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Effect of AM Fungi on Antioxidant Enzymes Activity in Tomato (*Solanum lycopersicum* L.)

Pushpa K. Kavatagi[1], H.C.Lakshman[2]

[1]Assistant Professor, Vijanagara College Hosapete, India
[2]Professor, Karnataka University Dharwad

Abstract

Arbuscular mycorrhizal fungi (AMF) have been used to enhance the plant growth and yield to maintain good health and fertility that contributes great extents to a sustainable product. The study was undertaken to identify the responses of antioxidant enzymes activities in tomato (*Solanum lycopersicum* L.Var PMK 1 and Vaibhav) inoculated with AM fungi. The Arbuscular mycorrhizal fungi (AMF) increased the antioxidant enzyme activities such as Superoxide dismutase (SOD) and Catalase (CAT) activity in roots of inoculated plants, indicating lower oxidative damage in the colonized plants. It can be concluded that the mycorrhiza helps tomato plants by enhancing the antioxidant activity compared to non-mycorrhizal plants. Therefore, inoculation of indigenous AM fungi is recommended at seedling stage.

Keywords: Arbuscular mycorrhizal fungi (AMF). *Solanum lycopersicum* L. Antioxidant. Peroxidase.

Introduction

Tomato is an important crop throughout the world and is grown under a wide range of production systems.In the areas with optimal climate for tomato cultivation. Tomato (*Solanum lycopersicum* L.) is a savory, typically red, edible fruits, as well as the plant which bears it, originating in South America. Oxygen free radicals induce damage due to peroxidation of biomembranes, which lead to tissue damage, thus cause occurrence of a number of diseases. Antioxidants neutralize the effect of free radicals through different ways and prevent the body from various diseases.

AM fungal symbiosis is complex biological interactions, their impact varies in different environmental conditions and depends on the specific combination of plant and fungus involved (Johnson et al., 1997; Burleigh et al., 2002; Smith et al., 2003). Consequently profitable use of AM symbioses in an agricultural context requires the selection of a suitable combination of plant host, fungal partner costs and benefits.

The induction of reactive-oxygen-species-scavenging enzymes, such as peroxidase (POX), detoxifying reactive-oxygen-species synthesized during stress responses (Mittler, 2002; Matsumura *et al.*, 2007). Arbuscular mycorrhizal fungi (AMF) are benefits to plants include improved mineral nutrition (Lakshman, 2010; Smith and Read, 1997).

Therefore purpose of the present study is to investigate the role of AM fungal species *Glomus fasciculatum* changes in physiological and biochemical (Antioxidant enzymes like CAT, POX and SOD) in Tomato (*Solanum lycopersicum* L.) as compared to non-treated control.

Materials and Methods

Pots experimental set up

Only two treatments were set as inoculated with *Glomus fasciculatum* (PMK-1 and Vaibhav) and non-inoculated control pots filled with 3kg of air-dried sterilized soil-sand (3:1 v/v) mixture before sowing seeds of *Solanum lycopersicum* L.,(Var PMK-1 and Vaibhav) a thin layer of inoculum *Glomus fasciculatum* were placed 2cm below the soil surface except in non-inoculated control pots. After a week, the germinated seedlings were made one plant per pot. The observations were recorded at a period of 45 days after planted. Root samples for each harvest were analyzed for the estimation of Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD) in the roots of *Solanum lycopersicum* L(Var. PMK 1 and Vaibhav). The roots were washed to remove the soil particles, cleaned and fresh roots were used for the assay.

Reagents and glass wares

EDTA, NBT (Nitro blue tetrazolium), Potassium phosphate buffer (PH 7.8), Riboflavin, Borosil pipettes and test tubes.

Assay of SOD

The activity of Superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of Nitro blue tetrazolium (NBT), according to Stewart and Bewley (1980). The reaction mixture (2ml) contained 0.1M EDTA, 1.5 mM. Nitro blue tetrazolium (NBT), 0.25ml of enzyme extract within 0.067M Potassium phosphate buffer (PH 7.8). The reaction was started with 0.12mM riboflavin by exposing the cuvette to a 15-W fluorescent tube for 10 min. the absorbance of each reaction mixture was measured at 560 nm using a UV-VIS spectrophotometer (Hitachi-Japan (U-3310) model). Results were calculated as a min^{-1} g^{-1} fresh weight during the first minute of the reaction.

