Short Communication

SYNERGISTIC EFFECT OF *RHIZOBIUM* AND AM FUNGI INTERACTION ON PHOTOSYNTHESIS, ROOT PHOSPHATASE ACTIVITY AND GRAIN QUALITY IN URD BEAN (*VIGNA MUNGO* (L.) HEPPER) UNDER RAINFED FIELD CONDITIONS

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Abstract : Two varieties of urd bean (PU-35, T-9) inoculated with *Rhizobium* and vesicular arbuscular mycorrhiza fungi (applied through layering technique) were raised under field conditions. The synergistic effect was noticed with the combined treatment over any of the bacteria or AM fungi, in terms of chlorophyll content, photosynthetic rate and root phosphatase activity. The interaction enhanced the dry matter production, grain yield and quality also. The carbohydrate, fat and protein content also increased in the *Rhizobium* inoculated seeds; however, it was higher when *Rhizobium* and mycorrhiza fungi were combined together. *Rhizobium* inoculation enhanced the nitrogen content in grain and straw where as *Rhizobium* + AM treated plants had enhanced phosphatase activity and nitrogen content.

Keywords : AM fungi, Photosynthetic rate, *Rhizobium*, Root Phosphatase activity and grain quality, Urd bean

INTRODUCTION

Legumes have unique quality in term of nodulation and biological nitrogen fixation for sustainable crop productivity with improved soil health and soil fertility. It is widely recognized that biological nitrogen fixation by legume-*Rhizobium* synthesis is an important component of productivity in tropical agriculture where it is grown in farm land that are marginal either in terms of distance from the market or small farm size and poverty of the farmers (Giller, 2001). The symbiotic association between leguminous plants and species of *Rhizobium* has been well known (Shiv Kumar and Shiva Dhar, 2008) through the process of biological nitrogen fixation. Now-a-days mycorrhizal roots are known to extract more nutrients from the nutrient deficient soils than are non-mycorrhizal roots because hyphae exploit greater volume of soil than root alone. The use of AM fungi increases the uptake of P as well as micronutrients particularly Zn, Cu and Mn (Pandey *et al.,* 2006, Panwar and Thakur 1995) and had been reported to enhance water extraction from the deeper zone of the soil (Marschner *et al.,* 2006, Panwar, 1993).

The combined inoculation of *Rhizobium* and AM fungi may be beneficial for plant growth and development. How does it affect the physiological processes and seed quality was the main objective of this study and hence was undertaken under rain fed field condition.

MATERIAL AND METHOD

The seeds of two varieties of urd bean (PU-35 and T-9) were obtained from the National Seed Corporation, Delhi and the *Rhizobium* culture and Arbuscular Mycorrhiza fungi (*Glomus sps*) were obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi. Before sowing, the seeds of both the varieties were inoculated with *Rhizobium* culture using jaggary solution and dried under shade except control and AM treated (alone) plants. The AM fungus was applied through layering technique. All the package and practices were followed for successful rising of the crop under field conditions. The data was collected on three representative plants and for grain quality tests; the harvested seeds from different treatments in all the three replications were collected. The chlorophyll content in leaves was measured using the DMSO (Dimethyl Sulphoxide) as per procedure followed by Hiscox and Israelstam (1979) and Photosynthetic rate was measured by LI-6200 portable photosynthesis system containing LI-6250 analyzer. The activities of root acid phosphatase enzyme were measured following the modified procedure as described by Tabatabai (1994) (Amaya-Carpio, *et al.,* 2009) based on the hydrolysis of p-nitro phenyl phosphate substrate to yield p-nitro phenol and inorganic phosphate. The protein content in seed was estimated following the procedure as adapted by Jackson (1958). Fat was estimated through cold percolation method (Kartha and Sethi, 1957) and carbohydrate content for reducing sugar was followed by Hodge and Hofreiter (1962) and non-reducing sugar through Nelson method (1944). The statistical analysis was done by adopting the Analysis of Variance as described by Panse and Sukhatme (1967).

