Arbuscular mycorrhizal fungi enhanced date palm tolerance to water deficit

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) has a high hardiness and adaptability to the climatic conditions of the arid and sub-arid areas where it may represent not only a biological tool to counteract processes of erosion and desertification, but also provide adequate microclimate for the establishment of other species, playing thereby the role of pioneer and productive species. However, low water availability and nutrients deficit exacerbated by the effects of climate fluctuations that are particularly marked in these areas severely limit agricultural production. During the last decades, the management of the arbuscular mycorrhizal (AM) fungi as providers of key ecological services has been at the forefront of generating and promoting agricultural production technologies. These soil microorganisms can form a symbiotic association with roots of most land plants and can participate in improving plant growth and nutrition, strengthening plant performance, restoring ecosystems and combating pests and pollution. Several studies have clearly highlighted the fundamental role that mycorrhizal fungi play at the interface between the soil and plant roots enhancing thereby the multitrophic and protective interactions that affect productivity, competitiveness and survival of the majority of plant species both in natural ecosystems and in managed field. In this presentation, we will discuss results of our recent investigations showing the positive effects of arbuscular mycorhizal symbiosis on date palm tolerance to water. We will discuss the most persuasive and effective use of these fungi as biofertilizers-biostimulative to improve date palm growth and physiology under low water availability.

Keywords: Sustainable agriculture, Mycorrhizal symbiosis, Arbuscular mycorrhizae,

INTRODUCTION

In last decades, the oasis ecosystem was subjected to different environmental constraint such as poor soils, water scarcity, sand invasion and desertification causing considerable economic, ecological and social damage. The recent worsening of these problems is the result of the detrimental effects of the global climate changes that are more pronounced in these arid and semi-arid areas. These constraints cause not only reduction in the production of dates, the principal food of humans and animals in the desert, but also an imbalance of the oasis ecosystem causing a serious threat to the plant resources and the long-term agricultural production in these difficult environments (Haddouch, 1997). Arbuscular mycorrhizas are highly evolved mutualistic associations formed between soil-borne fungi and plant roots. AM symbiosis is known to benefit mineral nutrition and to provide enhanced water relations thereby enhancing host plant protection against the detrimental effects of environmental constraints (Stutz *et al.,* 2000). In exchange, the plants supply mycorrhizal fungi with carbon fixed using photosynthetic process. The management of mycorrhizal fungi as providers of key ecological benefits has been at the forefront of generating and promoting agricultural production technologies. These microorganisms often referred to as “ecosystem engineers”, can participate in improving plant growth and nutrition, strengthening plant performance, restoring ecosystems and combating pests and pollution. In difficult areas AMF may represent not only a biological mean to counteract processes of soil degradation, but also a challenge for the development of the long-term agricultural production. The purpose of this paper is to review some of our recent research results that deal with the effect of AM symbiosis on promoting date palm tolerance to water deficit. We will discuss the most persuasive and effective effect of these fungi in improving date palm growth and physiology under low water availability.

MATERIALS AND METHODS

Seeds collected from the date palm population of the palm grove of Marrakech were surface-sterilized by immersing in 70 % alcohol for 5 min, rinsed three times with distilled water and germinated on wet paper in pans at 38°C. After two weeks seedlings were transplanted into plastic pots containing 1kg of autoclaved soil collected from the palm grove of Marrakesh. The experimental pots were placed in greenhouse under natural light condition. Mycorrhizal inoculum from our own stock culture consisted of soil spores and hyphae and infected root fragments from rhizospheric soil of mycotrophic plant. Ten grams of inoculum was used per pot and placed 5 cm below date palm roots. Non-mycorrhized seedlings received the same weight of autoclaved inoculum.

