influence N-fixation by legume plants. AM fungi are able to increase nodulation and N-fixation differently. The structure, functioning, and nutritional demand of nodules are different with plant roots. Nodules are produced by cortical cell division, in which rhizobia with high energy and P requirements reside and fix nitrogen (Mohammadi *et al*., 2011). Phosphorus application to legumes have a positive effect on nodulation in crops through enhanced root proliferation and thus help in increased nitrogen fixation by the crops. Similar effects of phosphorus application on nodulation have also reported (Khatik *et al*., 1994).

Table 4. Nodule number and nodule fresh and dry weight of *Vigna radiata* (L.) with mycorrhiza and different concentrations of P.

Treatment	Nodule no.	Fresh wt. (gm)	Dry wt. (gm)
Control	15.2	0.245	0.041
Phosphorus 5%	17.4	0.265	0.069
Phosphorus 10%	19.6	0.303	0.091
Phosphous 15%	23.6	0.335	0.123
Mycorrhiza	25.2	0.391	0.284

Protein content

The effect of AM treated plants showed a greater increase in protein content than in control and phosphorus treated plants. Mycorrhiza and phosphorus concentrations enhanced the protein content as compared to control (Table 5). Increases in protein concentration of mycorrhiza treated plants are more frequently found (Gianinazzi-Pearson and Gianinazzi, 1995). The application of 5%, 10% and 15% concentrations of phosphorus showed increased protein content over the control. The highest value of protein content was obtained at 15% phosphorus concentration. The increase in protein content was due to the fact that P is an important structural component of DNA and RNA. The Phosphate group in nucleic acids bridges the RNA or DNA. DNA is the carrier of genetic information and RNAs function in protein synthesis (Mengel and Kirkby, 2001).

Leghaemoglobin content

The most abundant and best characterized nodule specific proteins are the leghaemglobin (Lb), which are expressed in the infected cells just prior to the onset of nitrogen fixation.

Table 5. Biochemical analysis of *Vigna radiata* (L.) with mycorrhiza and different concentrations of P.

Treatment	Protein	Legheamoglobin	Soil Phosphorus Estimation	
			Before Sowing	After Sowing
Control	2.12	0.50	$2.00X10^{-2}$	$1.89X10^{-2}$
Phosphorus (5%)	2.31	0.51	$1.90X10^{-2}$	$2.0X10^{-2}$
Phosphorus(10%)	2.50	0.52	$1.98X10^{-2}$	$2.3X10^{-2}$
Phosphorus(15%)	2.79	0.54	$1.89X10^{-2}$	$2.5X10^{-2}$
Mycorrhiza	3.49	0.55	$2.10X10^{-2}$	$1.20X10^{-2}$

The Lb concentration was significantly higher in Mycorrhiza treated plants (Table 5). The leghaemoglobin content was maximum at 15% concentration of phosphorus when compare to 10%, 5% concentration of phosphorus and control. These oxygen binding heam proteins are supposed to be responsible for supporting the flux of oxygen to the nitrogen fixing bacteroids (Ajpleby, 1984). The pink color, typical of healthy and effective nodules, is due to the presence of leghemoglobin. The nodule became pink earlier, developed more quickly and became active sooner in response to phosphorus. Leghaemoglobin contribute to the higher nitrogen-fixing ability of AM plants (Evelin *et al*., 2009). Leghaemoglobin is the most abundant protein in the nodules. Leghemoglobin is a hemoprotein that has a high affinity for oxygen leading to low oxygen content in the bacteroids to protect the oxygen-sensitive nitrogenase in the nodules (Johnston *et al*., 2001). The better nodulation might be resulted in higher content of leghemoglobin in nodular tissues. Similarly, higher leghemoglobin content in mycorrhiza was mainly due to better root and nodules development (Sidhu *et al*., 1967).

