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Arbuscular Mycorrhizal Fungi Influences Oxidative Stress in Tomato Plants in Drought Stress

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Research Article

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ABSTRACT

The present investigation has several aspects related to drought tolerance in Arbuscular Mycorrhizal (AM) tomato plants. The study included both shoot and root tissues in order to reveal the preferred target tissue for AM effects against drought stress. Non-AM and AM soybean plants were grown under well-watered or drought-stressed conditions, and oxidative damage to lipids, and other parameters were determined. Results showed that AM plants were protected against drought, as shown by their significantly higher shoot and root biomass production. These results showed that AMF inoculation stimulated growth and enhanced drought tolerance of tomato plants through changes in morphological, physiological and biochemical traits. This symbiosis might be an effective cultivation practice in improving the yield and development for tomato plants.

INTRODUCTION

Drought stress is considered to be one of the most significant abiotic factors limiting plant development and yield in many areas. Drought stress is known to restrict the vegetative growth and yield, in addition to adversely affecting fruit quality and a huge economic loss to the vegetable growers. The host plants can be protected by the symbiosis of arbuscular mycorrhizal (AM) fungi against the detrimental effects of drought stress. Understanding how plants respond to drought stress it can play a major role in stabilizing crop performance in the protection of natural vegetation. Drought stress incurred a large set of parallel changes in the morphological, physiological and biochemical responses whereas arbuscular mycorrhizal (AM) fungi symbiosis can protect host plants against the detrimental effects [1,2].

According to Duan et al. acclimation of plants to water deficit is the result of adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defences [3]. The production of ROS (reactive oxygen species) in plants is an early event of plant defense response to water-stress. However, ROS levels increase dramatically resulting in oxidative damage to proteins, DNA and lipids and minimize the affections of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes (SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), APX (ascorbate peroxidise) [4]. The objective of this work was to investigate whether the AM symbiosis can help tomato plants to overcome the negative effects of drought stress.

MATERIAL AND METHODS

The experiment consisted of a randomized complete block design with two inoculation treatments i.e. control non-mycorrhizal tomato plants and mycorrhizal tomato plants inoculated with *Funnelifomis mosseae* (Nicolson and Gerd.) Walker and Schuessler. Eight replicates of each treatment were done totalling 16 pots and two plants per pot, so that half of them were cultivated under well-watered conditions (watered in alternate days) throughout the entire experiment while the other half were drought-stressed (watered periodically). Clay loam soil was collected from the nearby areas of Guru Ghasidas Vishwavidyalaya, Bilaspur (CG, India), sieved (2 mm), mixed with sand (<1 mm) (1:1, soil: sand, v/v) and sterilized by steaming (120˚C for 1 h d-1 for one week). The

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soil had a pH of 7.1; 3.72% organic matter, available nutrient concentrations (mg kg^{-1}): N, 3.1; P, 5.8 (NaHCO3-extractable P); K, 154.0. The soil texture was made up of 36% sand, 33% silt, and 31% clay.

Tomato (Solanum lycopersicum Mill. cv. Sadabahar) surface-sterilised in 2% sodium hypochlorite for 10 min, rinsed thoroughly with distilled water and germinated on moist filter paper in darkness at 24°C for 4 days. Four-day-old seedlings were transferred to plastic pots containing 900 g of the sterilized soil/sand mixture. Mycorrhizal inoculum was bulked in an open-pot culture of *Allium cepa* L. and consisted of soil, spores, mycelia, and colonized root fragments. Ten grams of inoculum were added to the appropriate pots at transplanting time just below the tomato seedlings. Plants were grown in a controlled environmental chamber with 70–80% relative humidity, day/night temperatures of 25/15˚C, and a photoperiod of 16-h photoperiod under fluorescent white light (175 µmol m^2 s).

At harvest (8 week after planting), the shoot and root systems were separated and the shoot and root dry weight (DW) measured after drying in a forced hot-air oven at 70°C for 2 days. The percentage of AM fungi root colonization was estimated by visual observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v), according to Phillips and Hayman (1970). The extent of mycorrhizal colonization was calculated according to the gridline intersect method [5].

Lipid peroxides were extracted by grinding 1g of roots or leaves in an ice-cold mortar and 10 ml of 100mM potassium phosphate buffer (pH 7). Homogenates were filtered through one two layer of nylon cloth and centrifuged at 12,000 g for 30 min. The chromogen was formed by mixing 200 ml of supernatants with 1 ml of a reaction mixture containing 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) 2-thiobarbituric acid (TBA), 0.1% (w/v) butyl hydroxytoluene, and 0.25 N HCl, and by incubating the mixture at 100°C for 30 min [6]. After cooling to room temperature, tubes were centrifuged at 1,000 g for 5 min and the supernatant was used for spectrophotometric reading at 532 nm. Lipid peroxidation was estimated as the content of 2-thiobarbituric acid-reactive substances (TBARS) and expressed as equivalents of malondialdehyde (MDA) according to Halliwell and Gutteridge [7].