Water treatments began 4 months after AM inoculation. Well watered pots (WW) were watered with 75% of field capacity and water stressed (WS) pots received 25% of field capacity. The water status of the pots was daily examined and the amount of water loosed was refilled into each pot. After 8 weeks of water treatments, plants were harvested and growth parameters (plant height and root length) and biomass production (shoot and root dry weights) were recorded. Water relation parameters were measured as previously described (Faghire *et al.,* 2010). Relative water content (RWC) was calculated using the technique described by Turner (1981). Leaf water potential (Ψw) was measured by the method of chamber pressure developed by Scholander et al. (1965). Osmotic potential at full turgor ($\Psi\pi100$), osmotic potential at turgor loss ($\Psi\pi0$), symplastic water (WS), and cell elasticity modulus (ξ) were obtained from the pressure-volume curve method (Tyree and Hammel, 1972).

Biochemical changes including superoxide dismutase (Beyer & Fridovich, 1987), catalase (Aebi, 1984), guaiacol peroxidase (Polle *et al.,* 1994) and ascorbate peroxidase (Amako *et al.,* 1994) activities were determined. Proline (Paquin and Lechasseur 1979), soluble protein (Bradford (1976) and total soluble sugar (Yemm and Willis 1954) contents were estimated as described by balsam *et al.,* (2009). Leaf antioxidant enzyme activities SOD, CAT, APX and GPOX were determined. Malondialdehyde 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).

The experimental treatments consisted of two watering regimes (well watered and water stress) and were arranged in a complete randomized block design. Each treatment was replicated ten times. 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 AND DISCUSSION

AM symbiosis increased growth and biomass production of date palm seedlings under both well watering and water restricted conditions. Plant height, root length and shoot and root dry weights were more sensitive to mycorrhizal colonization under water limiting regime. Improved growth under water stress is due to the action of AM fungi on 1) the improvement of water and nutrients absorption, 2) the maintenance of water relations such as increased relative water content and symplastic water content, maintained water potential and osmotic potential and the cell turgor, 3) the induction of osmotic adjustment through the accumulation of organic osmolytes such as sugar and proline and inorganic osmolytes such as K+, and 4) the protection of the plants against oxidative stress by reducing ROS production and increasing antioxidant enzymes activities responsible for the elimination of the ROS.

Our results showed that arbuscular mycorrhizal fungi had positive effects on nutrient concentrations analyzed in the tissues of date palm plants under water stress. Indeed tissue nutrients (P, K, Ca and Mg) concentrations were higher in mycorrhizal plants (AM-P) than in non-inoculated (N-AM) ones. Water stress induced a significant decrease of plant nutrient concentrations (Table 1). This negative effect was more pronounced in N-AM than in AM-P. These Results were in accord with the finding that has been reported for date palm (Oihabi, 1991; Al-Whaibi and Khaliel, 1994; Meddich *et al.,* 2004; Faghire et al. 2010) and other plant species (Ruiz-Lozano and Azcon, 1996; Wu and Xia, 2005). AMF have been shown to improve productivity in soils of low fertility and are particularly important for increasing the uptake of slowly diffusing ions such as PO_4^{3-}, immobile nutrients such as P, Zn and Cu, and other nutrients such as ammonium and potassium (Rhodes and Gerdemann, 1980; Liu *et al.,* 2002). Under drought conditions the uptake of highly mobile nutrients such as NO_3^- can also be enhanced by mycorrhizal associations (Subramanian and Charest, 1999). The most established benefits from mycorrhizal fungus to the host plant is through the widespread mycelial network which penetrates deeper and wider in the soil in search of water and nutrients thereby widening the zone of activity. Nutrient acquisition begins with the uptake of free nutrients from soil by fungal extra-radical hyphae that act as a bridge between the soil and plant roots (Bucher, 2007). Nutrients are then transferred through the periarbuscular membrane to the plant cytosol. The majority of this nutrients exchange is believed to occur within root cortical cells containing highly-branched hyphal structures termed arbuscules. The establishment of the mycorrhizal network offers a number of basic advantages for the acquisition of mineral nutrients: 1) fungal hyphae extend beyond the nutrient depletion zone that develops around the root. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion. AMF hyphae can readily bridge this depletion zone and grow into soil with an adequate supply of nutrients. 2) Fungal hyphae network greatly increase the surface area for the absorption of nutrients relative to non-mycorrhizal roots. 3) Due to their narrow diameter relative to roots, hyphae are able to extend into soil pores that are inaccessible to roots or even root hairs, 4) Mycorrhizal fungi can access forms of N and P that are unavailable to non-mycorrhizal plants, particularly organic forms of these nutrients. One mechanism for this access is the production by plant roots and the associated mycorrhizal fungi of organic acids and hydrolytic enzymes.

