



## EFFECT OF DUAL INOCULATION OF ARBUSCULAR MYCORRHIZAL FUNGUS AND RHIZOBIUM ON CHLOROPHYLL CONTENT OF PIGEON PEA [*CAJANUS CAJAN* (L.) MILL SP.]

HC Lakshman<sup>1</sup>, T Yeasmin<sup>2</sup> and J Puttaradder\*<sup>1</sup>

<sup>1</sup>Microbiology Laboratory, P.G. Department of Studies in Botany, Karnataka University, India

<sup>2</sup>Institute of Biological Sciences, University of Rajshahi, Bangladesh

Soil microorganisms are known to regulate mycorrhizal formation and function conversely. Mycorrhiza affects the establishment of rhizosphere population. Some interaction between mycorrhizae and soil microorganisms involve nutrient recycling. Hence, they have an impact on plant and nutrient (Kapoor and Mukerji 1998). The symbiotic relation between arbuscular mycorrhizal (AM) fungi and the host plants has been studied traditionally in terms of benefits to the individual plant and fungi (Smith and Smith 1996, Lakshman 1999). AM fungal association can affect the host plants in terms of stomata movement to increase chlorophyll content and the rate of transpiration and photosynthesis (Panwar 1991, Bheemareddy and Lakshman 2011). Mycorrhizal colonization is of particular value to legumes because it can increase the phosphorus uptake: nodulation and symbiotic nitrogen fixation by rhizobial require adequate supply of phosphorus, and restricted root system leads to poor competition for soil phosphorus (Carling et al. 1978, Bagyaraj 2006, Lakshman 2009).

Pigeon pea (*Cajanus cajan* L.) is probably a native of tropical Africa, and was introduced perhaps 3,000 years ago into India. It is chiefly grown in Madhya Pradesh, Bihar, Andhra Pradesh, Maharashtra, Uttar Pradesh, and Karnataka. Both the immature and ripen fruits are used for human food as a good source of protein. The leaves and twigs are used as fodder. The pericarp and husk, separated in threshing are used as cattle feed. The enzyme urease, obtained from it, is required for estimation of urea in blood, urine etc. Livestock and poultry are very much fond of it. It is chiefly consumed in South Indian homes. In fact, research on chlorophyll content of this plant with inoculation of AM fungus (*Glomus macrocarpum*) and *Rhizobium leguminosorum* is very meager, therefore the present study was undertaken. Seeds of Pigeon pea *Cajanus cajan* Mill sp. were obtained from the seed bank unit of University of Agricultural Science Dharwad – 580005, in Karnataka state. Only healthy seeds were selected for study. Seedlings were raised in earthen pots measuring 20 × 25 cm (length × breadth) diameter containing 4 kg sterile mixture sand and soil in equal proportion. Before sowing the seeds of Pigeon pea were inoculated with AM fungus *G. macrocarpum* (15 g) dry mixed inoculum was placed as a thin layer, just below 4cm soil surface of experimental pots. One seedling was left per pot and without inoculation as control. Leaves were collected for analyses of chlorophyll -A and chlorophyll -B after 30, 60, 90 days of inoculation. The chlorophyll content was estimated following the procedure of Arnan (1949). All the experiments were carried out in triplicate under green house condition.

---

\*Author for correspondence: j.puttaradder@gmail.com

**Table 1.** Showing the effect of AM fungus and *Rhizobium* on chlorophyll content of pigeon pea at different intervals of time (30, 60 and 90 days) in unsterile soil.

Treatment	Chlorophyll A (mg/g)/ Day			Chlorophyll B (mg)/ Day			Total Chlorophyll (mg/g)/ Day		
	30	60	90	30	60	90	30	60	90
Control	0.631	0.723	0.871	0.584	0.689	0.717	1.215	1.412	1.588
<i>Glomus macrocarpum</i>	0.891	0.930	1.124	0.721	0.749	0.803	1.612	1.679	1.927
<i>Rhizobium leguminosorum</i>	0.862	0.894	1.015	0.645	0.734	0.811	1.507	1.628	1.826
G. ma + Rh	0.941	1.842	2.019	0.672	0.739	0.823	2.783	2.581	2.842

**Table 2.** Showing the effect of AM fungus and *Rhizobium* on chlorophyll content of pigeon pea at different intervals of time (30, 60 and 90 days) in sterile soil.