Enzymes were extracted at 4˚C from 1 g fresh weight (FW) of root or shoot tissues in a mortar and pestle with 50 mg polyvinylpolypyrrolidone (PVPP) and 10 ml of the following optimized medium: 50 mM K-phosphate buffer pH 7.8 containing 0.1 mM EDTA for SOD, catalase (CAT), and ascorbate peroxidase (APX) (Gogorcena et al) [8]. The same medium supplied with 10 mM b-mercaptoethanol was used for glutathione reductase (GR) (Moran et al.). Extracts were filtered through five layers of nylon cloth and centrifuged at 12000 g, 30 min, 0–4˚C. The supernatants were kept at – 70˚C for subsequent enzymatic assays. Total SOD activity (EC 1.15.1.1) was measured according to Beyer and Fridovich based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated photochemically [9]. CAT activity (EC 1.16.1.6) was measured by the disappearance of H_2O_2. APX activity (EC 1.11.1.11) was measured in a 1 ml reaction volume containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM hydrogen peroxide, and 0.5 mM ascorbate. Adding the H_2O_2 started the reaction and the decrease in absorbance at 290 nm was recorded for 1 min to determine the oxidation rate of ascorbate (Amako et al.) GR activity (EC 1.6.4.2) was determined by the procedure of Carlberg and Mannervik [10].

Data were subjected to analysis of variance (ANOVA) with mycorrhizal treatment, water supply, and mycorrhizal treatment–water supply interaction as sources of variation, and followed by Duncan's multiple-range test.

RESULTS AND DISCUSSIONS

Arbuscular mycorrhizal fungi symbiosis has been shown to increase plant tolerance to water deficit, although the exact mechanisms involved are still a matter of debate. Under well-watered conditions, SDW (shoot dry weight) of AM and non-AM soybean plants were similar **(Table 1).** A decrease of 42% in AM plants and 54% in non-AM plants in plant growth was observed with drought stress in both treatments. The drought-stressed AM plants showed enhanced SDW compared to non-AM tomato plants. Mycorrhizal and non-mycorrhizal tomato plants did not show any significant difference in RDW at whatever water regime. Arbuscular mycorrhizal symbiosis has been shown to increase plant tolerance to water deficit and both root and shoot tissues are influenced by AM symbiosis by means of drought avoidance and drought-tolerance mechanisms [11]. AM fungi enhanced drought tolerance of *Casuarina equisetifolia* seedlings (Zhang et al.) [12]. No mycorrhizal colonization was observed in plants not provided with AM inoculum. Mycorrhizal plants showed about 74% of mycorrhizal root length under well-watered and 92% drought-stressed conditions (data not shown).

Table 1. Root and Shoot dry weight (DW), SOD, CAT, APX, and GR activities in roots and shoots of non-AM and AM fungal tomato plants grown under well-watered and drought stressed conditions.

Treatments	DW (g $plant^{-1}$)		CAT (U g^{-1}FW min^{-1})		SOD (U g^{-1}FW min^{-1})		APX (µmol ASA g^{-1} FW h^{-1})		GR (nmol NADPH g^{-1} FW m^{-1})	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Non-AM fungal Plants										
Well-Watered	1.22a	2.28a	5.6ab	2.3b	7.6b	4.2a	2.4a	9.5c	2.3b	5.2a
Drought	0.56b	1.44c	7.3a	1.9bc	9.2a	2.1c	1.8b	20.1b	4.5a	2.2b
AM fungal Plants										

Well-Watered	1.18a	2.37a	4.2b	2.7a	11.4a	2.5c	1.2c	15.5bc	1.9b	0.7c
Drought	0.68b	1.84b	6.5a	1.4c	7.3b	3.2b	1.5b	27.4a	0.8c	1.8bc
Significance of sources of variation										
AMF (M)	**	ns	*	**	ns	*	**	**	*	*
Water (W)	***	**	*	***	**	**	*	**	*	*
M × W	*	ns	ns	**	*	ns	ns	*	ns	ns

DW: Dry Weight, CAT: Catalase, SOD: Superoxide Dismutase, APX: Ascorbate Peroxidise, GR: Glutathione Reductase. Means followed by the same letter are not significantly different as determined by Duncan's multiple range test (n=4). Significance of the sources of variation is also displayed, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns: not significant.

