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Biochemical changes induced in tomato as a result of arbuscular mycorrhizal fungal colonization and tomato wilt pathogen infection

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ABSTRACT

A pot experiment was conducted in tomato to study the biochemical changes, the protective enzyme activities and disease resistance against the tomato wilt pathogen Fusarium oxysporum f. sp. lycopersici. Two dominant species of Arbuscular Mycorhizal (AM) fungi Glomus fasciculatum and Acaulospora laevis were isolated, mass multiplied and used for further studies. Tomato plants were grown in plastic pots filled with sterile soils and inoculated with AM fungi G. fasciculatum and A. laevis. There were six treatments along with the control. The effect of the interaction between the AM fungi and pathogen Fusarium oxysporum f. sp. lycopersici on tomato plants were monitored regularly. The morphological and biochemical modifications were observed in mycorrhizal, pathogen infected and mycorrhizal plants infected with pathogen and the results were compared with the control plants. Mycorrhizal colonization significantly increased the mineral nutrient concentration, chlorophyll, protein, amino acids, starch, sugars and phenolic content. Among the AMF, A. laevis proved to be the more effective strain compared to G. fasciculatum.

Key words: Arbuscular mycorrhizae, Tomato, *Fusarium* wilt, biocontrol, biochemical changes

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that predominate in the roots and soils of agricultural crop plants. The interaction between the AM fungi and other soil organisms are complex; they may be inhibitory or stimulatory. The AM fungi have been shown to promote plant growth mainly by enhancing nutrient acquisition[1]**.** Mycorrhizal fungi vary in their effect with host and environment and a specific species has to be prescribed according to host, soil type, location and pathogen. Arbuscular mycorrhizae is involved in the most universal intimate and important symbiosis[2]**.** Different mechanisms have been shown to play a role in plant protection by AM fungi namely: (i) enhancement of plant nutrition, (ii) competition with the pathogen for resources and space, (iii) plant morphological changes and barrier formation, (iv) changes in biochemical compounds related with plant response, (v) alleviation of physical stresses, and (vi) changes in antagonist and/ or deleterious microbe populations in the mycorrhizosphere[3]**.** Tomato is an important crop which is cultivated widely throughout the world and it is grown under a wide range of production system. It is rich in vitamins and is therefore used in salads, cooked as a vegetable or made into tomato paste and tomato sauce. Tomato consumption has been associated with decreased risk of breast cancer and might be strongly protective against neurodegenerative diseases[4,5,6] Tomato plants are affected by several diseases, including Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersic*i (Sacc.), a soil borne pathogen. This is a destructive disease of tomato worldwide[7]. It causes vascular wilt in tomato and even resistant varieties may be affected. The disease starts out as yellowing and drooping on one side of the plant. Leaf wilting, plant stunting, browning of the vascular system, leaf death, and lack of fruit production can also occur[8]**.**

MATERIALS AND METHODS

Soil

The experimental soil was sandy loam. It was autoclaved at 121^{o} C for 2h to eliminate naturally occurring endophytes. The same protocol was repeated twice on consecutive days and then mixed with sterile sand in the ratio of 2:1(v:v).

Mycorrhizal inoculums

The Arbuscular fungus *Glomus fasciculatum* and *Acaulospora laevis* isolated as described earlier [9] were maintained on onion (*Allium cepa* L.) to prepare pot cultures. The spores of the above AM fungi were inoculated into sand –soil mixture in polythene bags and were grown under controlled conditions. Two months after inoculation, the fibrous onion root were collected and mycorrhizal infection was assessed by modified clearing and staining technique[10]. Then the roots were chopped (2-3mm in length) and mixed with steam sterilized sand and loam soil. This mixture of soil, chlamydospores and segmented ,colonized roots was air- dried, packed in plastic bags, stored at 4^{o}C and used whenever required.

Maintenance of *Fusarium oxysporum* f. sp. *lycopersici (Sacc.)*

Fusarium oxysporum f. sp. *Lycopersic*i (Sacc.) was obtained from Dr. S. Beena, Professor Pathology Department, College of Horticulture, Thrissur, Kerala and then subcultured in synthetic nutrient agar which allowed sporulation of *Fusarium.*

Tomato Seeds

Seed of tomato variety Mukthi were obtained from Kerala Agricultural University, Kerala.

