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Response of Arbuscular mycorrhizal fungi and *Rhizobium* inoculation on growth and chlorophyll content of *Vigna unguiculata* (L) Walp Var. Pusa 151

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ABSTRACT: The aim of the present study was to investigate the effect of *Rhizobium* and Arbuscular mycorrhizal fungi inoculation, both individually and in combination on growth and chlorophyll content of economically important plant *Vigna unguiculata* L. A significant ($p < 0.05$) increase over control in root length (45.6 cm), shoot height (12.2 cm), dry weight of root (0.4 g) and shoot (1.8 g), total number of nodules (39.6 nos.), dry weight of nodules (0.5 g), percentage of mycorrhizal infection (96.6 %), chlorophylls *a* (0.83 mg/g fr.wt.), *b* (1.19 mg/g fr.wt) and total chlorophyll (2.24 mg/g fr.wt) was recorded in dual inoculated (AM fungi and *Rhizobium*) plants than plants with individual inoculation. Thus it is clear that the dual inoculation with AM fungi and *Rhizobium* biofertilizer is more effective than the individual treatment. @JASEM

The indiscriminate use of chemical fertilizer /pesticides leads to disastrous consequences of environmental quality, promoting the study of natural sources of fertilizer, bio-stimulants and soil amendments. Organic wastes and biofertilizers are the alternative sources to meet the nutrient requirement of crops. Arbuscular Mycorrhizal (AM) fungi are found in many soils around the w orld, and they form association with 80% of all terrestrial plant roots (Harley and Harley, 1987). The beneficial effects of AM fungi symbiotic association on the growth of plants are well known (Powell and Bagyaraj, 1984; Safir, 1987; Smith and Smith, 1996; Rajasekaran and Nagarajan, 2004; Francis and Read, 1995). AM fungi helps in water regulation of plants by extending their hyphae towards the available moisture zone for continuous water absorption and translocating them to plants. AM association can affect the host plants in terms of stomatal movement and photosynthesis of leaves and has been shown to increase the rate of transpiration, photosynthesis and chlorophyll content (Panwar, 1991; Bethlenfalvay *et al*., 1988).

Legumes are important pulse cultivated throughout Indian subcontinent, cost effective cultivation of this agricultural crops depends on effective symbiotic nitrogen fixation in their nodules that relies on the rhizobial populations of the soil (Postgate, 1982; Thevanathan, 1980; Bhavani, 1983). Mycorrhizal infection has particular value for legumes because nodulation and symbiotic nitrogen fixation by rhizobia require an adequate phosphorus supply and restricted root system leads to poor competition for soil phosphorus (Carling *et al*., 1978). *Vigna unguiculata* (L) is commonly called as cowpea, a climbing bushy annual, usually cultivated for its beneficial fruits and for soil reclamation. In the light of these, the present investigation was undertaken to study the dual inoculation of *Rhizobium* and AM fungi on the growth, nutrition and chlorophyll content of *Vigna unguiculata*.

MATERIALS AND METHODS

The present study was conducted during January 2005 in the Dept. of Botany, A.V.C. College, Mannampandal, Mayiladuthurai, Tamil Nadu, India. The seeds of *Vigna unguiculata* (L) Walp var. Pusa 151 (Cowpea) were obtained from the Agriculture Research Station, Vamban, Pudukkotai D istrict, Tamilnadu, India. Seeds free from visible defects and uniform size were surface sterilized and sown in circular earthen pots (40 cm height and 30 cm diameter) filled with a mixture of sterile garden soil and sand mixture at the ratio of 2:1 (v/v). Seeds were inoculated individually with *Rhizobium* and AM fungi (applied as layering on soil surface) and combination of both. One pot of seedlings was left without inoculation as control. The plants were irrigated with tap water as and when required. The leaves were collected from each replication for sampling on 15th, 30th, and 45th day after sowing and estimation of chlorophyll content was done following the method of Arnon (1949). Plant shoot and root length, dry weight of shoot and root, total number of nodules per plant, dry weight of nodules per plant and root colonization of AM was calculated at 45th day. Root colonization of AM fungi was estimated by tryphan blue technique (Phillips and Hayman, 1970). Data were analysed statistically using Student 't' test. All the measurements were carried out in triplicates and the results were expressed in the average of the three replication.

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RESULTS AND DISCUSSION

The plants inoculated either with *Rhizobium* or AM fungi significantly increased the shoot length and root length, dry weight of shoot and root, total number of nodules and dry weight of nodules when compare to control. The dual inoculation of AM fungi and *Rhizobium* showed maximum values in all the tested parameter than plants inoculated with individual endophytes (Table 1). Fewer nodules with increased biomass were formed in dual inoculated plants, compared to plants inoculated individually with *Rhizobium* and AM fingi and uninoculated control plants. This contradicts other reports, where more nodules are reported on dual inoculation than non-mycorrhial plants (Asimi *et al*., 1980; Hazarika *et al*., 2000). However Patterson *et al*, (1990) reported AM fungi due to indirect response of the host plants affected a similar observation in the lower parts of the roots.