Assay of CAT

Antioxidant activity was monitored using protocol set by Chance and Meahley (1955). The reaction mixture contained 1ml of potassium phosphate buffer (PH 7). 1.9ml of distilled water, 0.1ml of enzyme extract. The 1ml of 30% H_2O_2 was added to the previous mixture and immediately after adding H_2O_2 optical density was measured at 240 nm in spectrophotometer.

Poly Acrylamide Gel Electrophorosis(PAGE)

Reagents and glass wares:

- 30% acrylamide in 29.2g acrylmide 0.8g N, N’ methyle bis acrylamide solubilized in distilled water and made up to 100ml. filtered and stored in brown bottle.
- 1.5 M Tris-HCL, pH 6.8 and 8.8
- 10% Ammonium persulphate (APS)
- TEMED (N,N,N’,N’- tetramethyl ethylene diamine)
- Congo red
- Brosil beaker, pipettes and petriplate

Procedure

SOD and CAT activity was followed by the methods of Laemmli, U.K. (1970). Cleaned and dried grease free PAGE glass plates were assembled using spacer and clips. The bottom of the assembly was sealed using agar.

Prepared the separating and stalking following:

Contents	Volume separating Gel (5%, 10 ml)	Volume stalking Gel (4%, 5ml)
Distilled water	4.83	3.31
30% Acrylamide	1.33	1.0
1.5 M Tris-HCL Buffer	3.73 (pH 8.8)	0.630 (pH 6.8)
APS	0.100	0.05
TEMED	0.010	0.005

The stalking gel was casted on separating gel and immediately inserted comb. The gel was left for few min to polymerize and later removed the comb carefully. Assembled the gel into the PAGE unit and added electrophoresis buffer (Tris-3.g, Glycin 14.3 g, 0.1% carboxymethyl cellulose for 1 liter) into upper and lower tank. The gels were pre run with buffer for 1 h at 4^0 C. mixed the protein sample with sample buffer (1:1) (1.5 M Tris-HCL (pH 6.8) 0.625 ml, glycerol 1.0 ml, 0.2% bromophenol blue). The sample was loaded into the gel was run at constant current of 50-150 V for 3-4 h at 4^0 C. The gel was removed carefully and stained it for cellulose .The gel was inoculated in citrate phosphate buffer (50 mM, pH 5.0) for 13 h followed by incubation in 0.1% congo red for 1 h. The gel was destained with 1M NaCl.

The activities of the enzymes directly involved in AOS scavenging namely, SOD and CAT were analysed by SDS-PAGE in the same root extracts used for the above described spectrophotometric assays.

Results and observation

The SOD and CAT of *Solanum lycopersicum* L, varieties PMK-1 and Vaibhav inoculated with arbuscular mycorrhizal fungi and in the non-inoculated roots are shown (Table 1), SOD and CAT activity (Figure 1,2).

PMK-1

There was an increased SOD content in the inoculated *Glomus fasciculatum* (0.328mg/g) where as in non-inoculated control (0.211mg/g). The catalase activity were recovered higher inoculated with *Glomus fasciculatum* (0.49mg/g). In the non-inoculated control catalase activity were recovered lower (0.39 mg/g).

The enzyme was extracted from roots of *Solanum lycopersicum* L, and then analysed by SDS-PAGE. As visualized from SDS-PAGE intensity several bands of molecular weight 97, 66,43, 29, 20.1 and 14.3 kDa were screened against the standard protein markers. From staining it was cleared that the root part of *Solanum lycopersicum* L contained several molecular weight enzymes but the 97 kDa gave the thickest band comparing to other bands (Figure 2).

Vaibhav

There was an increased (0.50mg/g) SOD Content in the inoculated *Glomus fasciculatum* and in the non-inoculated control (0.31 mg/g) SOD. Increased in the (0.38 mg/g) CAT in the *Glomus fasciculatum* inoculated roots where as in non-inoculated control (0.21 mg/g) CAT.