Effect of inoculation on Post-Harvest Soil Phosphorus

The highest amount of P content was recorded in the treatment of P_2O_5 at (15%) concentration (Table 5). While mycorrhiza treated plot show uptake of low amount of P content because mycorrhiza significantly improve the nutrient uptake efficiency. Lowest amount of P was recorded in the control plot. Mycorrhizal inoculation on the host plant is improve in phosphorus uptake due to enhanced capacity to absorb more phosphorus from the soil which is otherwise unavailable to the plants (Bai *et al*., 2008). AM fungi supported nitrogen fixation by providing phosphorus to legumes and other immobile nutrients which are crucial for nitrogen fixation (Clark and Zeto 2000)

Conclusion

The study concludes that inoculation with mycorrhiza has increased the growth and nitrogen fixation in mung bean crop. Mycorrhiza treatment showed maximum positive effects on phosphorus concentration and their uptake by plant because arbuscular mycorrhiza (AM) has widespread symbiotic associations. The AM symbiosis, facilitate the uptake of nutrients such as phosphorus.

Acknowledgements

The authors are grateful to the Department of Botany, C.C.S. University, Meerut, for providing all facilities.

REFERENCES

Ajpleby, C.A. 1984. Leghaemoglobin and Rhizobial respiration. Annual Review of Plant Physiology. 35: 443-478.

Bai JF, Lin XG., Yin R., Zhang HY., Wang JH., Chen, X.M. and Luo, Y.M. 2008. The Influence of Arbuscular Mycorrhizal Fungi on As and p Uptake by Maize (*Zea mays* L.) from As-Contaminated Soils. *Applied Soil Ecology*. 38(2):137-145.

Basirat, M. Malboobi, M.A. Mousavi, A. Asgharzadeh, A. and Saeed, S. 2011. Effects of phosphorous supply on growth, phosphate distribution and expression of transporter genes in tomato plants. *Australian Journal of Crop Science*. 5(5): 537-543.

Bonfante, P. and Genre, A. 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends in Plant Science*. 13(9): 492-498.

Bradford, M.M. 1976 Analytical Biochemistry. **72** 248-258.

Chen J, 2006. The Combined Use of Chemical and Organic Fertilizers and/or Biofertilizer for Crop Growth and Soil

Clark R.B. and S. K. Zeto. 2000. Mineral Acquisition by Ar-buscular Mycorrhizal Plants. Journal of Plant Nutrition. 23: 867-902.

Earanna, N. Mallikarjuniah, R.R. Bagyaraj, D.J. and Suresh, C.K. 2001. Response of *Coleus aromaticus* to *Glomus fasciculatum* and other beneficial soil microflora. *Journal of Spices and Aromatic Crops*. 10: 141-143.

Evelin, H. Kapoor, R. and Bhoopander. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress**:** a review Annals of Botany. 104**:** 1263–1280**.**

Fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. Pp 16-20.

Gianinazzi-Pearson, V. and Gianinazzi, S. 1995. Proteins and proteins activities in endomycorrhizal simbioses. In: V Varna & Hock B (eds.), Mycorrhiza Springer-Verlag, Berlin Pp. 251-266.

Hartee, E.F. 1957. In: 'Modern Plant Analysis'. Verlag. 4: 197-245.

Hussain, A. Amjed, A. and Noorka, I.J. 2012. Effect of phosphorus with and without Rhizobium inoculation on nitrogen and phosphorus concentration and uptake by mungbean (*Vigna radiata*). *Journal of Agricultural Research*. 50(1): 49-57.

Jia, Y.S. Gray, V.M. and Straker, C.J. 2004. The influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. Annals of Botany. 94: 251-258.

Johnston, A.W. Yeoman, K.H. and Wexler, M. 2001. Metals and the rhizobial-legume symbiosis–uptake, utilization and signalling. *Advances in Microbial Physiology*. 45: 113-156.

Khatik, S.K. Dikshit, P.R. and Bhadorir, A.K.S. 1994. Infuence of oxalic acid industrial waste vis-avis inorganic and biofertilizer on nodulation and nitrogen fixation. *Journal of Soils and Crops*. 4(1) 31-35.

