EVALUATION OF ARBUSCULAR MYCORRHIZAL INTERACTIONS WITH *VIGNA CATJANG WALP.*

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ABSTRACT : The mutually beneficial relation between feeder roots of plants and fungi is called 'Mycorrhiza'. The term 'Mycorrhiza' was coined by Frank in 1885 to describe symbiotic association of plant roots and fungi. The word 'Mycorrhiza' originate from two greek words 'Mycos' meaning fungus and rhiza meaning root. Arbuscular mycorrhizae [AM] are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that association with AM fungi facilitates better nutrient uptake enhancing plant growth. Hence to exploit these biological tools, pot experiments were carried out and response on growth and yield of Vigna catjang Walp. was studied.AM inoculums brought from Tamil Nadu Agricultural university containing the mixture of Glomus species was directly used as an inoculum to study the effect of AM on Vigna catjang Walp. Pot experiments were conducted in mixture of sterilized garden soil and sterilized sand in the ratio 3:1. The experiment was conducted with AM [treated] and non AM [control] plants of Vigna catjang Walp. Soluble protein content, alpha amino nitrogen content, nitrate content and nitrate reductase activity in the leaves of treated and control plants of Vigna catjang Walp. were estimated at an interval of 15 days after sowing the seeds [DAS], 30DAS, 45DAS and at 60DAS. The association of AM fungi enhances the growth in all the treated plants. The significantly high growth rate and yield was observed in treated plants than control plants. Significantly higher amount of soluble protein content, alpha amino nitrogen content and nitrate reductase activity was observed in the leaves of treated plants of Vigna catjang Walp. than that in control ones while nitrate content was found to be more in the leaves of control plants.

Keywords : Mycorrhiza, Plant root, Symbiotic, Vigna

INTRODUCTION:

Arbuscular mycorrhizae [AM] are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that association with AM fungi facilitates better nutrient uptake enhancing plant growth. The association of AM fungi enhances the ability of leguminous plants to withstand the various stresses to some extent. When the nutrient uptake levels and growth rate were estimated in AM and control leguminous plants in drought and saline stresses, the AM associated leguminous plants showed more growth rate and nutrient levels than the ones without AM association. It was found that percentage variation in growth rate [i.e. root and shoot length and root and shoot dry weight] and nutrient uptake in leguminous plants under drought and different levels of salinity stress condition were directly proportional to the percentage of mycorrhization [Kumar and Muraleedhara, *2003]*.

MATERIALS AND METHODS :

AM inoculum AM inoculums was brought from Tamil Nadu Agricultural university which contained the mixture of *Glomus* species was directly used as an inoculum to study the effect of AM on *Vigna catjang* Walp.

Preparation of control and treated pots Twelve large sized plastic pots with holes at the bottom having an internal diameter of 18 cm were used for the experiment of which six were maintained as control and six were used for treatment with 'Mycorrhiza'.Garden soil was obtained in bulk from nursery suppliers. Similarly sand was procured from sea shore and was washed thoroughly in running water for several hours to remove soluble salts. Both garden soil and sand were mixed in proportion of 3:1 by volume in large trays. Sand help in improving aeration in pot and thereby help AM fungi to grow as mycorrhizae are aerobic microorganisms.This soil sand mixture was sterilized at a temperature of 200^0c for 2 hours in hot air oven, to kill soil microorganisms and insects. This sterilized mixture was used as a growth medium for pot experiments.Out of 12 pots six were maintained as treated and remaining six as control. Initially ¾th part of each pot was filled up with sterilized soil mixture. 10 g of AM inoculums was added to each treated pot the inoculum was distributed evenly in the pot and was covered with a layer of 4 cm. of sterilized soil mixture. Twelve water soaked seeds were sown in each pot and covered with a layer of soil. The pots were watered with watering can having small pores to avoid the disturbance of the soil surface.

Following physiological parameters from the leaves of the plants of *Vigna catjang Walp*., both control and treated were studied.

1) Soluble protein content
2) Alpha amino nitrogen content
3) Nitrate content,
4) Nitrate reductase activity.

Soluble protein contents in the fresh leaf material were analyzed by the method of Lowry *et al.,*[1951].

Alpha amino nitrogen was estimated in the fresh leaf material by the method of Moore and Stein [1948].

Nitrate content of fresh leaves was estimated by the method of Johnson and Ulrich, [1950].

The *in vivo* assay of nitrate reductase [NR] activity in the leaves was carried out according to the method of Kleeper *et al*. [1971].

All the parameters were studied on 15th, 30th, 45th and 60th day after sowing the seeds. The roots of *Vigna catjang Walp*. were screened to obtain percentage of AM colonization at 15, 30, 45 and 60 DAS. Isolation and quantification of spores from rhizosphere soil of *Vigna catjang Walp*. was also carried out before sowing the seeds and at 60 DAS.Screening of the roots was carried out to study the per cent of root association by AM fungi in treated pots by the method described by Grace and Stribley [1991]. The percent of root infection was calculated by using Nicolson's formula [1955].

Table 1 -Soluble protein content in the leaves of treated and control plants of *VignacatjangWalp*. [mg per 100mg fresh leaf]

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	1.430	2.49	4.106	3.153
Control	0.790	1.576	2.75	2.016
Calculated 't'	3.242	2.840	4.643	3.919
Level of significance	++	+	+++	++
Standard error (S.E.)	± 0.197	± 0.321	± 0.291	± 0.289

Table 2 -Alpha amino nitrogen content in the leaves of treated and control plants of *VignacatjangWalp*. [mg / 100 mg fresh leaf.]

