Nutrient uptake and promotion of growth by Arbuscular Mycorrhizal Fungi in Tomato and their role in Bio-protection against the tomato wilt pathogen

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ABSTRACT

AM fungi colonize the roots of host plants and promote plant growth due to improved uptake of nutrients. A pot experiment was conducted to investigate the effect of mycorrhizal fungi on growth of tomato plants and access their role in bioprotection against the tomato wilt pathogen Fusarium oxysporum f. sp lycopersici. Tomato seedlings were inoculated with AM fungi Glomus fasciculatum and Acaulospora laevis and their interaction and effects on tomato plant were observed over a period of three weeks. There were 5 treatments along with the control. A significant increase in plant height, leaf number, leaf area, fresh and dry matter of shoot and root were recorded. Height of mycorrhizal plants were approximately 53% than non mycorrhizal plants .The dry weight of shoot and root increased 40-55% in mycorrhizal plant. Mycorrhizal plant inoculated with tomato wilt pathogen Fusarium oxysporum f. sp lycopersici also showed considerable resistance to the wilt pathogen and increase in shoot and root dry weight , height of shoot and stem thickness compared to non mycorrhizal plants inoculated with the wilt pathogen.

Key words: AM, Tomato, Biocontrol, Fusarium wilt.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that predominate in the roots and soils of agricultural crop plants. AM fungi promote plant growth by improved uptake of nutrients with particular emphasis on phosphorous nutrition[1, 2]. Through their function in efficient exploration of soil mineral resources and their bio-protective role against number of soil borne pathogens, AM fungi are instrumental in the survival and fitness of many plant taxa in diverse ecosystems, including many crop species[3,4]. Tomato fruit 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[5] and might be strongly protective against neurodegenerative diseases[6,7,8].They contain lycopene, a natural and powerful antioxidant. Tomato plants are affected by several diseases, including Fusarium wilt caused by Fusarium oxysporum f. sp. lycopersici (Sacc.), a soil born pathogen. This is a destructive disease of tomato worldwide [9]. It causes vascular wilt in tomato and even resistant varieties may be affected[10]. The present study was carried out to test the role of AMF in promoting growth of tomato plants and assess the role of AMF in bioprotection against the tomato wilt pathogen.
MATERIALS AND METHODS

Soil
The experimental soil was sandy loam. It was autoclaved at 121°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* was obtained from Dr. Kumuda, Department of Microbiology, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu and *Acaulospora laevis* from Dr. T. Muthukumar, Root and Soil Biology Lab, Department of Botany, Bharathiyar University, Coimbatore, Tamil Nadu.

All the AM fungi were maintained on onion (*Allium cepa* L.) to prepare pot cultures(11). 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(12). 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°C and used whenever required.

Maintenance of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.)
*Fusarium oxysporum* f. sp. *lycopersici* 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[13] without phosphorous beginning 30days after planting. One month after transplanting the plants were inoculated with a conidial suspension of *Fusarium oxysporum* f. sp. *lycopersici* (25ml/pot), poured on the soil surface. There were total 6six treatments applied as follows; *Glomus fasciculatum*, *G. fasciculatum*+ *Fusarium oxysporum* f. sp. *lycopersici*, *Acaulospora laevis*, *A. laevis* + *F. oxysporum* f. sp. *lycopersici*, Control and control+ *F. oxysporum* f. sp. *lycopersici*. 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. Five plants were harvested from each treatment 5, 10, 15 and 25 days after transplanting. The collected root samples were washed under tap and suitably processed[12]to study the percentage of root colonization. The plants were washed free of soil, and the fresh weight and the dry mass of shoot and root were recorded after drying them in a hot air oven at 60°C for 48h. The morphological characters like plant height, stem diameter, leaf number and leaf area were also recorded. Disease severity was monitored and estimated using the following scale: 0= No symptoms; 1= yellowing of lower leaves(10-25%); 2= wilting of leaves(26-49%); 3= half plant wilted and stem dies(50-74%); 4= heavy infection(75-100%); 5 =Plant dead.

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

Plant height and stem diameter
In this study the use of AM fungi had a significant effect on height and stem diameter of tomato plant compared to control and pathogen infected plant. The maximum increase in plant height was obtained when soil was infested with *Acaulospora laevis*. The least height was observed in plants infected with pathogen only. Highest stem diameter was observed in plants infested with *Acaulospora laevis* (Table - 1).

Leaf Area(cm²) and Number of leaves
AM fungi increased the leaf area of all treated plants when compared to control plants and pathogen inoculated plants. After 25 days of pathogen infection the maximum leaf area was observed in tomato plant infected with *Acaulospora laevis* (Table - 1). *Acaulospora laevis* stimulated the number of leaf per plant also (Table - 1).

Percent of root colonization
The best result of root colonization was observed with *Acaulospora laevis* (90%). It is evident from Table -1 that, roots with no AM fungal colonization were rapidly infected with *Fusarium oxysporum f. sp. lycopersici*,

Dry weight of shoot and root
Mycorrhizal plants showed a considerable increase in dry weight of both shoot and root when compared to non-mycorrhizal, control and pathogen alone plants. Application of *Acaulospora laevis* showed a better result (Table - 2).