Louw-gaume, A. Idupulapati, M.R. Gaume, A. and Frossard, E. 2010. A comparative study on plant growth and root plasticity responses of two Brachiaria forage grasses grown in nutrient solution at low and high phosphorous supply. Plant Soil. 328:155-164.

Mengel, K. and Kirkby, E.A. 2001. Principles of plant nutrition (5^{th} ed.) Kluwer Academic Publishers, London

Minaxi. Saxena, J. Chandra, Sand and Nain, L. 2013 Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *Journal of Soil Science and Plant Nutrition*. 13(2): 511-525.

Mohammadi, K. Khalesro S, Sohrab, Y. and Gholamreza, H. 2011. A Review: Beneficial Effects of the Mycorrhizal Fungi for Plant Growth. *Journal of Applied Environmental and Biological Sciences*. 1(9): 310-319.

Neumann, E. and George, E. 2004. Colonization with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) enhanced phosphorus uptake from dry soil in sorghum bicolour (L.). Plant Soil. 261: 245-255.

Olsen, S.R. Cole, C.V. Watanabe, F.S. and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939**:** 1-19. Gov. Printing Office Washington D.C.

Pereira, P.A.A. and Bleis, F.A. 1989. A Selection of Common bean (*Phaseolus vulgaris* L.) for N2 fixation at different levels of available Phosphorus under field and environmental-controlled condition. Plant Soil. 115: 75-82.

Piotrowski, J.S. Denich, T. Klironomos, J.N. Graham, J.M. and Rilling, M.C. 2004. The effect of arbuscular mycorrhiza on soil aggregation depend on the interaction between plant and fungal species. New Phytol. 164: 365-373

Prabhu, D. Sankaralingam, S. Sathyavathe, V. and Shankar, T. 2013. Effect of *Glomus* sp and *Gigaspora* sp. on *Vigna radiata* (L.) Under Water Stress Condition. *American-Eurasian Journal of Agricultural & Environmental Science*. 13(7): 935-942.

Rajendran, K. and Devaraj, P. 2004. Biomass and Nutrient Distribution and Their Return of Casuarina equisetifolia Inoculated with Biofertilizers in Farm Land. Biomass and Bioenergy. 26**:** 235-249.

Rashid, M. and Iqbal, M. 2012. Effect of phosphorus fertilizer on the yield and quality of maize (*Zea mays* L) fodder on clay loam soil. *The Journal of Animal & Plant Sciences*. 22(1): 199-203.

Redecker, D. Vonberswordtwallrabe, P. Beck, D.P. and Werner, D. 1997. Influence of inoculation with arbuscular mycorrhizal fungi on stable isotope of nitrogen in Phaseolus vulgaris. *Biol Fertil Soil*. 24: 344-346.

Sidhu, G.S. Singh, N. and Singh, R. 1967. Symbiotic nitrogen fixation by some summer legumes in Punjab. Role of leghemoglobin in nitrogen fixation. *Journal of Research, Punjab Agricultural University*. 4: 244-248.

Smith, A.M. Coupland, G. Dolan, L. Harberd, N. Jones, J. Martin, C. Sablowski, R. and Amey, A. 2010. Plant Biology. Garland Science, UK

Tabassum, Y. Burni, T. and Hussain, F. 2012. Effect of Arbuscular Mycorrhizal inoculation on nutrient uptake, growth and Productivity of chickpea (*Cicer arietinum)* varieties. *International* journal of Agronomy and Plant Production. 3 (9): 334-345

Tien, C.Y. Feng, G. Li, X.L. and Zhang, F.S. 2004. Different effect of arbuscular mycorrhial fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Applied Soil Ecology* 26 143-148.

Vessey, J.K. 2003. Plant Growth Promoting *Rhizobacteria* as Biofertilizers. Plant Soil. 255: 571-586.

Wahl, S. and Ryser, P. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytol. 148: 459-471.

Zhao, B. Trouvelot, A. Gianinazzi, S. and Pearson, G.V. 1997. Influence of two legume species on hyphal production and activity of two arbuscular mycorrhizal fungi. Mycorrhiza.7 (4): 179-185.

****