The plants inoculated with individual treatment of AM fungi and dual inoculation only shows positive for AM fungi colonization in roots. The *Rhizobium* inoculated plants and uninoculated control plants do not have colonization. The high frequency of 96% of infection was observed in roots of dual inoculated plants roots and 91% in AM fungi inoculated plants roots (Table 1).

Table: 1. Effect of AM fungi and *Rhizobium* on growth, nodulation and mycorrhizal effect of *V. unguiculata*.

	Control	*Rhizobium*	AM fungi	AM fungi + *Rhizobium*
Shoot length (cm)	27.6±0.7	30.9±0.9*	41.0±1.1*	45.6±1.1*
Root length (cm)	8.3±0.3	9.5±0.4*	11.6±0.8*	12.2±0.5*
Dry weight of shoot(g)	1.4±0.02	1.6±0.05*	1.7±0.02*	1.8±0.02*
Dry weight of root(g)	0.3±0.01	0.4±0.01*	0.4±0.01*	0.4±0.01*
Total number of nodules/plant	1.3±0.02	32.6±2.5*	2.5±0.5	39.6±1.5*
Dry weight of nodules (g)	0.02±0.01	0.3±0.01*	0.02±0.01	0.5±0.01*
Mycorrhizal effect (%)	-	-	92.0±1.7	96.6±2.1

* Significant at $p < 0.05$

Plants inoculated with AM fungi, either alone or in combination with *Rhizobium*, brought about significant changes in chlorophyll a, b and total chlorophyll content. The maximum total chlorophyll content was noticed at 45th days old sampling leaves in dual inoculated plants followed by individual inoculation of AM fungi and *Rhizobium* (Table 2). A considerable increase in chlorophyll content of AM fungi and *Rhizobium* inoculated tissue of cowpea is in agreement with results reported elsewhere (Hayman, 1983). This increase may be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Hayman, 1983; Sampath and Ganesh, 2003; Rajasekaran *et al*., 2006) or due to the presence of large and more numerous bundle sheath chloroplasts in the inoculated leaves (Krishna and Bagyaraj, 1984).

Table: 2. Effect of AM fungi and *Rhizobium* on chlorophyll (mg/g fr.wt.) content of *V. unguiculata*.

	Control			*Rhizobium*			AM fungi			AM fungi + *Rhizobium*		
	Days after inoculation			Days after inoculation			Days after inoculation			Days after inoculation		
	15	30	45	15	30	45	15	30	45	15	30	45
Chl 'a'	0.63± 0.04	0.74± 0.03	0.83± 0.03	0.73± 0.02*	0.78± 0.02*	0.99± 0.02*	0.65± 0.02	0.83± 0.04*	0.90± 0.02*	0.79± 0.02*	0.93± 0.02*	1.09±0.02*
Chl 'b'	1.06± 0.02	1.13± 0.01	1.19± 0.02	1.12± 0.02*	1.22± 0.01*	1.34± 0.02*	1.15± 0.03*	1.26± 0.01*	1.22± 0.01*	1.23± 0.01*	1.26± 0.02*	1.65±0.02*
Total Chl	2.07± 0.02	2.16± 0.02	2.24± 0.02	2.15± 0.01*	2.32± 0.02*	2.43± 0.03*	2.17± 0.01*	2.35± 0.01*	2.28± 0.02*	2.28± 0.02*	2.35± 0.01*	2.73±0.04*

* Significant at $p < 0.05$

There are several reports indicates that the chlorophyll content was higher in the leaves of resistance varieties than those of susceptible varieties, as biochemical characters like phenol, proteins and chlorophyll may play a vital role in making plants resistant to pathogens (Bhavani, 1983; Rajasekaran and Nagarajan, 2005; Charitha and Reddy, 2001). It is reported that use of AM fungi inoculants saving fertilizer phosphorus to a tune of 25-30 kg P2 O5/ ha. Besides supplying p to plants, these fungi also serve as biocontrol agents against certain soil-borne plant pathogens and can survive under inhospitable soil environments. Thus the present study clearly reveals that the dual inoculation with AM fungi and *Rhizobium* biofertilizer is more effective in increasing growth, nutrition, chlorophyll content and biomass production of legumes. It is also essential to study the possibility of inoculating the legume crops with selected strains of AM fungi and *Rhizobium* to for higher pulse production.

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