Experimental condition

Tomato (*Lycopersicon esculentum* Mill.) seeds were surface sterilized in 70% ethanol for 2 min followed by 2min in 0.6% mercuric chloride, rinsed three times in sterile distilled water, then sown in plastic pots (13cm diameter) containing potting mix and vermiculate (2:1).Two weeks after germination the seedlings were transplanted to 13cm diameter plastic pots. Each pot contained 1g of mycorrhizal inoculum(500 spores per 1 g soil) of either *G. fasciculatum* or *A. laevis.* Inoculum was distributed in one layer, 5cm, below the soil surface. The seedlings were placed 2.5cm above the mycorrhizal inoculum. Non mycorrhizal pots with one gram of non mycorrhizal onion roots served as control. Seedlings were watered with distilled water and fertilized with 50ml/week of 10%Hoaglands nutrient solution[11] without phosphorous beginning 30days after planting. One month after transplanting the plants were inoculated with a conidial suspension of *Fusarium oxysporum* f. sp. *lycopersic*i (25ml/pot), poured on the soil surface.

There were a total of six treatments which were as follows; *Glomus fasciculatum(GF), G. fasciculatum*+ pathogen (GF+P), *Acaulospora laevis* (AL), *A. laevis* + Pathogen(AL+P), Control(C) and Control+ Pathogen(C+P), All pots(5replicates per treatment) were grown under controlled conditions .After inoculating with pathogen the plants were covered with polythene bag in order to maintain humidity and to prevent contamination from other sources. Eight weeks after transplanting, four plants from each treatments were harvested, washed and were used in further experiments.

Experimental Assays

Biochemical parameters like total sugars[12], reducing sugars and non-reducing sugars[13], proteins[14]**,** starch[15] aminoacids[16]**,** peroxidase[17] and phenolic content[18] were determined in the root and leaf tissues.. The Phosphorus content was determined calorimetrically by Vandomolybdate method[19] and total nitrogen was also determined[20]**.** Total chlorophyll and carotenoid contents of the leaves were estimated according to Lichtenthaler[21]**.**

Extraction of free amino acids was performed by first homogenizing leaves and roots with blender in 80% ethanol; the solution, treated with chloroform and concentrated under vacuum. The residue was dissolved with picric acid and then absorbed on a Dowex 50 X 80, 100-200 mesh. The fraction was eluted with 4 N NH_4OH, dried under vacuum, esterified, and then acylated with heptafluorobutyric anhydride.

Amino acid composition was determined using a Carlo Erba "Fractovap 2200' gas chromatograph equipped with a flame ionization detector. The column (2 mm X 2 m) was packed with 3 % SE 30 on Chromosorb HP 80-100 mesh. Oven temperature was maintained at 100 °C for 6 min and programmed up to 260 °C at 2 °C/min.

Statistical analysis

Mean and standard deviation values were calculated according to standard procedure using Windows Excel software.

RESULTS

AM fungal inoculation significantly affected the overall growth, nutrient content and disease severity of the tomato plant. In this study the use of AM fungi had a significant effect on total sugar content of tomato plant compared to control and pathogen infected plant. The maximum increase in total sugar content was obtained when soil was infested with *Acaulospora laevis*. The least result was observed in plants infected with pathogen only (Table -1). Mycorrhizal plants showed a considerable increase in both reducing and non reducing sugar content when compared to non -mycorrhizal, control and pathogen alone infected plants. The ratio of reducing sugars was more than that of non reducing ones. Application of *Acaulospora laevis* showed a better result (Table -1).

AM fungi increased the chlorophyll a, b and carotenoid content of all treated plants as compared to control plant and pathogen alone. *Acaulospora laevis* stimulated the maximum production of chlorophyll and carotenoid in tomato plant (Table-1). With regard to nitrogen and phosphorous content, all the AMF inoculated plants had significant increase than uninoculated control plants. Among the isolates tested, AL inoculated plants had higher content of both phosphorus and nitrogen. Uninoculated pathogen affected plant showed the least content. A similar trend was also observed in phenol content (Table -1). Inoculation of soil with GF and AL alone enhanced the protein content in tomato plant. AL showed the maximum result (Table -1).

