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Mycorrhizal colonisation improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions

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Abstract

The effect of arbuscular mycorrhizal (AM) colonisation by *Glomus clarum* on fruit yield and water use efficiency (WUE) was evaluated in watermelon (*Citrullus lanatus*) cv. Crimson Sweet F1 under field conditions. Treatments were: (1) well-watered plants without mycorrhizae (WW-M), (2) well-watered plants with mycorrhizae (WW+M), (3) water- stressed plants without mycorrhizae (WS-M) and (4) water-stressed plants with mycorrhizae (WS+M). When soil water tension readings reached −20 and −50 kPa for well-watered (WW) and water-stressed (WS) treatments, respectively, irrigation was initiated to restore the top soil to near field capacity. Water stress reduced watermelon shoot and root dry matter, fruit yield, water use efficiency but not total soluble solids (TSS) in the fruit, compared with the non-stressed treatments. Mycorrhizal plants had significantly higher biomass and fruit yield compared to nonmycorrhizal plants, whether plants were water stressed or not. AM colonisation increased WUE in both WW and WS plants. Macro- (N, P, K, Ca and Mg) and micro- (Zn, Fe and Mn) nutrient concentrations in the leaves were significantly reduced by water stress. Mycorrhizal colonisation of WS plants restored leaf nutrient concentrations to levels in WW plants in most cases. This is the first report of the mitigation of the adverse effect of water stress on yield and quality of a fruit crop.

Introduction

Water stress limits crop production throughout the world (Kramer and Boyer, 1995) and, contrary to other factors (e.g., acid, alkaline or saline soils), is highly variable within a given growing season and from year to year (Gutierez-Boem and Thomas, 1999). Even under supplemental irrigation crop growth and yield are affected during low rainfall months because water stress reduces flowering and increases fruit drop (Nagel, 1995). Optimisation of irrigation is particularly crucial for production and profitability of horticultural crops (Hardeman et al., 1999; Smittle et al., 1994; Wierenga and Saddiq, 1985). When water supply is limited, crop management practices that enhance drought resistance, plant water-use efficiency and plant growth are particularly beneficial (Egilla et al., 2001; Kirnak et al., 2001; Sangakkara et al., 2000; Figueiredo et al., 1999; Sangakkara et al., 1996; Abdul-Baki et al., 1992).

Mycorrhizal fungi improved water use efficiency in *Rosa hybrida* plants (Henderson and Davies, 1990) and in safflower and wheat (Bryla and Duniway, 1997a). Mycorrhizal inoculation may directly enhance root water uptake providing adequate water to preserve physiological activity in plants, especially under severe drought conditions (Faber et al., 1991; Smith and Read, 1997). Mycorrhizal inoculation also improves root P uptake, particularly under dry soil conditions (Jakobsen, 1995). Mycorrhizae, therefore, are likely to be important for increasing P acquisition during drought periods (Bryla and Duniway, 1997b).

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A strategy for management of drought through improvement of nutrient uptake would be to inoculate soils with appropriate AM fungi immediately prior to transplant of a horticultural crop or to adopt cultural practices that encourage native populations of AM fungi in the field soil. In this paper we report effects of mycorrhizal inoculation on transplanted watermelon subjected to water and nutrient stress conditions in the field.

Materials and methods

Plant culture, experimental design and irrigation treatments

Experiments were conducted at the Research Station of the Agriculture Faculty, University of Harran (Turkey) from May to September 2001 with watermelon (*Citrullus lanatus* Thunb. cv. Crimson Sweet F1). Temperature ranged from 23 to 40 °C during the trial period. The average daily pan evaporation during the experiment was 12.5 mm. Water content at field capacity, water content at the permanent wilting point, dry bulk density, pH, organic matter content and electrical conductivity (EC) of the top 0.3 m of soil were 30.8, 24.4%, 1.38 kg/m^3, 7.1, 1.2% and 1.10 dS/m, respectively. Soil texture was silty clay (Harran series) described as fine, smectitic, thermic and aridic (Aydemir, 2001). Electrical conductivity and pH of the irrigation water were 0.48 dS/m and 7.0, respectively.

