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Response of *Solanum melongena* L. to Inoculation with Arbuscular Mycorrhizal Fungi under Low and High Phosphate Condition

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Abstract

Solanum melongena L. a medicinally and economically important crop plants were grown in polythene bags. The effect of mycorrhizal inoculation (*Glomus mosseae*) and increasing phosphate levels on the expression of the photosynthetic activity in terms of chlorophyll content. Antioxidant enzymes like peroxidase, polyphenol oxidase, root acid and alkaline phosphatase activity of *Solanum melongena* were evaluated. The experimental design was entirely at CRBD with eight treatments with three levels of phosphate (50,100,150 mg kg^{-1} of soil). Root colonization ranged from 50.33% to 67.33% . The activity of the studied antioxidant enzymes were found to be increased in arbuscular mycorrhizal (AM) *Solanum* plants. Root phosphatase activity was greater in 100 and 150 mg phosphate level in AM treated than non AM treated *Solanum* plants. Besides, only AM treated plants of *Solanum* reflected increase in total chlorophyll content as compared to non AM plants. This work suggests that the mycorrhiza helps *Solanum* plants to perform better in low and high phosphate level by enhancing antioxidant enzyme activity, acid and alkaline phosphatase activity and total chlorophyll content.

Keywords: antioxidant enzymes, chlorophyll, *Glomus mosseae*, root phosphatase, *Solanum melongena*

Introduction

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) fungi (*Phylum Glomeromycota*). The mutually beneficial relationship between feeder roots of plants and fungi is called mycorrhiza. Mycorrhizae are active living components of the soil and supply the basic enzymatic machinery for absorption, translocation and assimilating major mineral ions like phosphate and inorganic nitrogen required for symbiosis while as the plant provides the peculiar ecological niche that is necessary for fungal growth and development (Martin and Hilbert, 1991).

Phosphorous is one of the essential mineral elements that frequently limits plant growth and is taken in the form of inorganic orthophosphate (Pi) by plants (Miyasaka and Habte, 2001). Pi is the only form directly accessible to plants. Plant roots colonized with AMF hyphae can utilize source of P in soil that are not readily available to non AMF roots because the fungal hyphae have an enhanced ability to mineralize organic P (Joner and Johansen, 2000; Koide and Kabir, 2000; Feng *et al*., 2003).

Several groups of enzymes that may hydrolyze phosphorous esters are commonly called Phosphatase (Tabatabai and Bremner, 1969), which are directly involved in the acquisition of phosphorous by plants. They are present in mature arbuscules and intraradical hyphae of AM fungi (Ezawa *et al*., 1995). It has been shown that colonization of rape and onion by *Glomus geosporum, Glomus mosseae* and *Glomus monosporum* resulted in significant increase in root acid phosphate activity (Dodd *et al*., 1987). Similarly colonization of *Carica papaya* L. by *Glomus mosseae* showed enhanced root surface alkaline phosphatase activity by 25-114%.

Thus the objectives of this study was to investigate the role of AM fungal species *Glomus mosseae* (*Gm*) under three phosphate levels and its effect on antioxidant enzymes, phosphatase enzyme activity and total chlorophyll content in *Solanum melongena* L. as compared to non treated control ones.

Material and methods

Plant material and experimental site

The experiment was conducted in polythene bags (12x14") in Botany department, University of Pune, Pune - 411007. The seeds of *Solanum melongena* L. local variety Kantakari were obtained from Agricultural College Pune, Maharashtra, India. Seeds were germinated in sterilized soil (121°C and 103.42 k pa pressure for 1 h) garden sandy loamy soil. One month old seedlings were transplanted to pots containing 8 kg of autoclaved soil. Three month old, 30 g of *Glomus mosseae* containing AM colonized roots, rhizospere soil having extramatrical mycelium and spores (10-15) was taken as inoculum.

Experimental design

The experimental design consisted of Completely Randomized Block design with eight treatments having four mycorrhizal and four non mycorrhizal control ones. K_2HPO_4 was used as source of phosphate which was given after one month of AM inoculation and it was given weekly till

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the last observation was taken. The control plants were untreated. Plants were harvested after 60, 90 and 120 days of AM inoculation.

Rhizosphere soil analysis

Before the beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. The rhizosphere soil samples (100 gram) of garden soil used for the experiment was collected from a depth of 10-15 cm, which was air dried at room temperature and further analyzed for the determination of pH (pH meter), EC (conductivity meter Elico), mineral constituents like organic carbon, P_20_5, K_2O, Cu, Zn, Mn, Fe (Rao, 1993), on AAS (Perkin- Elmer 3100, USA). Details of soil properties are shown in Tab. 1.