When compared to un-inoculated control, peroxidase activity was higher in root and leaves of AM inoculated plants, as well as in the AM+P plants. AL showed the maximum activity (Fig. 1). The use of AM fungi had a significant effect on aminoacid content of tomato plant compared to control and pathogen infected plant. The maximum increase in aminoacid content was observed when soil was infested with *Acaulospora laevis*. The least result was observed in plants infected with pathogen only. There was about three fold increase in the content of arginine and a two fold increase in proline and lysine content in roots of AL+pathogen inoculated plants especially in the roots. Phenylalanine and serine level was also high in these plants (Fig. 2).

Table -1. Effect of different Arbuscular mycorrhizal fungi on P uptake and changes in sugars, starch, amino acids, protein and phenols as a result of AM infection & Tomato wilt pathogen infection

	Control	***Glomus fasciculatum***	***Acaulospora laveis***	***Control + Pathogen***	***G.F + Pathogen***	***A.F + Pathogen***
VAM colonization (%)	0	40.2 ± 0.57	74.2 ±1.1	0	46.1 ±1.3	70.0 ±1.1
Phosphorous content (mg/g dry wt)	0.19 ± .002	0.34 ± 0.002	0.60 ± .003	0.11 ± 0.005	0.39 ± 0.005	0.51 ± .003
Nitrogen (mg/plant)	2.1 ± 0.17	4.30 ± 0.18	7.7 ± 0.15	0.9 ± 0.17	3.8 ± 0.17	7.1 ± 0.17
Total Sugar (%)	3.5 ± 0.18	6.59 ± 0.17	9.5 ± 0.08	2.5 ± 0.1	6.2 ± 0.09	8.6 ± 0.07
Reducing sugar (%)	2.2 ± 0.28	4.15 ± 0.19	6.4 ± 0.25	1.7 ±0 .28	4.3 ± 0.13	5.9 ± 0.25
Non-reducing sugar (%)	1.3 ± 0.02	2.44 ± 0.06	3.1 ± 0.10	0.8 ± 0.02	1.9 ± 0.12	2.7 ± 0.10
Starch (%)	1.6 ± 0.04	1.94 ± 0.03	2.9 ± 0.04	1.1 ± 0.03	1.8 ±0.05	2.4 ± 0.09
Phenols *(mg g^{-1} dry wt)*	9.4 ± 1.4	11.22 ± 1.23	18.6 ± 1.8	12.3 ± 2.1	12.3 ± 2.1	18.6 ± 1.8
Carotenoids *(mg g^{-1} f wt)*	5.13 ± 0.14	5.59± 0.14	6.41 ± 0.12	4.56± 0.09	5.02± 0.07	5.93± 0.13
Chlorophyll a*(mg g^{-1} f wt)*	0.57±0.002	0.65±0.002	0.99 ±0.005	0.43 ±0.003	0.58±0.002	0.92 ±0.003
Chlorophyll b*(mg g^{-1} f wt)*	0.431±0.002	0.521±0.005	0.772 ±0.002	0.380 ±0.003	0.464±0.003	0.893 ±0.003
Protein *(mg g^{-1} dry wt)*	2.51 ± 0.03	3.53 ± 0.02	4.8 ± 0.02	1.6 ± 0.04	3.6 ± 0.04	4.8 ± 0.02
Total free amino acids (*mg g^{-1} dry wt*)	11.2 ± 1.2	22.42 ± 1.82	42.5 ± 2.0	19.2 ± 2.2	28.4 ± 2.5	56.5 ± 2.0

Note: Each figure represents the mean of five replicates.