RESULT AND DISCUSSION

The two varieties of urd bean grown under rain fed condition following the *Rhizobium*, AM either alone or combined inoculation enhanced the leaf area per plant. The leaf area at 60 Days stage was more in T-9 as compared to PU-35. The *Rhizobium* and AM treated plants attained more leaf area, however, it was significantly higher with combined inoculation

in both the varieties (Table 1). T-9 has higher leaf chlorophyll content as compared to PU-35 but *Rhizobium* inoculation either alone or with AM had significantly higher chlorophyll content than AM alone or untreated control. The plants supplied with AM fungi had higher chlorophyll content than control in both the varieties.

The net photosynthetic rate as measured by Infrared Gas Analyzer (IRGA), it was found that variety PU-35 had attained higher photosynthetic rate at 60 DAS as compared to T-9. The *Rhizobium* treated plants either alone or with AM maintained higher photosynthetic rate than AM treated plants and untreated control. However, the AM treated plants maintained significantly higher photosynthetic rate than control plants. The total biomass production was higher in variety T-9, than PU-35. The combined inoculation of *Rhizobium* and AM has significantly higher biomass over *Rhizobium*/AM treated plants as single inoculation, there was not much significant difference were not observed between *Rhizobium* and AM treated plants but were significantly higher over untreated control in both the varieties. The grain yield was significantly higher in variety T-9 as compared to PU-35. Though, *Rhizobium* and AM treated plants attained higher grain yield over untreated control but the synergistic effect on yield was noticed with combined inoculation. The harvest index was significantly higher in *Rhizobium* and AM treated plants either alone or in combination that was significantly higher over uninoculated controls in both the varieties. Our results are in conformity with the earlier reports for enhancing photosynthetic rate (Sinha *et al.,* 1988, Cooper, 1984, Thakur, 1994 in mung bean),the combined inoculation of *Rhizobium* and AM inoculation in pigeon pea in presence of Rock phosphate (Jain *et al.,* 2008), Panwar and Thakur , 1995 in mungbean, Hodge, 2000, Srivastava *et al.,* 1998, in pea. Under rainfed conditions the hyphae of AM fungi extract more moisture and different nutrients like P, Zn, Fe and Cu (Thakur and Panwar, 1997) helped in maintaining the water status of the plant as well the *Rhizobium* fix nitrogen and made it available to the plant during later stages of growth and grain development has helped in terms of enhanced leaf area, photosynthetic rate, chlorophyll content for better growth and development. The enhanced root phosphatase activity has also helped in mitigating the energy requirement of the plant. The combined inoculation of AM and *Rhizobium* has not only helped the yielding potential of the plants, but also enhanced the quality of the grains.

It can be seen from Table-2 that the AM fungi either alone or with *Rhizobium* enhanced the phosphorus uptake measured in terms of root phosphatase activities in both the varieties and has significantly higher values than *Rhizobium* alone or untreated control. Radersma and Grierson, 2004, and Marschner *et al.,* 2006, had shown that the AM treated plants revealed the increase in the root phosphatase activity under P deficient soil in wheat, rye and triticale and a significant positive correlation between root phosphatase activity and root, shoot and total dry matter production (Pandey, 2006).

The grain analysis revealed that the N and protein content (%) was more in T-9 as compared to PU-35 and combined inoculation of *Rhizobium* and AM had significantly higher N and protein content than uninoculated control. There was marginal increase in the fat and carbohydrate content but it was superior in the dual inoculation as compared to either of the inoculants which was significantly higher over uninoculated control. Our results are in accordance to the earlier reports, Jain *et al.,* 2007 and 2008 in mung bean, Jain *et al.,* (2008) in pigeon pea. It may be concluded that the *Rhizobium* and AM has shown higher leaf area, chlorophyll content, net photosynthesis resulting higher biomass in both the varieties, but the combined (dual) inoculation was superior to either of the inoculated plants in both the varieties. The combined inoculation not only enhanced the physiological processes and root phosphatase activity but improves the grain yield and quality also in urdbean. The application of *Rhizobium* along with AM fungi will enable the farmers to have better yield advantage with economical and ecofriendly cropping system under rainfed conditions.

