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Effects of arbuscular mycorrhizal fungi on tree growth, leaf water potential, and levels of 1-aminocyclopropane-1-carboxylic acid and ethylene in the roots of papaya under water-stress conditions

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Abstract Seedlings of papaya (*Carica papaya* L. var. Solo) were transplanted to pots with or without an arbuscular mycorrhizal (AM) fungus (*Gigaspora margarita* Becker and Hall). After 3 months, half the plants were subjected to water stress by withdrawing irrigation. The leaf water potential (LWP) was measured during 20 days of water-stress treatment and then the plants were harvested. Root ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations were measured and plant fresh weight determined. The LWP decreased during the water-stress treatment and this decrease was more severe in the non-AM plants. Plant fresh weight was higher for AM than non-AM plants under both conditions. Under well-irrigated conditions, the ethylene concentration in the roots was increased by the presence of AM, although there was no significant difference between AM and non-AM roots in ACC levels. ACC increased in both AM and non-AM roots under water-stress conditions. The water-stress treatment resulted in a marked increase in ethylene concentration in non-AM roots but the concentration in AM roots was slightly lower than under normal conditions.

Keywords Arbuscular mycorrhizal fungi · 1-Aminocyclopropane-1-carboxylic acid · Ethylene · Water stress

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Introduction

The beneficial effects of arbuscular mycorrhizal (AM) symbioses in improving water-stress tolerance are well documented. Under drought-stress conditions, the extension of the root surface by the extraradical AM fungal mycelia may enhance the acquisition of nutrients and water (Koide 1985; Bethlenfalvay et al. 1988; Fitter 1988; Ruiz-Lozano and Azcón 1995; Ruiz-Lozano et al. 1995). However, the improvement in water status may be attributable to mechanisms not directly related to phosphorus nutrition or water uptake (Augé and Duan 1991; Davies et al. 1992). In addition to nutritional advantages and the improvement of water uptake, change in the balance of plant growth regulators may contribute to water-stress tolerance in AM plants (Sánchez-Díaz and Honrubia 1994).

Abeles et al. (1992) suggested that AM fungi alter the ethylene balance in plants. Some plant roots colonized by mycorrhizal fungi are known to suffer morphological changes induced by ethylene (Blake and Linderman 1992). Ishii et al. (1996) demonstrated that approximately 0.05 ppm of ethylene in the soil stimulated spore germination and hyphal growth of AM fungi in vitro, and AM formation in trifoliate orange roots. 1-Aminocyclopropane-1-carboxylic acid (ACC) is the precursor of ethylene (Yang and Hofmann 1984) in higher plants but the pathway of ethylene synthesis has not been documented in AM associations.

Papaya (*Carica papaya* L) is a tropical fruit known for its high nutritive value. Mohandas (1992) reported that AM inoculation of papaya seedlings increased growth, P concentration and acid phosphatase activity in leaves. The growth of and fruit production by papaya plants is affected by drought stress (Marler and Mickelbart 1998). However, no detailed reports exist on the effects of AM fungi on growth and tolerance to water-stress conditions in papaya or on the mechanisms involved. The present research aimed to investigate the improvement of water-stress resistance in the presence of AM fungi in papaya plants and the possible involvement of ethylene metabolism.

Materials and methods

Seeds of papaya (*C. papaya* var. Solo) were sown in trays with vermiculite. One month later they were transplanted to pots containing 8 l of sterilized sand substrate. The substrate was amended with 140 ppm of N, 10 ppm of P, 140 ppm of K and 50 ppm of Mg. The plants were inoculated by adding 5 g of an inoculum containing approximately 70 spores of *Gigaspora margarita* Becker and Hall obtained from Central Glass Co. Ltd., Japan.

The experiment was designed as a 2×2 factorial, which examined two conditions (normal irrigation and water stress) and two kinds of inoculation (AM and non-AM plants). Each treatment was replicated three times to give a total of 12 plants and these plants were randomized in a plastic greenhouse with no temperature-controlling system.

Three months after transplantation, tensiometers were inserted into pots to be water stressed in order to measure soil water potential (pF). The pots were covered with a polyethylene sheet tied around the base of the stem to keep the soil dry. Stressed plants were not watered for 20 days whereas irrigated plants were well watered during this period. To evaluate water stress during the period of 20 days, the leaf water potential (LWP) was measured using a pressure chamber with pressure supplied by nitrogen (Hellkvist et al. 1974).

Plants were harvested after 20 days when the pF of the water-stressed pots was approximately 2.7. Root and total fresh weights were measured. AM colonization was measured on root samples according to Phillips and Hayman (1970) and Ishii and Kadoya (1994). The ethylene concentration was measured in roots using a Hitachi 063 gas chromatograph equipped with a flame ionization detector (Ishii et al. 1982). The chromatogram of ethylene was obtained under the following operating conditions: glass column (3 mm×2 m) with activated aluminum of 60–80 mesh, N_2 carrier gas rate of 25 ml/min.; column temperature 120 °C; injector 150 °C; detector 150 °C. ACC was determined according to Lizada and Yang (1979).

Results

Colonization of papaya roots by *Gigaspora margarita* was higher under water-stress conditions (72.6% ±3.6) than under normal conditions (54.3% ±4.4). No root colonization of the non-AM control plants was observed in either condition.

Fresh weight increased in AM plants under both conditions (Table 1). Water stress reduced the yield of non-AM plants by 23.7% and of AM plants by 15.2%, so that mycorrhizal effects on fresh weight were greater under water stress (36.6%) than under irrigated conditions (29.6%). LWP decreased during the 20 days of water-stress and the decrease was more severe in non-AM than AM plants (Fig. 1).