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	0.12	0.17	0.256	0.130
Control	0.066	0.106	0.17	0.096
Calculated 't'	4.929	5.303	5.206	3.199
Level of significance	+++	+++	+++	++
Standard error (S.E.)	± 0.0109	± 0.0120	± 0.0165	± 0.0106

Table 3 - Nitrate content in the leaves of treated and control plants of *VignacatjangWalp* [mg per 100 mg fresh leaf]

	15 DAS	30 DAS	45 DAS	60 DAS
Treated	0.064	0.080	0.068	0.048
Control	0.052	0.056	0.088	0.064
Calculated 't'	0.961	2.130	2.248	1.350
Level of significance	0	0	+	0
Standard error (S.E.)	± 0.0124	± 0.0112	± 0.0088	± 0.0118

Table 4 Nitrate reductase activity in the leaves of treated and control plants of *VignacatjangWalp*. [NR activity is expressed as micromole nitrate g^{-1} fresh leaf $hour^{-1}$.]

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	3.0	1.35	3.475	1.625
Control	2.7	2.375	2.475	0.8
Calculated 't'	0.807	4.276	3.063	2.845
Level of significance	0	++	+	+
Standard error (S.E.)	± 0.371	± 0.239	± 0.326	± 0.289

Level of significance 'o' = Difference of mean not significant, '+' = Difference of mean significant (P=0.05), '++' = Difference of mean significant (P=0.01), '+++' = Difference of mean significant (P=0.001), DAS = Days after sowing. Each value is a mean of six replicates

Table 5 Results of the screening of roots of *VignacatjangWalp*. to obtain percentage of AM colonization

Days after sowing	% colonization of AM roots	AM structures observed in the root cortex
15	19	Mycelium
30	48	Mycelium + vesicles + Spores
45	79	Mycelium + vesicles + Spores
60	94	Mycelium + vesicles + Spores

DISCUSSIONS :

As percentage of AM colonization increases in root system of AM plants, the soluble protein content and *Alpha* amino nitrogen content of leaves also increases. At 60 DAS both soluble protein content and *Alpha* amino nitrogen content of the leaves shows reduction in both AM and non AM plants. This may be due to the utilization of these nutrients by plants for flowering and fruiting. At 15 DAS the nitrate content of leaves of AM associated *Vigna catjang Walp*. plant was less than that in the leaves of non AM plants but, no significant difference was observed in two values. Nitrate reductase activity was significantly high in AM plants than non AM plants at 15 DAS. At 30 DAS, nitrate content in leaves of AM plants was significantly higher than that in the leaves of non AM plants. NR activity at this stage was significantly higher in non AM plants than that in AM plants. As NR activity is high, utilization of nitrate is also higher. Again at 45 DAS and at 60 DAS nitrate content of leaves of AM plants was less than that of leaves of non AM plants. This can be correlated with high NR activity in AM plants than that in non AM plants at this stage. More nitrate might have been utilized for flowering and fruiting in AM plants.

Lactuca sativa (lettuce) when inoculated with *Glomus fasciculatum* showed increased growth, nitrate reductase activity and protein content compared to that of non AM plants. [Azcon*et al.,* 1996].

Cliquet and Stewart [1993] have reported higher amino acid concentration in AM inoculated Maize roots as compared to that of non AM inoculated maize roots. Major component of the free amino acid pool were glycine, glutamic acid, alanine, serine, aspargine and 4-amino-n-butyric acid.

AM inoculated *Ziziphus mauritiana* showed increase in soluble protein concentration in both roots and shoots. Different AM species varied in their efficacy to increase soluble protein concentration in both the organs. *Glomus fasciculatum* can increase soluble protein concentration most efficiently in both the organs. [Mathur and Vyas, 1995].

The effect of VAM fungus *Glomus fasciculatum* on growth and nitrogen assimilation was measured on *Allium cepa* grown under drought conditions. Under water limitations, the effectiveness of *Glomus fasciculatum* to increase nitrate reductase activity in plant was enhanced. The proportion of nitrate assimilation into root was increased in VAM plants. AM plants reached a high specific and total Glutamine synthetase activity in shoots and roots. AM plants can utilize nitrate form more efficiently than ammonium form. Lettuce, when inoculated with AM fungi, *Glomus deserticola, Glomus fasciculatum or Glomus mosseae* showed higher nitrate reductase activity than the plants not inoculated with AM fungi, particularly under water stress conditions. Control plants had 57 per cent less nitrate reductase activity than that in *Glomus deserticola* colonized plants under well watered conditions, with a reduction in nitrate reductase activity by 79 per cent when the plants were subjected to drought stress. It was suggested that either the AM fungi increased the nitrate reductase activity in the host plant or that AM fungi have enzymatic activity per se. Drought stress decreased the nitrate reductase activity but the decrease was less in AM inoculated plants [Singh, 2007].

AM inoculated *Vignasinensis*L.,when grown in saline soil, showed significant increase in nitrogen content. In this case also the nitrogen uptake was directly propotional to the percentage of mycorrhization [Kumar and Muraleedhara, 2003]. Increased nitrate reductase activity has also been reported in mycorhiza inoculated plants [Manoharachary*et al*., 2009].

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