The oxidative damage to lipids increased as a significance of drought only in non-AM fungal tomato plants. AM fungal tomato plants showed similar levels of lipid peroxidation under both well-water and drought stress conditions **(Figure 1).** However, under drought stress conditions roots of AM plants exhibited 19% less lipid peroxides than roots of non-AM plants. In shoots, the different behaviors of AM fungal and non-AM fungal tomato plants were shown different results. Drought enhanced lipid peroxidation in non-AM tomato plants while lipid peroxidation in shoots of AM fungal tomato plants remained unaffected. Under drought conditions shoots of AM fungal tomato plants had 59% less lipid peroxides than shoots of non-AM fungal plants. The oxidation of membrane lipids is a reliable indication of uncontrolled free-radical production and hence of oxidative stress (Noctor and Foyer) and lipid peroxides were 55% lower in shoots of drought mycorrhizal soybean plants than in non-mycorrhizal plants was observed [12,13]. The amount of lipid peroxides was quantified in roots and shoots. In roots, the lipid peroxidation in AM fungal tomato plants subjected to drought was 19% lower than in drought stressed non-AM fungal tomato plants. In shoots, lipid peroxidation was 59% lower in drought stressed AM fungal plants than in drought stressed non-AM fungal **(Figure 1).**

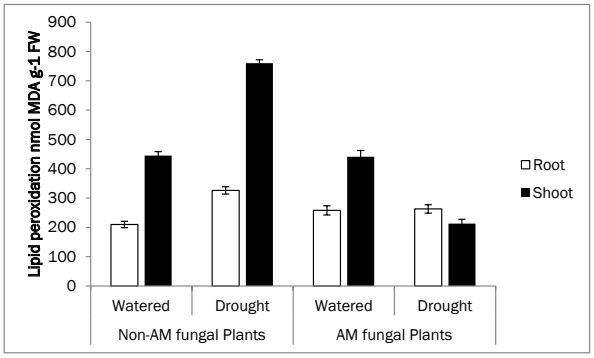

Figure 1. Oxidative damage of lipids in shoots and roots of non-AM and AM tomato plants under well-watered and drought stressed conditions.

The activities of four antioxidant enzymes were measured for correlation with the oxidative damage to lipids. Results showed that there was no relationship between the antioxidant activities and the decrease in lipid peroxidation in roots and shoots of drought stressed AM tomato plants. CAT activity showed a contradictory behavior in roots and in shoots **(Table 1).** In shoots, the CAT activity of non-AM fungal plants was lower than in AM fungal plants under well-watered conditions, but under drought stress conditions the CAT activity of AM fungal plants decreased, reaching a value comparable to that in non-AM fungal plants. In roots, CAT activity decreased in non-AM fungal plants as a significance of drought. An improved drought tolerance results from direct water supply by extra-radical fungal hyphae but also form improved nutrient status, and increased antioxidant levels in AM fungal plants [14-16]. AM fungi might indirectly increase water uptake by improving root conductance to water flow and extraradical mycorrhizal hyphae might transport water to colonized roots directly [17,18].

In shoots, non-AM fungal plants had higher SOD activity than AM fungal plants when cultivated under well-watered conditions and higher activity when cultivated under drought stress conditions. In roots, SOD activity was similar in the different treatments, apart from for drought-stressed AM roots, which had significantly lower SOD **(Table 1).** APX was always higher in non-AM plants than in AM plants. Mycorrhizal roots had significantly lower APX despite the consequences of whether they had been grown under well-watered or drought-stressed conditions. Drought stress increased the APX activity in shoots of both AM and non-AM plants compared with well-watered conditions **(Table 1).** Only shoot SOD and shoot APX activities showed a significant interaction between mycorrhization and water regime, while no significant interaction was observed for the other enzyme activities. AM fungi colonization could alleviate the damage of ROS, protect the plants against damage by oxidation and finally improve the drought tolerance of tomato. In general, the results obtained for the four antioxidant activities agree with the roots of soybean plants inoculated with *Glomus mosseae* [11].

Drought stressed AM and non-AM fungal shoots had similar GR activities, while under well watered conditions the GR activity increased by 86% in non-AM fungal plants compared with AM fungal plants. GR activity was significantly increased by drought stress in roots of non-AM fungal plants and decreased in roots of AM fungal plants **(Table 1).** GR activity, lower oxidative damage to lipids in the AM plants seems to be a consistent effect of AM fungal symbiosis, regardless of the fungal species involved in the association [11,19]. The increase in the activities of APX in drought stressed AM fungal plants suggests increased production of

H_2O_2, whilst the increase in GR activity may be related to the maintenance of the intracellular levels of reduced glutathione which is required for phytochelatin biosynthesis [20]. The consistently higher contents of glutathione in AM fungal plants than in non-AM fungal plants may have contributed to protecting tomato plants against the oxidative stress generated by drought. A similar reduction of oxidative damage to lipids by AM symbiosis has been observed in tomato plants subjected to salt stress [21,22].

This study investigated physiological and biochemical aspects related to water relations and drought tolerance in AM and non-AM fungal plants to drought stress. Drought stressed antioxidant enzymes mainly in plants associated with *F. mosseae* and the accumulation of peroxidation levels was the most evident response to drought in non-AM fungal plants. It was also clear in the present study that at drought stress condition, mycorrhizal tomato plants exhibited well-water conditions in root and shoot and those inoculated with *F. mosseae* exhibited better growth. AM fungal plants showed a higher tolerance to the drought stress than non-AM plants, as shown by their enhanced shoot biomass production (27%), and lower lipid peroxidation.

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