Mycorrhizal symbiosis alleviated reactive oxygen species accumulation in date palm under water deficit

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ABSTRACT

The scarcity of water is producing a generalization of drought effects in most of plant species in arid and sub-arid areas. Reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{1-}) are generated in plants undergoing water deficit and can cause oxidative damage to lipids, proteins and nucleic acids leading to loss of membrane integrity and cell death. The induction of ROS-scavenging enzymes, such as SOD, CAT, APX and GPX is the most common mechanism for detoxifying plant tissue. The present study was focused on determining the effect of arbuscular mycorrhizal AMF on antioxidant enzymes activites and non-enzymatic antioxidants production in leaves of date palm seedlings subjected to well-watered (75% of field capacity) and water stressed (25% of field capacity) conditions. Obtained results showed that water stress steeply decreased plant growth (shoot height) and biomass production (shoot and root dry weights) in non-inoculated date palm seedling compared to AM-plants. Leaves of water stressed mycorrhizal plants showed lower malondialdehyde (MDA) and H2O2 and O2 concentrations than their relative non-inoculated plants. Moreover, mycorrhizal symbiosis notably increased the activities of guaiacol peroxidase (G-POD) and ascorbate peroxidase (APX) under water stress. Soluble sugar and prolin contents were also enhanced in leaves of mycorrhizal plants subjected to water stress. Our results suggest that the increased concentrations of antioxidant enzymes activities and non-enzymatic antioxidants contents found in mycorrhizal plants may enhance date palm protection against oxidative damage enhancing thereby drought tolerance.

Key words: Arbuscular mycorrhizal fungi, date palm, drought, antioxidant metabolism, oxidative damage.

INTRODUCTION

The lack of adequate soil moisture leading to low water availability is the most common characteristic in arid and semi-arid regions. The water scarcity produces a generalization of water stress effects in most plant species in arid land. Water stress is often associated with increased levels of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{1-}) which are generated in most plants undergoing water deficit and can seriously destroy the normal metabolism of the plant causing oxidative damage to lipids, proteins and nucleic acids leading to loss of membrane integrity and cell death. To maintain growth and productivity plants have to prevent accumulation of these harmful species as rapidly as possible. The induction of ROS-scavenging enzymes, such as SOD, CAT, APX and GPX is the most common mechanism for detoxifying plant tissue.

It well established that Arbuscular mycorrhizal fungi (AMF) can form symbiotic association with the vast majority of land plants including those of the arid areas. AMF play a critical role in plants mineral nutrition and terrestrial ecosystem functioning. Once established, AM association benefits host plants not only by improving water uptake and mineral nutrition (Aqqua et al. 2010), but also by increasing plant resistance to drought (Faghire *et al.,* 2010; Baslam *et al.,* 2013), soil salinity (Giri *et al.,* 2003) and pathogens (Garmendia *et al.,* 2004). In date palm, AM symbiosis was recognized as positively significant for growth, nutrients and water status, and plantlets establishment and survival especially on poor soil (Oiahabi 1991; Meddich, 2000; Baslam *et al.,* 2008, 2009; Aqqua *et al.,* 2010; Faghire *et al.,* 2010; Baslam *et al.,* 2013). In earlier studies we have shown that AM fungi allow for greater uptake of nutrients and play an important role in improving water relations thereby enhancing date palm growth under water deficiency (Faghire *et al.,* 2010; Aqqua *et al.,* 2010; Baslam and al., 2008; 2009). In the present study the effect of AMF on biomass production, osmoregulation and antioxidant metabolism was investigated in date palm seedlings under water deficit.