Treatment	Chlorophyll A (mg)/ Day			Chlorophyll B (mg) Day			Total Chlorophyll (mg/g)/ Day		
	30	60	90	30	60	90	30	60	90
Control	0.631	0.721	0.783	0.489	0.593	0.616	1.12	1.31	1.40
<i>Glomus macrocarpum</i>	0.841	0.924	1.116	0.521	0.633	0.782	1.37	1.55	1.90
<i>Rhizobium leguminosorum</i>	0.852	0.896	1.014	0.508	0.612	0.774	1.36	1.51	1.79
G. ma + Rh	0.937	1.841	2.115	0.678	0.738	0.823	1.61	2.52	2.94

G. ma- *Glomus macrocarpum*, Rh- *Rhizobium*

The analysis of chlorophyll A, B, and total chlorophyll content of leaf revealed a significant variation, due to Pigeon pea plants inoculated with AM fungus (*Glomus macrocarpum*) alone or in combination with *Rhizobium*. The total chlorophyll content was highest in dual inoculated plants grown in sterilized soil than that of plants grown in unsterile soil (Table 1 and 2). Such an increase might be due to transpiration or increased growth (Hayman 1983, Sampathkumar and Ganeshkumar 2003) or due to the presence of a large number of chlorophyll in the bundle sheath of inoculated leaves (Krishna and Bagyaraj 1984, Rajashekharan and Nagarajan 2005). Our study is par with earlier studies of other workers (Bhavani et al. 1998, Katiyar et al. 1998, Baqual et al. 2005, Rajashekharan and Nagarajan 2005), that chlorophyll content is higher in the leaves of bio inoculants inoculated plants compare to noninoculated (Control) plants, as biochemical characters like phenols, proteins and chlorophylls may play a vital role in making plants resistant to

pathogens. Similar reports are also available that the AM fungi association with the N- fixing bacteria can increase the N-fixing capacity of many crop plants, mainly legumes (Patterson et al. 1990).

## References

- Arnon DI (1949). Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1-15.
- Bagyaraj DJ (2006). Arbuscular mycorrhizal fungi in sustainable agriculture. In: *Techniques in Mycorrhizae* Eds. Bukhari, MJ and BF Rodrigues: 1-8.
- Baqual MF, Das PJ and Katiyar RS (2005). Effect of arbuscular mycorrhizal fungi and other microbial inoculants on chlorophyll content of mulberry (*Morus* spp.). Mycorrhiza News 17(3): 12-14.
- Bheemareddy VS, and Lakshman HC (2011). Effect of AM fungus *Glomus fasciculatum* on the accumulation of metabolites in four varieties of *Triticum aestivum* under short term water stress. Vegetos 24(1): 41-49.
- Carling DE, Richle NE and Johnson DR (1978). Effect of VAM on nitrate reductase and nitrogenase activity in nodulation and non-nodulating soybean. Phytopathology 68: 1590-1596.
- Hayman DS (1983). *The physiology of VA endomycorrhizal symbiosis*. Canadian Journal of Botany 61: 944-963.
- Kapoor R and Mukerji KG (1998). Microbial interactions in mycorrhizosphere of *Anethum graveolens* L. Phytomorphology 48: 323-325.
- Katiyar RS, Das PK and Choudhury PC (1998). VA- Mycorrhizal inoculation of established mulberry garden through maize (*Zea mays*) intercropping: An effective technique. National Conference on Mriculture: Physiological, Biochemical and Molecular Aspects of Stress Tolerance in Mulberry, Trichy, 854 pp.
- Krishna KR and Bagyaraj DJ (1984). Growth and nutrient uptake of peanut inoculated with mycorrhizal fungus *Glomus fasciculatum* compared with uninoculated ones. Plant and Soil 17: 405-408.
- Lakshman HC (1999). Dual inoculation of VAM and *Rhizobium* is beneficial to *Petrocarpus marsupium* Roxb., timber tree species. Ecology Environment and Conservation 5(2): 133-136.
- Lakshman HC (2009). Growth response and nitrogen fixation of *Phaseolus lunatus* (Lima bean) with the inoculation of AM fungi and *Rhizobium*. Asian Sciences 4: 37-41.
- Panwar JDS (1991). Effect of VAM and *Azospirillum brasilense* on Photosynthesis, nitrogen metabolism and grain yield in wheat. Indian Journal of Plant Physiology 34: 357-361.
- Patterson NA, Cheit I and Kapulink Y (1990). Effect of Mycorrhizal inoculation on nodule initiation activity and contribution to legume productivity symbiosis. Annals of Biology 8: 9-20.
- Rajashekharan S and Nagarajan SM (2005). Effect of dual inoculation (AM fungi and *Rhizobium*) on chlorophyll content of *Vigna unguiculata* (L). Walp. Var. Pusa 151. Mycorrhiza News 17(1): 10-11.
- Sampathkumar G and Ganeshkumar A (2003). Effect of AM Fungi and *Rhizobium* on Growth and Nutrition of *Vigna mungo* L. and *Vigna unguiculata* L., Mycorrhiza News 14(4): 15-18.
- Smith FA and Smith SE (1996). Mutualism and parasitism: diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. Advanced Botany Research 22: 1-43.
- Venkatasubbaiah BV, Rao S and Saigopal DVR (1998). Studies on mosaic diseases of sunflower biochemical changes and growth response. Indian Phytopath 51: 357-358.