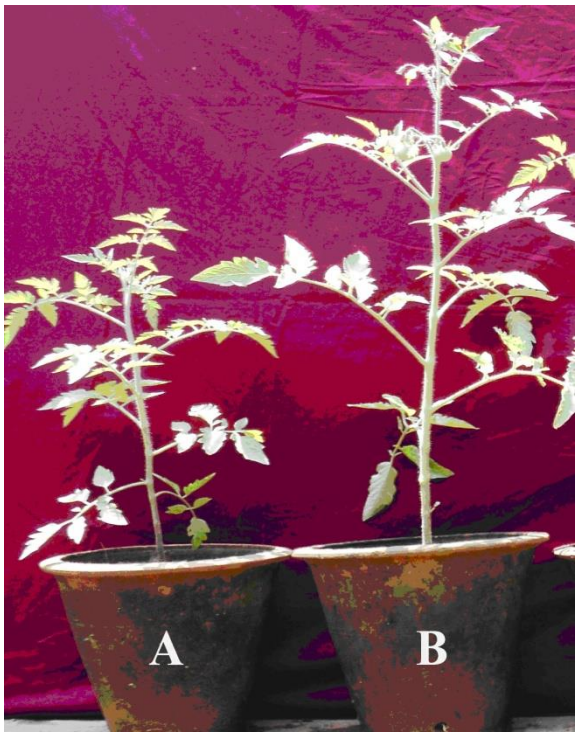
As visualized from SDS-PAGE intensity several protein bands of molecular weight 97, 66,43, 29, 20.1 and 14.3 kDa were screened against the standard protein markers. From the staining it was cleared that the root part of *Solanum lycopersicum* L (Var,Vaibhav) contained several molecular weight enzymes but the66 kDa gave the thickest band comparing to other bands (Figure 2).

Table1: Showing Superoxide dismutase (SOD) and Catalase (CAT) activities of *Solanum lycopersicum* L., varieties PMK-1 and Vaibhav.

Treatments	Superoxide dismutase (SOD) (µmoles/ml/min)	Catalase (CAT) (µmoles/ml/min)
PMK-1		
Noninoculated Control	0.16±0.01	0.32±0.01
Glomus fesciculatum	0.25±0.05	0.43±0.02
Vaibhav		
Noninoculated Control	0.32±0.01	0.21±0.01
Glomus fesciculatum	0.54±0.01	0.36±0.01

Figure 1: Showing non-inoculated and AMF inoculated of *Solanum lycopersicum* L., varieties (1) PMK 1 and (2) Vaibhav.

(1)



(2)

Figure 2: Showing the Enzyme Activity in non-inoculated control and AMF inoculated roots of *Solanum lycopersicum* L., varieties PMK-1 and Vaibhav.

A,B –Inoculated with *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend Walker & Koske., Superoxide dismutase (SOD) and Catalase (CAT) activities of *Solanum lycopersicum* L., varieties PMK-1.

C,D- Inoculated with *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend Walker & Koske., Superoxide dismutase (SOD) and Catalase (CAT) activities of *Solanum lycopersicum* L., varieties Vaibhav.

E,f,G,H- Non-inoculated control of *Solanum lycopersicum* L., varieties PMK-1 and Vaibhav.