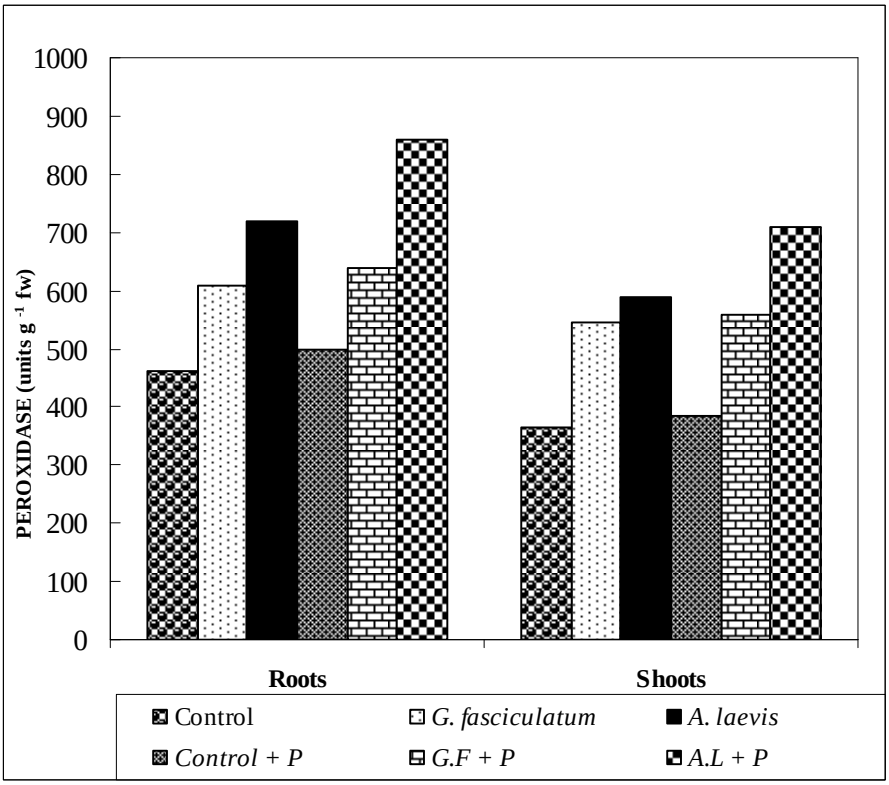
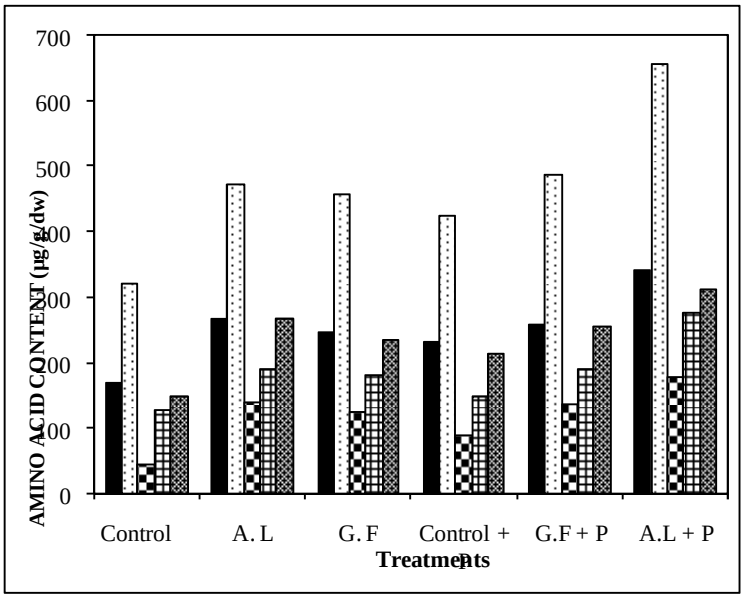


Fig. 1. Peroxidase activity in Roots and Shoots as a result of AM infection & Tomato wilt pathogen infection

A



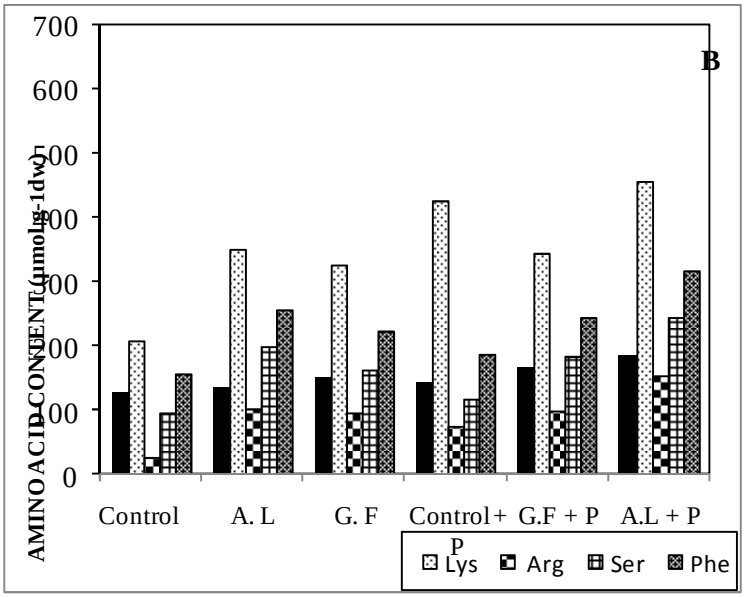


Fig. 2. Content of Aminoacids in Roots (A) and shoots (B) as a result of AM infection & Tomato wilt pathogen infection