AM colonization

The percentage of AM colonization in roots was analyzed by clearing and staining of roots by Phillips and Haymans (1970) method and percent AM colonization in root was determined by Gridline intersect method Giovannetti and Mosse (1980).

Antioxidant enzyme activity assay

Enzyme extracts were prepared in extraction buffer containing 0.1 M phosphate buffer (pH 7) by grinding 0.5 mg leaf or root sample in extraction buffer. Extracts were centrifuged at 18,000×g at 5°C for 15 minutes (POD) and 16,000×g for 10 minutes (PPO) and the supernatant was used as source of enzymes. The POD activity was assayed by Putter's (1974), method and expressed as M Kat gm^{-1} fresh weight. The PPO activity was assayed by Mahadevan and Shidhar (1982), method and expressed as ΔO.D min^{-1} gram of fresh weight.

Physiological parameter

The total leaf chlorophyll content was measured by the method of Arnon (1949). The amount of chlorophyll present in the extract mg chlorophyll per gm tissue was calculated using the following equations:

mg chlorophyll g-1 tissue=20.2(A645)+8.029(A663) *V/1000*W

where A=Absorbance at specific wavelengths, V=final volume of chlorophyll extract in 80% acetone, W=fresh weight of tissue extracted.

Phosphatase activity assay

Enzyme extracts were prepared in extraction buffer containing 50 mM Citrate buffer (pH 5.3) for acid and 50 mM Glycine NaoH buffer (pH 10.3) for alkaline phosphatase by grinding 1 gram root samples in 10 mL extraction buffer. Extracts were centrifuged at 10000×g for 10 min and the supernatant was used as source of enzyme. Phosphatase activity was determined by Lowry *et al*. (1954) method and was expressed in moles of PNP released g^{-1} fresh weight of roots.

Statistical analysis

Data were analyzed by One Way ANOVA followed by Duncan's multiple new range test (DMRT) at $p \leq 0.05$ level.

Results and discussion

As shown in Fig. 1a, after 60 days of AM inoculation, the mycorrhizal *Solanum* plants showed 25.00% of AM colonization, at second phosphate level the AM colonization was higher (34.67%) than first and third level of phosphate (31.33%, 31.67%). After 90 days, AM colonization increased with increasing level of phosphate but after 120 days of AM inoculation at second phosphate level *Solanum* AM plants showed 78.33% AM colonization which was higher than first and third level of phosphate. That means at second phosphate level, the percent of root colonization increased and at third level it was found to be inhibited. Similar results were obtained by Amijee *et al.* (1989), Martenson and Carlgen (1994). It is believed that a high soil phosphate level reduces both intra as well as extraradical AM development and thus inhibit AM colonization (Abbott and Robson, 1984; Liu *et al*., 2000).

As shown in Tab. 2 after 60 days of AM inoculation, the POD activity was higher in AM inoculated *Solanum* plants as compared to non mycorrhizal *Solanum* plants at first, second and third phosphate level. After 90 and 120 days of AM inoculation, POD activity was found to reduce in latter stages of AM colonization in all levels of phosphate. As shown in Tab. 3, PPO activity increased significantly in mycorrhizal *Solanum* plants than non mycorrhizal *Solanum* ones. PPO activity was higher in early stages of AM colonization i.e, after 60 days and later on the PPO activity was found to decline after 90 and 120 days of AM colonization in all treatments. In initial stages of AM fungal colonization, the enzyme activities were maximum but decreased in highly colonized roots. The increased activities may be due to the contribution by fungus or due to the biochemical activities as a result of infection or both. It may also be attributed to protect the plant from oxidative stresses or it may be due to the influence of AM

Tab. 1. Physical and chemical soil properties of experimental site

Physical	Depth	Sand	Silt	Clay					
	10-15cm	80%	15%	5%					
Chemical	Depth	pH	EC	OC	P_2O_5	Zn	Fe	Cu	Mn
	10-15cm	7.8	0.1 mmho/m	0.52%	12 kg/acre	80 kg/acre	3.7 ppm	11.00 ppm	31.0 Ppm

EC: electrical conductivity, OC: organic carbon, P_2O_5: phosphorous oxide, Zn: zinc, Fe: iron, Cu: copper, Mn: manganese