Disease severity
AM fungi increased resistance to soil pathogens. The introduction of AM fungi either *G. fasciculatum* or *A. laevis* with tomato plants reduced the disease severity. The data shown in (Table -2) suggest that disease severity was significantly higher on leaves of non mycorrhizal plants than on those of mycorrhizal plant. *Acaulospora laevis* infested plants were more resistant to infection compared to all other treatments.

| TABLE – 1 | Effect of Arbuscular Mycorrhizal fungi and *Fusarium oxysporum f. sp. lycopersici* on growth of tomato plants 20 days after infection (Values are mean ± SD) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Treatment                      | % of colonization | Stem diameter (cm) | Plant height (cm) | Leaf Area (cm²) | No of leaves |
| Control                        | 0                  | 1.2 ± 0.02          | 40 ± 3.2          | 15.5 ± 0.99     | 7 ± 1.0       |
| Control + Pathogen             | 0                  | 0.4 ± 0.01          | 32±3.5            | 2.63 ± 0.05     | 5 ±0.18       |
| *Glomus fasciculatum*          | 82 ± 4.8           | 1.7 ± 0.04          | 49.8±4.2          | 8.5 ± 1.3       | 9 ± 0.4       |
| *G. fasciculatum* + Pathogen   | 64 ± 3.1           | 1.2 ± 0.03          | 40±3.6            | 6.24 ± 1.2      | 7 ±1.2        |
| *Acaulospora laevis*           | 90 ± 4.5           | 1.9 ± 0.03          | 53.9±4.6          | 9.5 ± 3.2       | 10 ±1.5       |
| *A. laevis* + Pathogen         | 78 ± 3.7           | 1.3 ± 0.02          | 42±2.2            | 7 ± 1.1        | 7 ± 1.2       |

| TABLE – 2 | Effect of Arbuscular Mycorrhizal fungi on Biomass of tomato plants and disease severity of tomato wilt pathogen and *Fusarium oxysporum f. sp. lycopersici* |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Treatment                      | Shoot Dry weight (gm) | Root Dry weight (gm) | Disease Severity Scale |
|                                | 5       | 10     | 15     | 20     | 5     | 10     | 15     | 20     | 5   | 10   | 15     | 20     |
| Control                        | 0.45    | 0.52   | 0.58   | 0.61   | 0.13  | 0.16  | 0.21  | 0.23  | 0   | 0    | 0      | 0      |
| Control + Pathogen             | 0.43    | 0.43   | 0.36   | 0.2    | 0.11  | 0.1   | 0.09  | 0.07  | 1   | 2    | 4      | 5      |
| *Glomus fasciculatum*          | 0.70    | 0.76   | 0.89   | 0.92   | 0.21  | 0.29  | 0.39  | 0.47  | 0   | 0    | 0      | 0      |
| *G. fasciculatum* + Pathogen   | 0.57    | 0.63   | 0.64   | 0.64   | 0.18  | 0.23  | 0.26  | 0.26  | 0   | 1    | 2      | 3      |
| *Acaulospora laevis*           | 0.75    | 0.80   | 0.93   | 1.1    | 0.28  | 0.34  | 0.48  | 0.56  | 0   | 0    | 0      | 0      |
| *A. laevis* + Pathogen         | 0.69    | 0.71   | 0.71   | 0.72   | 0.24  | 0.28  | 0.29  | 0.29  | 0   | 0    | 1      | 2      |

Disease severity - 0= No symptoms; 1= yellowing of lower leaves; 2= wilting of leaves; 3 = half plant wilted and stem dies; 4= heavy infection Plant wilted; 5 = Plant dead.

DISCUSSION

AM fungi are an interesting group of microorganisms that form a symbiotic association with a wide range of plant species, and effectively reduce root diseases caused by a number of soilborne pathogens, particularly nematodes and fungi [14,15]. AM fungi symbiosis with host plant has an improved growth effect also. In the present study inoculation of *G. fasciculatum* and *A. laevis* showed a positive influence on plant height, stem diameter, number of...
leaves and leaf area of tomato plant. However there were differences 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. Plant weight had increased only slightly in the controls and plants inoculated *Fusarium oxysporum* f. sp. *Lycopersici*. By day 15, the control plants and the plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*, only showed very poor growth which may be attributed to nutrient deficiency, e.g. the lack of available P in the unfertilized soil. In addition, sterilization of the soil killed the native microflora which assisted plant growth and nutrient uptake. AM fungi colonize the root cortex, developing intercellular hyphae and extensively branched intracellular hyphae called arbuscules [16]. Arbuscules are thought to be the site of nutrient exchanges, i.e. the fungus provides the plant with mineral nutrients (i.e. phosphorus, nitrogen)[17]. In several studies it has been reported that a local bioprotective effect depends on the degree of AM root colonization [18,19]. The obtained result indicated that percentage of root colonization was considerably reduced in *Fusarium* infected mycorrhizal plant than that of non infected mycorrhizal plant. Percentage of root colonization was high in *Acaulospora laevis* infected plant and disease severity was lower in same plants. Our results are in accordance with earlier reports made by some workers on role of AM fungi in bio – protection [20,21]. Further characterization of the bio-chemical mechanism underlying the bio-protection is being carried out.

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