DISCUSSION

It was obvious from the present study that the integration of AMF has a significant effect on *Fusarium oxysporum* f. sp. *lycopersic*i population in tomato rhizosphere. Inoculation with AM fungi not only improved the growth, chlorophyll content, N_2, P level but also reduced the incidence of wilt disease. However there was difference in the growth promoting efficiency of the different AM fungi. *Acaulospora laevis* was found to be more efficient than *G. fasciculatum* in increasing the total biomass production and disease resistance. This is in accordance with the previous reports that symbiotic effectiveness depends on the interaction between mycorrhizal plant, mycorrhizal fungus and soil characteristics. The results obtained indicated that in AL inoculated plants the level of biochemicals and macronutrients were high. It may be due to increased photosynthetic rates in mycorrhizal plants than the non mycorrhizal one [22, 23]**.** It supports the previous reports, that increased photosynthetic rate is directly correlated with the amount of chlorophyll content in the mycorrhizal plants as reported by Gemma *et al*., [24]; Neelima Ratti *et al.*, [25]**.** The amount of chlorophyll was high in the AM fungi inoculated plant than the control. AM fungi inoculation in the present study resulted in significant increase in both N_2 and P content of tomato plant. Increased nitrogen content in mycorrhizal plants might be due to the increased nitrate reductase activity in consequence of improved P- nutrition provided by AM symbiosis as suggested by Oliver *et al.*, [26]**.** The AM fungi inoculated tomato plant had higher phosphate content than uninoculated plant. The phosphorus with in the soil is taken up via a phosphate transporter located in the extra-radical hyphae of this fungus[27]**.** Results of the present study reveals that there was marked increase in starch and ratio of non reducing sugars to reducing sugars of AM fungi inoculated tomato plant as compared to uninoculated control. Increase in aminoacid contents was observed in roots and leaves of AM fungi inoculated tomato plant. Content of arginine and proline was considerably high. These aminoacids are known to play an important role in providing resistance to the mycorrhizated plant against pathogens. The proline content has been reported to get increased in resistant cultivars, which indicates its disease resisting capability in host plants. Other authors have suggested that tobacco resistance to infection by *Thielaviopsis basicola* may be caused by changes in the free aminoacid content of mycorrhizal root. In particular, the inhibition of chlamydospore formation by *T. basicola* has been attributed to the high content of arginine in mycorrhizal roots[28]**.** The protein content was high in AM fungi inoculated tomato plant than uninoculated control. Increased levels of protein in the inoculated plants could be attributed to either the presence of fungal proteins or post infectional stimulation of protein synthesis in the host plant[29].

The obtained results demonstrate that AM colonization led to significant increase in the phenolic content of tomato plant than uninoculatd control. Many plant phenolic compounds are known to be antimicrobial because increased phenol synthesis of the plants brings about an increase in phenyl propanes, which are lignin precursors. The increase

in total phenols in AM fungi inoculated plant could be due to the general triggering of aromatic biosynthesis[30]**.** Accumulation of lignin and phenolic compounds has been correlated with disease resistance in a number of plant-pathogen interactions. These include wheat-*Fusarium graminearum* and cucumber-*Pythium aphanidermatum*[31]. Significant increase in peroxidase activity was found in AM fungi infected plants than uninoculated control. The increased peroxidase activity by AM fungi may be due to AM inoculation which also resulted in increased activity of phenol oxidase enzyme. This increased phenol oxidase activity might be responsible for increased phenolic contents in the plants. Peroxidase and phenol oxidase are important enzyme of the defence mechanism of plants against pathogens. Both these enzymes are involved in the oxidation of phenolic components into quinines, which are toxic to the pathogen [32].

From the present study it is concluded that AM colonization increased plant growth and resistance to Fusarium wilt infection in tomato plants. Among the AM fungi tested, *Acaulospora laevis* was more effective in controlling the tomato Fusarium wilt pathogen.

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