Tab. 2. Peroxidase activity in *Solanum melongena* L. inoculated with *G. mosseae* after 60, 90 and 120 days of AM inoculation

Peroxidase activity M Kat gm^{-1} fresh weight			
Treatments	60 days	90 days	120 days
C	0.0121±0.0022d	0.0084±0.0001d	0.0025±0.0019d
C+1P	0.0121±0.0032d	0.0089±0.0021d	0.0032±0.0001d
C+2P	0.0130±0.0023d	0.0106±0.0002cd	0.0046±0.0001d
C+3P	0.0162±0.0020d	0.0147±0.0021bc	0.0084±0.0001c
Gm	0.0173±0.0000cd	0.0149±0.0021b	0.0089±0.0021bc
Gm+1p	0.0202±0.0009bc	0.0167±0.0030b	0.0120±0.0001b
Gm+2p	0.0214±0.0009b	0.0173±0.0001ab	0.0167±0.0040a
Gm+3p	0.0247±0.0036a	0.0214±0.0009a	0.0173±0.0004a

Tab. 3. Polyphenol oxidase activity in *Solanum melongena* L. inoculated with *G. mosseae* after 60, 90 and 120 days of AM inoculation

Polyphenol oxidase activity Δ O.D $min^{-1}gm^{-1}$fresh weight			
Treatments	60 days	90 days	120 days
C	0.668±0.289d	0.585±0.433d	0.443±0.126d
C+IP	0.817±0.878cd	0.650±0.471c	0.502±0.144d
C+2P	0.846±0.382bc	0.679±0.520c	0.585±0.433d
C+3P	0.870±0.670bc	0.796±0.027bc	0.650±0.471c
Gm	1.276±0.804abc	1.193±0.866bc	0.679±0.520bc
Gm+1P	1.341±0.289abc	1.228±0.191ab	0.776±0.577ab
Gm+2P	1.556±0.382ab	1.341±0.289ab	0.876±0.607ab
Gm+3P	1.590±0.750a	1.473±0.901a	0.903±0.140a

on the composition of amino acids and carbohydrates in host plants. Similar results are reported by Pacovsky *et al*. (1990) in *Phaseolus vulgaris* infected with *G. etunicatum*.

The chlorophyll is the essential component for photosynthesis and it increases with mycorrhizal colonization (Colla *et al*., 2008). The results indicated that total chlorophyll content was found to increase in mycorrhizal *Solanum* plants in all treatments after 60 and 90 days of AM inoculation. But after 120 days at third level of phosphate mycorrhizal *Solanum* plants showed slight decrease in chlorophyll content as compared to first and second phosphate level (Fig. 1b). Thus AM symbiosis enhanced the chlorophyll content of *Solanum* leaves which was in agreement with the results of other studies (Elahi *et al*., 2010; Kapoor and Bhatnagar, 2007). At first and second phosphate levels, mycorrhizal *Solanum* plants showed more chlorophyll content that means mycorrhization or P fertilization influenced the concentration of photosynthetic pigments.

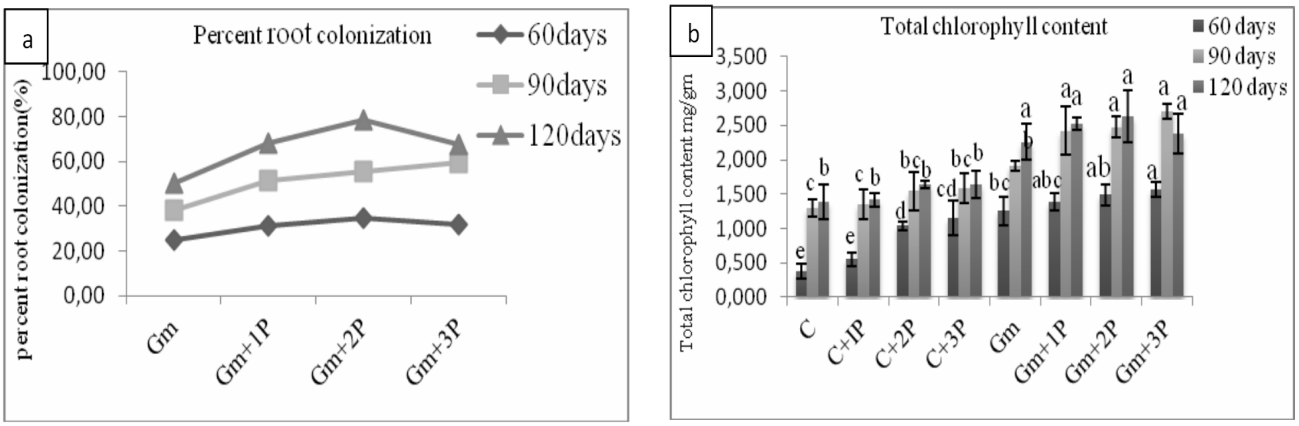

Fig. 1. Percentage of AM colonization and total chlorophyll content of *Solanum melongena* L. after 60, 90 and 120 days of AM treatment