Under irrigated conditions, the ethylene concentration in roots of AM plants was higher than that in non-AM plants, although the difference in ACC levels between AM and non-AM roots was not significant. The water-stress treatment resulted in an increase in ACC in both AM (0.35 nmol/mg fresh wt.) and non-AM (0.48 nmol/mg fresh wt.) roots. The ethylene concentration was markedly increased in non-AM roots by water-stress treatment but slightly reduced in AM roots (Table 2).

Table 1 Biomass yield (g) of papaya trees inoculated with an arbuscular mycorrhizal (*AM*) fungus, *Gigaspora margarita*, and non-inoculated (*Non AM*) trees under irrigated and water-stress conditions. The data are means±standard error (SE) ($n=3$) (*RFW* root fresh weight, *TFW* total fresh weight)

Treatment	Biomass yield			
	Irrigated		Water stressed	
	RFW	TFW	RFW	TFW
Non AM	55.2±5.8	99.4±9.8	44.0±5.4	75.8±7.3
AM	85.9±6.5	141.1±10.5	66.4±4.9	119.6±6.6

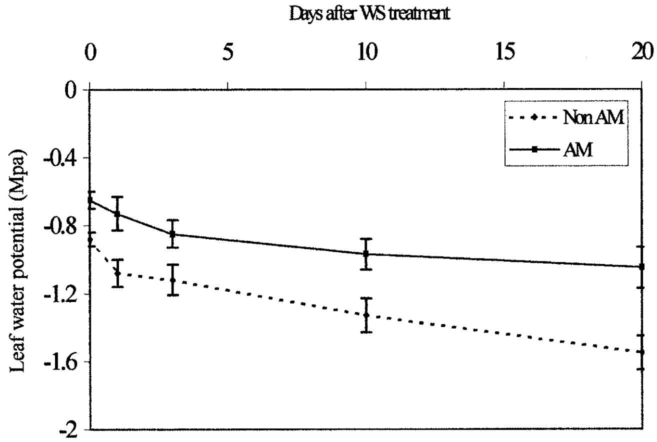

Fig. 1 Leaf water potential of papaya trees inoculated with an arbuscular mycorrhizal (*AM*) fungus, *Gigaspora margarita*, and non-inoculated (*Non AM*) trees during period of water stress (*WS*). *Vertical bars* indicate SE ($n=3$)

Table 2 Concentrations of 1-aminocyclopropane-1-carboxylic acid (*ACC*) and ethylene in papaya roots inoculated with an arbuscular mycorrhizal (*AM*) fungus, *Gigaspora margarita*, and non-inoculated (*Non AM*) trees under irrigated and water-stress conditions. The data are means±SE ($n=3$)

Treatment	ACC (nmol/g fresh wt.)		Ethylene (ppm)	
	Irrigated	Water stressed	Irrigated	Water stressed
Non AM	0.14±0.04	0.62±0.04	0.93±0.04	1.41±0.04
AM	0.06±0.01	0.41±0.04	1.35±0.04	1.23±0.03

Discussion

AM fungi such as *Glomus* spp. have been found to improve the growth and nutrition of papaya (Ramirez et al. 1975; Mohandas 1992). This was confirmed in the present investigation, especially when papaya plants were exposed to water stress. High levels of root colonization by *Gigaspora margarita* were associated with this improved water-stress tolerance. Positive mycorrhizal effects on LWP of papaya were also observed under water-stress conditions.

Ruiz-Lozano and Azcón (1995) suggested that water taken up by the hyphae in AM plants explains the improved water relations of AM plants. Shrestha et al. (1995) reported that photosynthetic and transpiration rates of AM satsuma mandarin trees (*Citrus unshiu* Marc.) were higher than non-mycorrhizal trees. Furthermore, the rate at which soil water deficits arise or the size of the plant may modify the influence of drought on plant physiology (Marler and Mickelbart 1998).

The higher levels of AM colonization in water-stressed roots may be related to a decrease in ethylene concentration during the water-stress period. This hypothesis is supported by results of Ishii et al. (1996), who showed that approximately 0.05 ppm of ethylene in the soil stimulated spore germination and hyphal growth of *Gigaspora ramisporophora* and *Glomus mosseae* in vitro and mycorrhizal formation in trifoliate orange trees. These authors suggested that a low level of ethylene promotes the activity of AM fungi in the rhizosphere. Mycorrhiza establishment may result in the control of ethylene levels as one mechanism of reducing damage by water stress in papaya plants. Besmer and Koide (1999) showed that mycorrhizal colonization can decrease ethylene concentration in flowers, which might explain the increased vase-life of cut flowers.

Drought stress is generally associated with ACC and ethylene production (Abeles et al. 1992). Our results showed that both ACC and ethylene levels were lower in AM than in non-AM roots under water stress. AM colonization may act as an inhibitor of ethylene biosynthesis by influencing ACC conversion to ethylene. MacArthur and Knowles (1992) reported that substances leached from AM roots of potato inhibited ACC oxidase activity and that endogenous ethylene production was reduced in potato roots after AM colonization.

In conclusion, water stress can induce the production and the release of ethylene from papaya roots, which may stimulate AM colonization and maintain mycorrhizal growth effects. The presence of AM fungi may influence ethylene production in roots in a way which prevents concentrations high enough to damage the plants. Further investigations are needed to understand the mechanism by which AM fungi control ethylene biosynthesis in plants subjected to water stress.

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