Parameters related to water status (Table 2) showed that water stressed mycorrhizal date palm seedlings maintained better water relations in terms of relative water content, water potential and turgid potential compared to non-inoculated seedlings. Similar results were reported by Porcel and Ruiz-Lozano (2004) who showed that leaf water potential determined at the end of the drought stress period decreased larger in non-AM plants than in AM plants. Maintenance of favorable plant water relations is vital for the development of drought adaptation in crop plants (Auge RM, 2001). The higher RWC and Ψw and the lower WS and ξ of AM plants, compared with non-AM plants, were propitious to moving liquid water through the plants to the evaporating surfaces in the leaves (Nelsen and Safir, 1982). Also, the difference between $\Psi\pi$ at full and zero turgid for a given tissue tended to be smaller when cells have more rigid walls. The reverse was observed in mycorrhizal date palm seedlings. Although low ξ values (corresponding to flexible cell walls) have been correlated with drought-adaptation and may provide cells with a high resistance to water stress (Zimmermann, 1978; Robichaux, 1985; Goicoechea *et al.,* 2004). Mycorrhizal associations improve water uptake by increasing the hydraulic conductivity of the roots either by modifying root morphology and root anatomy or indirectly by hormonal and structural changes in the host plant. The survival of mycorrhizal plants in extremely dry condition is the result of a better root performance and the ability to explore water in wider zones of soil by extension of the fungal mycelium into non-rhizospheric soil (Kehri and Chandra, 1990).

Biochemical analysis emphasized the role of AMF in plants water stress tolerance by increasing antioxidant enzymes (SOD, P-POD, APX and PPO) activities and sugars and protein and accumulation and reducing MDA and H2O2 accumulation (table 3, 4). Compared to non-inoculated plants, mycorrhizal plants subjected to water stress accumulated more proteins, showed high level of soluble sugars and low accumulation of malonyldialdehyde and hydrogen peroxide. Similarly, mycorrhizal plants showed an increase in the activities of superoxide dismutase (SOD) and ascorbate and guaiacol peroxidase and polyphenol oxidase (Baslam et al. 2010; Faghire et al 2010; 2013; Fouad et al. 2012, 2013). In addition, oxidative damage, estimated as the ratio of malondyaldehyde / protein was higher in non inoculated seedlings and lowest in mycorrhizal seedlings (Baslam et al. 2010; Faghire et al 2010; 2013; Fouad et al. 2012, 2013). Our results suggest that the increased activity of antioxidant enzymes and decreased concentration of ROS compounds found in AM plants may serve to protect them against oxidative damage, enhancing thereby plants tolerance to water stress.

CONCLUSION

Contribution of the AM symbiosis to date palm drought tolerance is the result of their action on several plant functions including nutritional, physiological and biochemical processes. This appears to be due in many instances to differences in tissue hydration between AM-P and N-AM plants. However, AM symbiosis enhance drought tolerance of host plants through many mechanisms, such as increased water and nutrients absorption, high stomatal regulation by hormonal signals, enhanced osmotic adjustment, higher root hydraulic conductivity and leaf hydration, and reduced oxidative damage caused by the reactive oxygen species (ROS) generated during drought. A greater osmotic adjustment has also been reported in leaves of mycorrhizal plants than in non-mycorrhizal ones during drought period. In the same way, AM plants had postponed declines in leaf water potential during drought stress. Farther more, mycorrhizal plants have operated special biochemical mechanisms that prevent plant cell from oxidative damage through accumulation of some antioxidants compounds and enhancing antioxidant enzymes activities in leaves and roots of water stressed plants, and this was correlated to plant protection against drought.