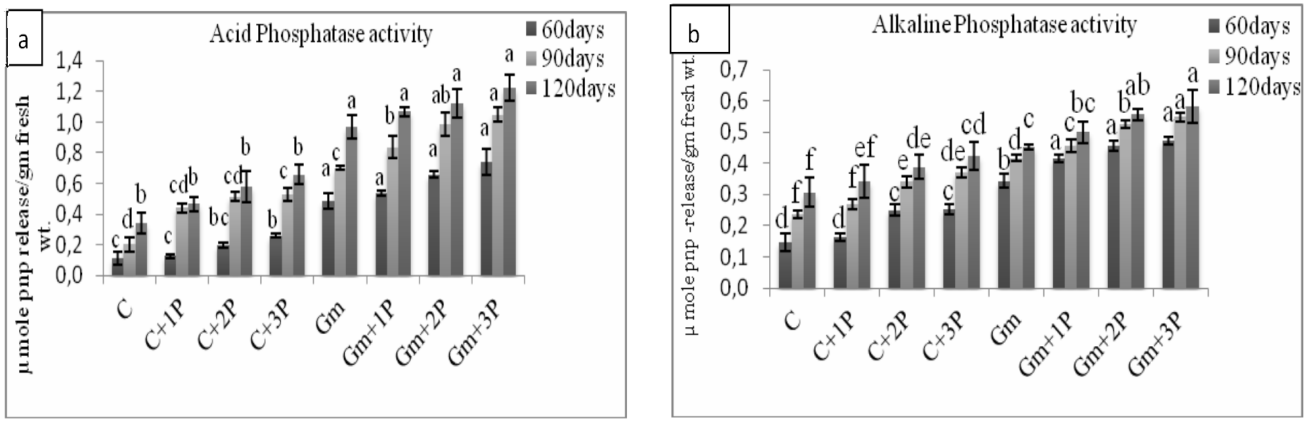

Fig. 2. Acid and alkaline phosphatase activity of *Solanum melongena* L. after 60, 90 and 120 days of AM treatment

In the present experiment after 60, 90 and 120 days of AM inoculation acid and alkaline phosphatase activity increased significantly with increasing phosphate levels which led to increase in phosphate accumulation by mycorrhizal *Solanum* plants through intraradical mycelium as previous studies have shown that intra radical mycelium have higher proportion of phosphatase active hyphae than extraradical hyphae. Thus the study revealed the fact that mycorrhizal *Solanum* plants showed enhanced acid and alkaline phosphatase activities in different phosphate levels which leads to higher uptake of phosphate from soil as shown in Fig. 2a and Fig. 2b. Similar results were reported by Garcia-Gomez *et al*. (2002), who observed higher acid phosphatase activity in *Glomus claroideum* inoculated *Carica papaya* L. plants. In the present experiment mycorrhizal *Solanum* plants showed significant higher phosphatase activity at all levels of phosphate, which suggests that acid phosphatase may be associated with the growth and development of the fungus within the host tissue (Gianiazzi *et al*., 1979), as well as with phosphorous acquisition in the rhizosphere whereas alkaline phosphatase is closely linked to both the mycorrhizal growth stimulation and the arbuscular phase of the colonization (Gianiazzi-Pearson and Gianiazzi, 1978).

Conclusions

In conclusion, the application of AM fungi may be helpful for plant survival in moderate phosphate levels and management of mycorrhizae in field condition through proper agricultural practices could reduce fertilizer application required for most economical yield. The results of this experiment showed that AM fungi helps the *Solanum* plants in increment of antioxidant enzyme activities which could help the plant to protect from oxidative effects of reactive oxygen species (ROS) and protect the plants from harmful effects of stress conditions. This study also states that AM fungi help the *Solanum* plants by increasing photosynthetic activity and root phosphatase activity by enhancing nutrient status.

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