MATERIALS AND METHODS

Pre-germinated seeds of date palm were transferred in pots containing 1 kg of sterilized soil collected from date palm grove and grown under greenhouse conditions. Half of plantlets were inoculated (AM-plant) with 10g of rhizospheric soil containing hyphae, mycorrhizal root fragments, and spores of the AM fungus *Glomus intraradices* recognized from earlier investigations as efficient for promoting date palm growth and nutrition (Baslam *et al.,* 2009). The same amount of autoclaved inoculum was added to non-inoculated plants (N-AM). Water stress treatments consisted of two watering regimes: 75% of field capacity (well water) and 25% of field capacity (water stress). Water status of the pots was daily examined and the amount of water loosed was refilled into each pot. The experiment was arranged in a completely randomized block design. Each treatment was replicated twenty times. Eight weeks after water stress application, plants were harvested and roots were washed free from soil under a stream of cold tap water. Root colonization (%M) was evaluated according to Trouvelot et al. (1986). Shoot height (SH) and root length (RL) were measured and shoot (SDM) and root (SDR) dry matters were recorded by drying in oven at 70ºC to constant weight. Biochemical changes including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) activities and osmoregulation matters including proline, soluble protein and total soluble sugar (TSS) were determined according to balsam *et al.,* (2009). Leaf antioxidant enzyme activities, SOD (Beyer et Fridovich, 1987), CAT (Aebi, 1984), APX (Nakano and Asada, 1981) and GPX (Maehly and chance, 1954) were determined. Malondialdehyde (MDA) was measured by the thiobarbituric acid method as described by Heat and Packer (1981) and H_2O_2 by using titanium method according to Patterson and al. (1984). TSS was analyzed with the anthrone method (Irigoyen *et al.,* 1992) and proline with the ninhydrin reaction according to Bates et al. (1973). All data were analyzed statistically by an analysis of variance using ANOVA modules of the Statistica software program (Statsoft, 1995). Mean comparisons were conducted using Newman-Keuls test at $P < 0.05$.

RESULTS

Date palm plants inoculated with AMF (AM-plants) showed mycorrhizal structures in roots while these al structures are never seen in roots of non-inoculated plants (N-AM). Water restriction disfavored the colonization of date palm roots by AMF (Table 1). Percentages of mycorrhizal colonization reached 61 % in well watered (WW) plants comparing to 42% in water stressed (WS) ones. The mycorrhizal efficiency index (ME)I increased in AM-plants cultivated under reduced irrigation regime compared with their respective WW N-AM plants (table 1). AMF inoculation notably increased date palm shoot height (SH) and root length (RL) and shoot (SDM) and root dry matter (RDM) regardless water regime (Table 1). Water stress significantly decreased plants growth (SH and LR) and biomass production (SDM and RDM), this decrease was more important in N-AM plants.

Water stress increased the MDA concentration of leave of both N-AM and AM-plants (Table 2). The increase of MDA content in response to WS was more relevant in N-AM (23%) than in AM-plant (9%). There was higher total soluble sugar and proteins and high proline concentration in leaves of AM-plant under WS than in their respective N-AM plants. Analyses of hydrogen peroxide revealed that the level of H2O2 was increased by drought in N-AM plants. In contrast, concentrations of H2O2 were similar in leaves of AM-plants under both WW and WS conditions (Table 2). Whether WS or not, AM symbiosis notably increased GPX and APX activities of leaves (Table 2). AM colonisation also markedly increased SOD activity and slightly decreased CAT activity of WS leaves (Table 2).

DISCUSSION

AM symbiosis increased growth and biomass production of date palm plants under both well water presence and water stress condition, confirming earlier findings (Meddich, 2000; Baslam and al., 2008, 2009; Aqqua *et al.,* 2010; Faghire *et al.,* 2010; Baslam *et al.,* 2013). The positive effect of AMF is likely attributed to the improvement of mineral nutrition, the enhancement of water uptake and the increase of root length density.

Osmotic adjustment due to the accumulation of certain organic and inorganic molecules osmotically active in plant cells is one of the mechanisms of the tolerance of water stress. Our results showed that soluble sugar and proline levels in leaves were higher in water stressed AM-plants than those in corresponding N-AM. Such elevated level of proline and TSS accumulation in water stressed AM-plants was reported by many previous investigations (Fouad *et al.,* 2012, 2013; Baslam *et al.,* 2013). These authors showed that proline and sugars accumulation played a role in osmotic adjustment and allowed cells to maintain turgid and the processes that depend on it, such as cellular expansion and growth.