Watermelon seeds were sown in small (100-mL) plastic pots (one seed per pot) containing equal volumes of pasteurized field soil (silty clay), animal manure and acid-washed sharp sand. At the first true leaf stage, seedlings selected for size uniformity were transplanted to the field. Soil was fumigated before planting. Ten plants per treatment replicate (or plot) were spaced 1.0 m apart within rows and 2.0 m apart between rows. Each treatment was replicated three times in a randomised split-block design. Each block contained 30 plants. A drip irrigation system (T-Systems, San Diego, CA) was controlled manually, with single laterals laid out for each row on the soil surface. Drippers with a constant discharge of 2.0 L h^{-1} were spaced 1 m apart (one dripper per plant). Irrigation frequency was controlled using tensiometers placed at 0.45 m depth within each subplot. Each plot had a separate flow meter to monitor water input. There was no rainfall during the experimental period. The plants were sprinkler irrigated from 11:00 to 16:00 hours each day for a week after field planting at 4 mm h^{-1} to promote root establishment without stress.The amount of irrigation water applied to plants was calculated by multiplying pan evaporation by a pan coefficient of 0.65 and a variable crop coefficient (K_c) based on the growth stage. Crop coefficient values were obtained from Smajstrla (2000) and updated for growth stage using unpublished local data. Crop coefficients used for irrigation monitoring for initial (20 days), crop development (30 days), mid-season (35 days), and late season (20 days) were 0.30, 0.60, 0.90, and 0.70 respectively.

Treatments were: (1) well-watered plants without mycorrhizae (WW-M), (2) well-watered plants with mycorrhizae (WW+M), (3) water-stressed plants without mycorrhizae (WS-M) and (4) water-stressed plants with mycorrhizae (WS+M).

Fertilisation, mycorrhizal inoculation and irrigation regime

All treatments received for the season N (180 kg/ha), P(44 kg/ha)and K (102 kg/ha) as dry and liquid fertiliser in the form of ammonium sulphate, triple superphosphate and potassium sulphate, respectively. Forty percent of the N and K fertiliser was surface applied and 100% of the P fertiliser was banded into the soil prior to planting. The remaining 60% of N and K was added at weekly intervals through the drip irrigation system starting 2 weeks after transplanting until 2 weeks before the last harvest. A regular spray programme for disease and insect control was followed throughout the growing period.

Plants were inoculated with *Glomus clarum* Nicolson & Schenck obtained from Soil Science Department of Cukurova University, Turkey. The isolate was originally supplied by Nutri-Link, USA (Ortas et al., 2002). The isolate was propagated on maize grown in a greenhouse for 8 weeks on a perlite/vermiculite medium. Colonised maize roots were used as an inoculum (30 g fresh weight per plant) placed at 15–20 cm soil depth immediately prior to transplanting of the watermelon seedlings into the field plots.

Water stress treatment started 4 weeks after inoculation to allow time for mycorrhizal colonisation to establish in the roots of the watermelon seedlings. When the soil water tension readings reached −20 and −50 kPa for the WW and WS treatments respectively, irrigation was initiated to restore the top soil to near field capacity.

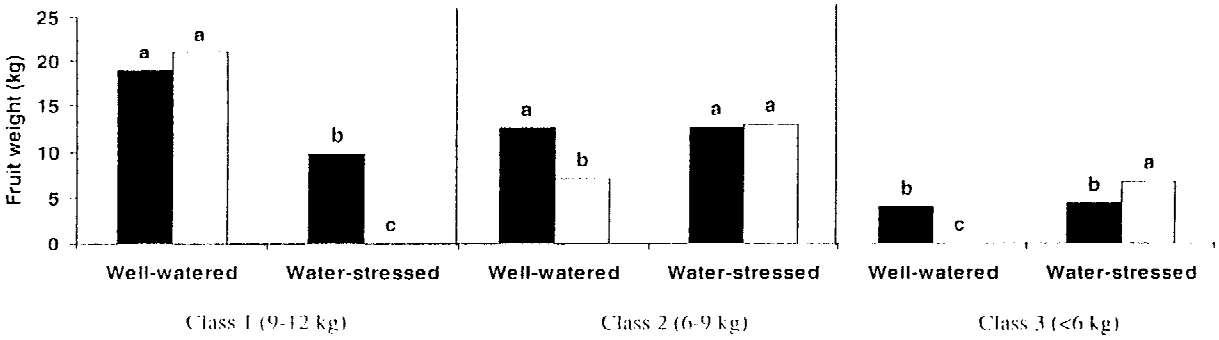

Figure 1. Size class distribution of fruit harvested from mycorrhizal (■) and non-mycorrhizal (□) watermelon plants grown under well-watered and water-stressed conditions. Within fruit class, same letter indicates no significant difference between treatments ($P < 0.05$).

Fruit harvest, dry weight, mycorrhizal colonisation and nutrient analysis

Fruits were harvested over a period of 4 weeks from mid August to September. Fruits were classified into three groups: class 1, 9–12 kg; class 2, 6–9 kg; and class 3, <6 kg. Fruit yield data are the means of eight randomly chosen plants per replicate. Total soluble solid (TSS) in fruit juice was measured at room temperature in three fruits per plant and two plants per replicate (chosen at random) using a hand refractometer. The water use efficiency (WUE) was expressed as yield per unit of plant water use.