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Tables

Table 1: Mycorrhizal colonization, Plant height, Root length, shoot dry weight and root dry weight and nutrients contents of non-mycorrhizal (Non-AM) or mycorrhizal date palm seedlings grown under well watered (WW) or water stress (WS) conditions.

Water régime	AMF status	M (%)	SDW (g)	RDW (g)	SH (cm)	RL (cm)	P (mg)	Ca (mg)	Mg (mg)	K (mg)
WW	N-AM	0	3,16b	1,76b	22,5c	27,9c	1,69b	0,5b	0,72b	1,36c
	AM-P	61,4a	6,41a	3,38a	44,7a	60,5a	6,08a	0,88a	1,94a	2,44a
WS	N-AM	0	1,43c	0,81c	13,3d	18,9d	1,62b	0,23c	0,39b	0,69d
	AM-P	42,6b	3,21b	2,97a	26,6b	46,9b	5,78a	0,60b	2,16a	1,97b

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2: Relative water content (RWC), Leaf water potential (Ψ_w), Symplastic water (W_S), Osmotic potential at full turgor ($\Psi\pi^{100}$), Osmotic potential at turgor loss ($\Psi\pi_0$) and cell Modulus of Elasticity (ξ) of mycorrhized (AM) and non-mycorrhized (Non-AM) date palm seedlings subjected to two watering treatments.

Water regime	AMF Status	RWC (%)	Ψ_w (Mpa)	W_s	$\Psi\pi^{100}$ (Mpa)	$\Psi\pi^0$ (Mpa)	ξ
WW	N-AM	98.62 a	-30.5 a	5.6 a	-4.8 b	-15.3 b	1.77 b
	AM-P	99.11 a	-27.2 b	5.3 b	-6.9 a	-25.0 a	3.52 a
WS	N-AM	93.48 b	-33.6 b	2.5 a	-20.0 a	-28.6 a	1.32 a
	AM-P	96.96 a	-37.0 a	1.7 b	-13.3 b	-25.0 b	0.54 b

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 3: Malonyldialdehyde (MDA), hydrogen peroxide (H_2O_2), soluble sugar and proline contents and oxidative damage in leaves of non-mycorrhizal or mycorrhizal date palm seedlings grown under well water or water stress conditions.

Water régime	AMF status	MDA (nmol.g^{-1} DM)	H_2O_2 (µmol.g^{-1} DM)	TSS (mg.g^{-1} DM)	Proline (nmol.g^{-1} DM)	OD (nmol MDA mg^{-1} prot)
WW	N-AM	53.2b	24.1b	54.6c	4105.2c	12.42b
	AM-P	38.6d	24.2b	78.7a	9193.2a	6.88c

Water régime	AMF status	MDA (nmol.g^{-1} DM)	H_2O_2 (µmol.g^{-1} DM)	TSS (mg.g^{-1} DM)	Proline (nmol.g^{-1} DM)	OD (nmol MDA mg^{-1} prot)
WS	N-AM	65.5a	28.4a	47.5d	3570.2d	20.47a
	AM-P	42.1c	27.3a	68.5b	4070.4c	6.35c

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 4: Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (G-POD) activities in leaves of non-mycorrhizal or mycorrhizal date palm seedlings grown under well water or water stress conditions.

Water statut	AMF statut	SOD (USOD g^{-1} DM min^{-1})	G-POD (mmol.g^{-1} DM min^{-1})	CAT (nmol g^{-1} DM min^{-1})	APX (mmol g^{-1} DM min^{-1})
WW	N-AM	652,3a	3,07c	116,7cd	2,11d
	AM-P	447,7c	4,20b	136,8c	2,89d
WS	N-AM	596,1b	4,44b	225,5a	4,68c
	AM-P	667,4a	5,87a	104,41d	7,05a

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).