In higher plants, ROS production and removal are strictly controlled under amply watered conditions (Apel and Hirt, 2004). When higher plants are subjected to water stress, the equilibrium between production and scavenging of ROS is broken, resulting in oxidative damage to proteins, DNA and lipids. The oxidation of membrane lipids is a reliable indication of uncontrolled free-radical production and hence of oxidative stress (Noctor and Foyer, 1998). Many reports have emphasized the importance of AMF in increasing antioxidant activity and reducing oxidative damage (Ruiz-Lozano 2003; Alguacil et al. 2003). Accordingly, H_2O_2 accumulation and oxidative damage estimated as the ratio of malondyaldehide to proteins in mycorrhizal date palm seedlings subjected to drought was three times lower than in their respective non inoculated seedlings. Additional biochemical responses including enzymatic defense is an important component of the protective systems that minimize the deleterious effect of water stress. SOD catalyses the dismutation of O2 to H2O2, CAT dismutates H2O2 to oxygen and water, and APX reduces H2O2 to water. Our result showed that AM symbiosis notably increased the activity of GPX, APX and SOD and decreased the activity of CAT in date palm seedlings under water. Our finding suggest that the increased activity of antioxidant enzymes and decreased concentration of ROS compounds found in AM plants may serve to protect the date palm against oxidative damage, enhancing drought tolerance. These results are in good agreement with previous investigations showing that AMF inoculation markedly enhances the antioxidant enzyme activities (GPX, SOD and APX) and steeply reduces MDA and H_2O_2 accumulation (Alguacil *et al.,* 2003). Arafat and He (2011) associated the lower accumulation of H_2O_2 and lipid peroxidation, evaluated by MDA production, with the greater activity of antioxidant enzymes in AMF compared to non AMF plants. Other authors have shown a positive correlation between tolerance to water deficit and increased antioxidant activities (Ruiz-Lozano, 2003; Alguacil and al., 2003).

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Tables

Table 1: Root colonization (%), Plant height (cm), Root length (cm), shoot and root dry matters (g) of non-mycorrhizal (N-AM) or mycorrhizal (AM-plant) date palm seedlings grown under well water (75%FC) or water stress (25%FC) conditions.

Water regime	AM status	M	SDM	RDM	SH	RL	MEI
75%FC	N-AM	0	3.16c	1.76d	22.50c	27.90d	ND
	AM-plant	61.43a	6.41a	3.38a	44.67a	60.50a	49.7b
25%FC	N-AM	0	1.43e	0.81e	13.33d	18.95d	ND
	AM-plant	42.6c	3.21c	2.97b	26.60c	46.90c	63.8a

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2: Total soluble sugar (mg.g^{-1} DM), hydrogen peroxide (mmol.g^{-1} DM), proline (nmol.g^{-1} DM), malonyldialdehyde (nmol.g^{-1} DM) and protein (mg.g^{-1} DM) contents in leaves of non-mycorrhizal (N-AM) or mycorrhizal (AM-plants) date palm plants grown under well watered (75%FC) or water stress (25%FC) conditions.

Water regime	AMF status	TSS	H_2O_2	Prolin	MDA	Protein
75%FC	N-AM	54.76c	25.54b	4105.2c	53.23b	6.14a
	AM-plant	78.76a	26.2b	9193.2a	38.61d	5.75a
25%FC	N-AM	47.53d	28.58a	3570.2d	65.51a	3.3c
	AM-Plant	68.51b	26.53b	4070.4c	42.10c	4.21b

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).

Table 3: Catalase (nmol mg^{-1} prot), superoxide dismutase (U mg^{-1} prot), ascorbate peroxidase (mmol.mg^{-1} prot), and guaiacol peroxidase (mmol.mg^{-1} prot) activities in leaves of non-mycorrhizal (N-AM) and mycorrhizal (AM-plants) date palm plants grown under well water (75%FC) or water stress (25%FC) conditions.

Water regime	AMF status	SOD	GPX	CAT	APX
75%FC	N-AM	452.3c	3.07c	62.7cd	2.11d
	AM-plant	447.7c	4.20b	136.8c	3.89c
25%FC	N-AM	596.1b	4.44b	225.5a	4.68b
	AM-plant	667.4a	5.87a	114.41d	7.05a

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).