Total dry matter accumulation at final harvest was estimated using three plants minus their fruit from each replicate plot. Root samples were collected from a depth of 45 cm soil and 50 cm apart from the plant shoot. The shoots were separated from roots at the soil surface and the dry weights of root and shoot (leaves + stem) determined after drying at 70 °C for 48 h to constant weight.

Root samples were cleared with 10% KOH solution and stained with 0.1% Trypan blue before estimation of mycorrhizal colonisation (Rufyikiri et al., 2000). Arbuscular mycorrhizal (AM) colonisation was estimated using a modified line intersect method from McGonigle et al. (1990), where a minimum of 100 line intersections per root sample, replicated three times, were scored for presence of AM structures. These observations were made using light microscopy to rate the degree of root infection by AM fungi in one plant per replicate (three plants per treatment). The percentage of AM infection was calculated from the following equation:

$$\% \text{ of AM infection} = (\text{Root length infected}/\text{Root length observed}) \times 100$$

Leaf samples from two randomly chosen plants (fifth leaf from the apex) were collected at early fruit stage. Leaves were washed, dried to constant weight, ashed at 550 °C, acid extracted and the extract made up to constant volume (Kaya and Higgs, 2002). All chemical elements except N were determined in these sample solutions. Total Zn, Fe, Mn, Ca and Mg (with additional lanthanum for Ca and Mg) were determined using an atomic absorption spectrophotometer (Unicam Solar 929, UK). Total N was measured in samples of 0.1 g dry weight using the Kjeldahl method. P was analysed by the vanadate-molybdate method (Chapman and Pratt, 1982) . K in the sample solution was analysed using a flame photometer (Corning 400, UK) (Chapman and Pratt, 1982).

Statistical analysis

Differences among treatments were analysed for main effects (irrigation and mycorrhizae) and their interaction by two-way ANOVA using the Minitab statistical software package (Minitab Inc., State College, PA). Treatment effects were considered significant at $P < 0.05$.

Results

Mycorrhizal colonisation was successful in both mycorrhizal treatments while no root colonisation was present in either the WS-M or WW-M treatments (Table 1). The highest percentage root colonisation occurred in the WW+M treatment, but there was substantial AM colonisation (68%) in the WS+M plants as well.

Water stress significantly reduced root and shoot dry matter compared with the WW treatments. Mycor-

Table 1. Growth and yield response of mycorrhizal (+M) and non-mycorrhizal (−M) watermelon plants grown under well-watered (WW) and water-stressed (WS) conditions

Treatments		Water	Root	Shoot	Root	Yield (kg plant^{-1})		TSS	Water use
Irrigation	Inoculation	applied (mm)	colonization (%)	dry matter (g plant^{-1})	dry matter (g plant^{-1})	Marketable	Unmarketable	(%)	efficiency (g mm^{-1})
WW	+M	701a	87a	134.9a	13.7a	35.26a	0.04c	10.9b	50.3a
WW	−M	701a	0c	125.2a	12.4a	27.96b	0.04c	10.5b	39.9c
WS	+M	567b	68b	98.4b	10.7b	26.13b	0.57b	11.1b	46.1b
WS	−M	567b	0c	73.6c	8.7c	17.60c	1.91a	12.6a	31.0d
Interaction									
Irrigation × Mycorrhizae		ns	**	**	**	*	**	**	**

Two-way ANOVA test: within each column, same letter indicates no significant difference for the different treatments at the $P < 0.05$ level. Interaction with two-way ANOVA test: ns – not significant, * $P < 0.05$, ** $P < 0.01$.

rhizal colonisation significantly improved these parameters in the water- stressed plants but they remained lower than values for well-watered plants in all cases. Mycorrhizal colonisation did not significantly affect most parameters in WW plants but did improve both marketable yield and WUE (Table 1). Interactions between irrigation and mycorrhizal colonisation were significant for both shoot and root dry matter ($P < 0.01$).

Fruit yield was significantly reduced by WS compared to WW plants (Table 1). Mycorrhizal colonisation significantly mitigated the detrimental effect of water stress on fruit yield with higher total yields (which were similar to WW-M treatment) than in the nonmycorrhizal treatment (Table 1). Mycorrhizal colonisation also improved numbers of fruit in classes 2, 3 and total fruit yields in the WW plants (Table 1, Figure 1). No class 1 fruits were produced in the WS treatment; WS+M treatment resulted in a significant level of class 1 fruit (Figure 1). Total soluble solids increased in the fruit of water stressed plants compared to other treatments. Unmarketable fruit yield (due to cracked, misshapen and small fruit and those with blossom end rot) was significantly higher in WS treatments and marketable fruit yield was at its lowest in WS-M treatment. Mycorrhizae in water stressed plants significantly reduced unmarketable fruit and increased marketable fruit yield to a level similar to WW values (Table 1). The highest marketable fruit yield was achieved in WW+M plants.