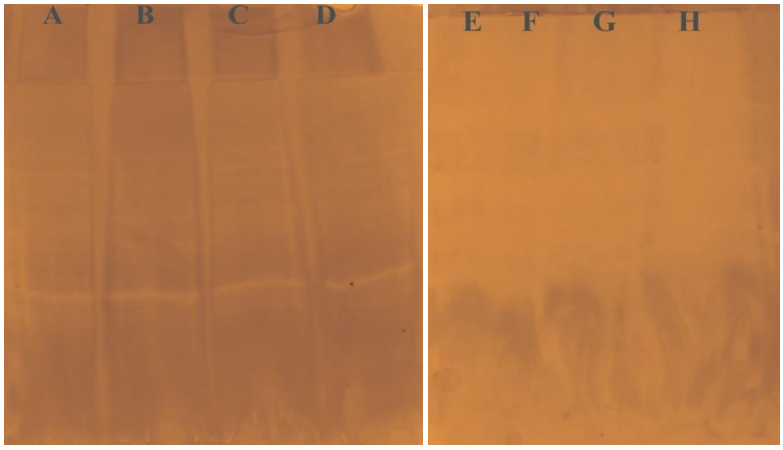
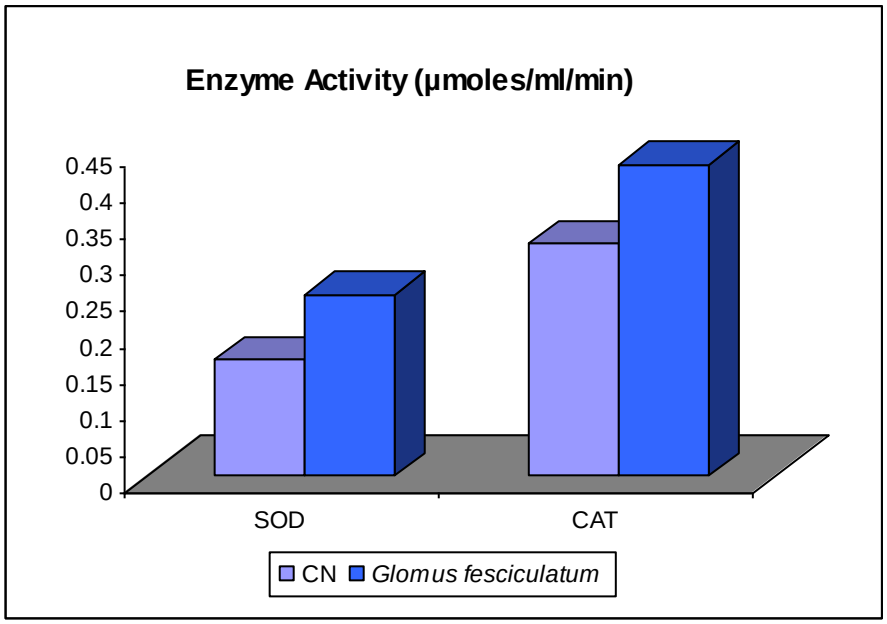
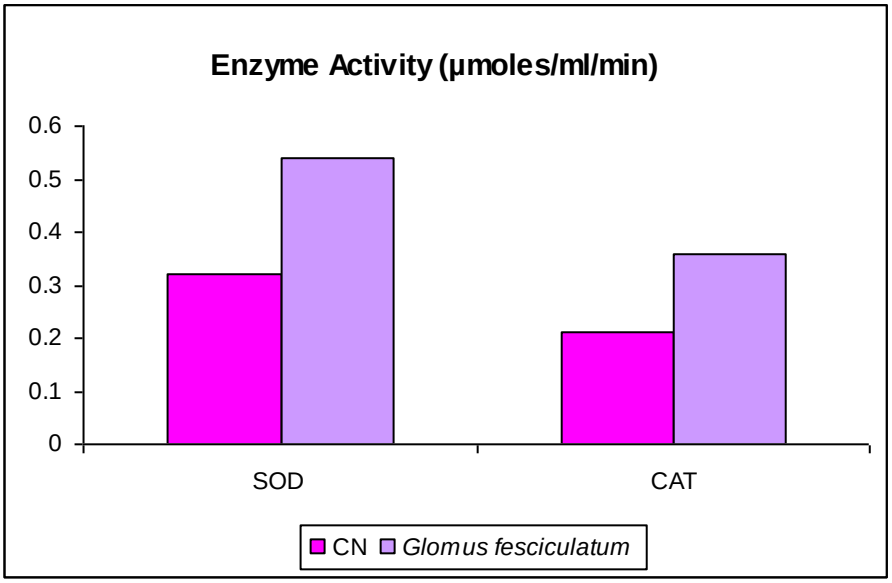


Figure 3: Showing the Superoxide dismutase (SOD) and Catalase (CAT) activities in noninoculated control and AMF inoculated roots of *Solanum lycopersicum* L., varieties (a) PMK-1 and (b) Vaibhav.



(a)



(b)

Discussion

The SOD content in PMK-1 and Vaibhav varieties increased in the inoculated arbuscular mycorrhizal fungi than non-inoculated control roots. The activities of antioxidant enzymes are usually stimulated on exposure to oxidative stress (Tanak, 1994). Acevedo *et al* (2001) showed that rapid and continued increased in CAT activity might indicate that CAT is a major enzyme detoxifying hydrogen peroxide in barley. SOD is the key enzyme in the active oxygen scavenger system and considered to be the first line of defense against ROS (Hamilton and Heckathorn, 2001). Previous studies show the positive effects of mycorrhiza on plant growth (Wu and Zou, 2010).

In most of the previous studies mycorrhizal application enhanced the antioxidant system (Ruiz-Lozano *et al.*, 1996). These findings are similar to our results. The antioxidant activity patterns studies were unspecific and showed variations with the colonization level of AMF strains. In summary the results of our study reinforce the idea that one of the services provided by AM symbiosis to the

ecosystem is the bioregucation of plant development and increase in plant quality for human health due to the ability of AMF for modifying plant metabolism and physiology (Gianinabzi *et al.*, 2010).

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