RESEARCH ARTICLE

Effect of Arbuscular Mycorrhizal interactions on chlorophyll content and per cent productivity of *Pisum sativum* L.

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Ayare Komal S and Golatkar VV (2015) Effect of Arbuscular Mycorrhizal interactions on chlorophyll content and per cent productivity of *Pisum sativum* L., *Int. J. of Life Sciences,* Special Issue, A5: 27-31.

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 *Pisum sativum* L. 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 *Pisum sativum* L. 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 *Pisum sativum* L. Soluble protein content, alpha amino nitrogen content, nitrate content and nitrate reductase activity in the leaves of treated and control plants of *Pisum sativum* L. 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, nitrate content and nitrate reductase activity was observed in the leaves of treated plants of *Pisum sativum* L. than that in control ones.

Key word: Arbuscular mycorrhizae (AM), chlorophyll content, *Pisum sativum* L, symbiotic association, growth rate, *Rhizobium,*

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*).* Am symbiosis can affect stomatal behaviour and photosynthesis of host leaves and have been shown to increase both transpirational and photosynthetic rates as well as chlorophyll concentration (Devi and Reddy, 2004). They observed that innoculation with AM fungus alone or in combination with *Rhizobium* brought about significant increase in chlorophyll "a", chlorophyll "b" and total chlorophyll content in ground nut, thereby increasing the rate of photosynthesis.

MATERIALS AND METHODS

AM inoculums

AM inoculums was brought from Tamil Nadu Agricultural university which contained the mixture of *Glomus* species (*Glomus fasciculatum, Glomus aggregatum, Glomus multicaule, Glomus dimorphicum, Glomus microcarpum*, etc) was directly used as an inoculum to study the effect of AM on *Pisum sativum* L.

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 *Pisum sativum* L., both control and treated were studied.

1)Chlorophyll content
2) Phosphate content
3) Per cent productivity.

The method of Arnon (1949) was used for estimating the chlorophyll content of the leaves.
Phosphate in the oven dry leaf material was extracted by the method of wet digestion byJackson, (1967).

All the parameters were studied on 15th, 30th, 45th and 60th day after sowing the seeds. The roots of *Pisum sativum* L. were screened to obtain percentage of AM colonization at 15, 30, 45 and

60 DAS. Isolation and quantification of spores from rhizosphere soil of *Pisum sativum* L. 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).

RESULTS AND DISCUSSION

Chlorophyll content, phosphate phosphorus content in the in the leaves of treated plants of *Pisum sativum* L. was higher than the control ones throughout the period of experiment and per cent productivity of the treated plants was higher than the control ones.

Table 1: Chlrophyll 'a' content in the leaves of treated and control plants of *Pisum sativum* L. (mg per 100 g fresh leaf)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	9.276	9.732	9.651	9.305
Control	9.001	9.629	9.531	9.182
Calculated 't'	13.75	6.058	8.571	12.3
Level of significance	+++	+++	+++	+++
Standard error (S.E.)	± 0.02	± 0.017	± 0.014	± 0.01

Table 2 –Chlorophyll 'b' content in the leaves of treated and control plants of *Pisum sativum* L. (mg / 100 g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	9.518	10.807	10.674	9.626
Control	9.239	10.605	10.344	9.414
Calculated 't'	3.065	9.181	13.75	10.6
Level of significance	+	+++	+++	+++
Standard error (S.E.)	± 0.091	± 0.022	± 0.024	± 0.02

Table 3: Total chlorophyll content in the leaves of treated and control plants of *Pisum sativum* L. (mg / 100 g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	23.029	20.169	16.691	15.736
Control	20.831	18.958	15.855	15.489
Calculated 't'	6.820	6.465	4.146	2.523
Level of significance	+++	+++	+++	+
Standard error (S.E.)	± 3.220	± 1.871	± 2.016	± 0.977

Table 4: Phosphate content in the leaves of treated and control plants of *Pisum sativum* L. (mg / g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	0.625	1.325	1.666	0.791
Control	0.466	0.533	1.158	0.583
Calculated 't'	4.009	8.020	5.907	2.518
Level of significance	++	+++	+++	+
Standard error (S.E.)	± 0.0392	± 0.0980	± 0.085	± 0.0274

Table 5 –Analysis of fruits of treated and control plants of *Pisum sativum* L.

	Fruit length in cm.	**No. of seeds**	**Fresh wt. of seed**	**Dry wt. of seed**	**Per cent of production**
Treated	6.533	2.166	0.687	0.181	71.11%
Control	4.466	1.166	0.250	0.071	53.333%
Calculated 't'	3.256	2.301	2.364	2.322	2.410%
Level of significance	++	+	+	+	+
Standard error (S.E.)	± 0.634	± 0.433	± 0.184	± 0.0473	± 7.379

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 6 – Results of the screening of roots of *Pisum sativum* L. to obtain percentage of AM colonization

Days after sowing	**% colonization of AM roots**	**AM structures observed in the root cortex**
15	16	Mycelium
30	45	Mycelium + vesicles
45	72	Mycelium + vesicles
60	87	Mycelium + vesicles + spores

Before sowing the seeds 10 g of AM inoculums were found to contain 57 AM spores while at 60 DAS it showed presence of around 78 spores. The results are tabulated in following tables.

Chlorophyll chlorophyll 'b' and total chlorophyll content in terms of mg per 100 g fresh leaf, has seen to be significantly higher in the leaves of treated plants than that in the control ones throughout the period of experiment.

Similar results has been reported by Devi and Reddy (2004) while working with *Arachis hypogaea* L., incoulation with AM fungus, either alone or in combination with *Rhizobium,* brought about significant increase in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. This increase may be due to the increase in stomatal conductance, photosynthesis, transpiration, enhanced plant growth (Levi and Krikun, 1980;) or due to the presence of larger and more numerous bundle sheath chloroplasts present in AM inoculated leaves (Krishna and Bagyaraj, 1984).

Growth of the mycorrhizal plant is usually high when the potential for active photosynthesis is high. The high rate of photosynthesis by mycorrhizal plants may be evoked by a number of changes such as an increase in plant hormones (Miller, 1971). Stomatal opening, enhanced ion transport, and regulation of chlorophyll level (Johnson, 1984). Increased chlorophyll accumulation was observed in AM inoculated papaya plant as compared to that in control papaya plants by Shivaputra *et al*. (2004). Higher phosphorus levels in tissues as a result of root colonization by the AM can be expected to increase the chlorophyll content, as phosphorus is one of the important component of chlorophyll. Increased uptake of nitrogen, phosphorus, potassium, copper, manganese, iron and zinc and increased tolerance to biotic stresses may be contributed to the production of more leaves and greater leaf area, and thereby higher chlorophyll, higher photosynthetic capacity even during reproductive phase and translocation of carbohydrates from other plant parts to reproductive parts might have resulted in increased yield and yield attributes (Sabarad *et al*., 2007).

Khare *et al*. (2008) working with *Cyamopsis tetragonoloba* observed that absorption of phosphorus and its supply to the root system of the AM plants is a major contribution of AM fungi. Mycorrhizal inoculation also enhances magnesium uptake and reduces sodium concentration in plants. This in turn helps in increasing the chlorophyll content and improves the overall growth performance of mycorrhizal plants.

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