Table 1: Effect of Rhizobium and AM inoculation on leaf area, chlorophyll content,net photosynthetic rate and yield in two different varieties of urd bean

Variety	Treatment	Leaf Area per Plant (cm^2) 60DAS	Chlorophyll Content(mg/g m fresh weight) 60 DAS	Net Photosynthetic Rate (µ.moles/m^2 s) 60DAS	Yield per Plant (g) at harvest	Harvest Index (%)
PU-35	**Controlled**	374.71	1.111	7.00	2.41	27.10
	Rhizobium	539.12	1.199	8.32	3.26	31.46
	AM	635.63	1.166	7.24	3.55	32.06
	***Rhiz.*+AM**	840.61	1.489	12.14	4.24	32.02
T-9	**Controlled**	459.39	1.361	4.91	2.65	29.23
	Rhizobium	655.75	1.557	6.94	3.72	31.80

	AM	885.32	1.495	4.45	3.90	31.77
	***Rhiz.*+AM**	946.61	1.643	9.11	4.81	32.35
CD at 5%						
Variety		21.31	0.0600	0.2784	0.080	0.2510
Treatment		30.13	0.0848	0.3936	0.112	0.2880
VxT		42.62	N.S.	0.557	0.159	0.3780

Table 2: Effect of Rhizobium and AM inoculation on root phosphatase activity and protein, fat and arbohydrate content in the seeds of two different varieties of urd bean.

Variety	**Treatment**	**Root Phosphatase activity (m.mole.kg^{-1} FM)**	**Grain Nitrogen content per plant(%)**	**Protein content(%)**	**Carbohydrate content(%)**	**Fat content (%)**
PU-35	**Controlled**	2.600	2.74	16.77	52.11	1.53
	Rhizobium	2.503	2.94	18.19	53.06	1.66
	AM	3.377	2.88	18.62	52.97	1.69
	***Rhiz.*+AM**	3.853	3.08	19.85	54.73	1.77
T-9	**Controlled**	2.800	2.83	17.68	52.33	1.53
	Rhizobium	2.677	2.96	18.47	53.68	1.59
	AM	3.640	3.07	19.18	54.34	1.61
	***Rhiz.*+AM**	3.813	3.27	20.31	55.16	1.660
C.D. at 5%						
	Variety	0.0185	0.0826	0.5164	0.4294	0.0116
	Treatment	0.0261	0.1165	0.7538	0.6073	0.0165
	V x T	0.0370	0.1650	N.S.	N.S.	0.0230

REFERENCES

Amaya-Carpio, L., Daries, F.T., Jr., Fox, T. and He, C. (2009). Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition and growth of *Ipomoea carnea* ssp. fistulosa. *Photosynthe* **47**(1):1-10.

Cooper, K.M. (1984). Physiology of VA-Mycorrhizal Association *pp 155-203. [In] VA mycorrhiza. Ed. C.L. Powell, D.J., Bagyaraj; pp. 113-130. Boca Roton, Fla: CRC Press,London.*

Giller, K.E. (2001). Nitrogen fixation in Tropical Cropping System. *2nd edition, CABI publishing. pp. 423.*

Ghosh, G. and Poi, S.C.(1998). Response of *Rhizobium* ,PSB and mycorrhizal organisms on some legume crops. *Environ Ecol.***16**(3):607-610.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57** (7-12); 1332-1334.

Hodge, A. (2000). Microbial ecology of arbuscular mycorrhiza. *FEMS. Microbiology Ecology.* **32**:91-96.

Hodge, J.E. and Hofreiter, B.T. (1962). In : Method in Carbohydrate Chemistry (Ed. Whister, RL. and Bemiller, J.N.) *Academic Press, New York.*

Jain, A.K., Kumar, Sudhir and Panwar, J.D.S. (2007). Response of mung bean (*Vigna radiata*) to phosphorus and micronutrients on N and P uptake and seed quality. *Legume Res.* **30**(3):201-204.

Jain, A.K., Sudhir Kumar, O.P. Singh, J.D.S. Panwar and Vinod Kumar (2008). Studies in relation to *Rhizobium* and vesicular arbuscular mycorrhizal (VAM) inoculation with phosphorus in a Green Gram (*Vigna radiata*). *Plant Archives. Vol.* **8**. No.2, pp 935-936.

Jain, A.K., Sudhir Kumar and J.D.S. Panwar. (2008). Role of vesicular arbuscular mycorrhiza (VAM) and Rock phosphate in pigeon pea. *Ad. Plant Sci.***21**(11):411-412.

Jackson, M.L. (1958). Soil Chemical Analysis, *Prantice Hall, Englewood Cliffs, N.J., USA.*

Kartha, A.R.S. and Sethi, A.S. (1957). Cold percolation method for oil estimation in seeds. *Indian J. Agril. Sci*, **27**:211.

Marschner, P., Solaiman, Z. and Rengel, Z. (2006). Rhizosphere properties of Poaceae genotypes under P-limiting condition. *Plant Soil.***107**: 11-18.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153**:375-380

Panday, R. (2006). Root-exuded acid phosphatase and ^{32}Pi-uptake kinetics of wheat, rye and triticale

under phosphorus starvation. *Journal Nuclear Agric. Biol.* **35** (3-4): 168-179.

Pandey, R., Singh, B. and Nair, T.V.R. (2006). Effect of arbuscular mycorrhizal inoculation in low phosphorus soil in relation to P urtilization efficiency of wheat genotypes. *India J. Agric. Sci.* **76**(6): 349-353.

Panse, V.G. and P.V. Sukhatme. (1967). In "Statistical methods for agricultural workers", *Ed. Jaiswal, P.L., ICAR, New Delhi.*

Panwar. J.D.S. (1993). Response of VAM and *Azospirillum* inoculation to water status and grain yield in wheat under water stress condition. *Indian J. Plant Physiol.*, **36** (1):41-43.

Panwar, J.D.S. and Thakur, A.K. (1995). Physiological and biochemical studies in vesicular arbuscular mycorrhizae-*Rhizobium* inoculated mungbean plants under field condition. Proc Mycorrhizae biofertilizers for the future.*Tata Energy Res. Institute. pp 472-477.*

Radersma, S. and Grierson, P.F. (2004). Phosphorus mobilization in Agro forestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. *Plant Soil.* **259**:209-219.

Shiva Kumar, B.G. and Shiva Dhar. (2008). Microbial Transformation of nitrogen in Rhizosphere and its impact on soil fertility *[In]*. *Rhizosphere: strategies for augumenting soil fertility and productivity. Division of Agronomy, IARI (Eds.) Shiva Dhar; Majaiah, K.M., Annapurna, K. and R.K. Rai. pp. 56-63.*

Sinha, S. K., Bhargava, S. C. and Baldev, B. (1988). Physiological aspects of pulse crops. [In]. *Pulse Crops. Eds. Baldev, B., Ramanujam, S. P. and Jain, H. K. Pub. Oxford and IBH, New Delhi.*

Srivastava, T.K., Ahalawat, I.P.S. and Panwar, J.D.S. (1998). Effect of phosphorus, molybdenum and biofertilizers on productivity of Pea (*Pisum sativum* L). *Ind.J.Pl.Physiol.* **3**(3):237-239.July-Sep.1998.

Tabatabai, M. A. (1994). *Soil Enzymes.[In]: Methods in soil Analysis.Part 2(ed).Weaver,R. V.,Angle, J. S., Botomley, P.S. pp.775-833.Madison.*

Thakur, A.K. (1994). Physiological studies on *Rhizobium*- VAM symbionts in mung bean. *M.Sc. Thesis P.G. School, IARI, New Delhi.*

Thakur, A.K. and Panwar, J.D.S. (1997). Response of *Rhizobium* – vesicular arbuscular mycorrhizal symbionts on photosynthesis, nitrogen metabolism and sucrose translocation in green gram (*Phaseolus radiatus*).Indian J.Agric Science.**67**(6):245-248.June, 1997.