The total amount of water applied during the experiment was 567 and 701 mm for water-stressed and well-watered plants, respectively. Water use efficiency was highest in the WW+M treatment and lowest in WS-M treatment but WUE in the WS+M treatment was significantly higher than WW-M treatment (Table 1). Interaction between irrigation and mycorrhizal colonisation was significant ($P < 0.01$) for WUE.

Mycorrhizal colonisation in the WW plants resulted in the highest nutrient concentrations in most cases (Table 2). Leaf concentrations of all macro- and micro-nutrients tested were reduced in the WS treatment compared to the WW. Nutrient concentrations in the WS+M treatment were comparable to those in WW-M (except for Fe and Mn). Interaction between irrigation and mycorrhizal colonisation was significant ($P < 0.05$) for K, Ca, Fe, Mn and Zn and not significant for N, P and Mg. Yield was positively correlated with all macro- and micro-nutrients in leaves (Table 2).

Discussion

In this field study, irrigation limitation significantly reduced dry biomass of roots and shoots of watermelon. Similar effects of water stress on dry matter production have been reported in many different fruit species, e.g., in kiwifruit (Chartzoulakis et al., 1993), citrus (Huang et al., 2000) and strawberry (Kirnak et al., 2001).

Substantial root colonisation by *G. clarum* of watermelon seedlings resulted after inoculation of the soil in the root zone before transplant. Thus, differences in growth, fruit yield and other measured characteristics between −M and +M treatments are reasonably linked to the presence and function of mycorrhizae. A variety of mechanisms have been proposed for how mycorrhizae may ameliorate the effects of water stress in plants. For example, mycorrhizal colonisation may increase root length density or al-

Table 2. Macro- and micro-nutrient concentrations in leaves of mycorrhizal (+M) and non-mycorrhizal (−M) watermelon plants grown under well-watered (WW) and water-stressed (WS) conditions

Treatments		Macronutrients (mg g^{-1})					Micronutrients ($\mu g\ g^{-1}$)		
Irrigation	Inoculation	N	P	K	Ca	Mg	Fe	Mn	Zn
WW	+M	48.9a	4.3a	49.8a	28.7a	8.7a	198a	216a	41a
WW	−M	42.2b	3.5b	45.6b	22.5b	6.7b	174b	198a	35ab
WS	+M	40.2b	3.1b	42.4b	19.8b	6.0b	154c	176b	30b
WS	−M	34.9c	2.5c	34.5c	14.6c	4.2c	106d	121c	19c
Interaction									
Irrigation × Mycorrhizae		ns	ns	*	*	ns	*	*	*
Correlations									
Yield		0.989**	0.982**	0.986**	0.988**	0.995**	0.980**	0.965**	0.970**

Two-way ANOVA test: within each column, same letter indicates no significant difference for the different treatments at the $P < 0.05$ level.
Interaction with two-way ANOVA test: ns – not significant, * $P < 0.05$.
** Yield positively correlated at $P < 0.01$ for each nutrient.

ter root system morphology, enabling colonised plants to explore more soil volume and extract more water than uncolonised plants during drought (Kothari et al., 1990; Davis et al., 1996; Bryla and Duniway, 1997a). Mycorrhizal hyphae may also directly enhance root water uptake, increasing water supply which would help sustain physiological activity within plants (Allen, 1982). Mycorrhizal colonisation enhances stomatal control in rose plants and reduces water loss during drought (Auge et al., 1986). Moreover, improved drought tolerance and better drought recovery by mycorrhizal plants has been linked to improved P uptake (Nelsen and Safir, 1982; Graham et al., 1987; Fitter, 1988). Our findings support all of these views because mycorrhizae enhanced uptake of P and root growth of water-stressed watermelon plants compared to those without mycorrhizal colonisation. This is the first report of mitigation of the adverse effect of water stress on yield and quality of a fruit crop although similar results to ours were obtained in potato tubers (Duffy and Cassells, 2000).

Reduction in macro- and micro-element concentrations in the leaves after water stress of watermelon is supported by our previous work with strawberry (Kirnak et al., 2001). The finding that mycorrhizae increased leaf macro- and micro-nutrient concentrations irrespective of water stress is typical of mycorrhizal plants under mineral stress conditions (Smith and Read, 1997). The most commonly reported mineral nutrient enhanced in host plants with mycorrhizae is P (Bolan, 1991; Jakobsen, 1995; Marschner and Dell, 1994), but many other mineral nutrients (e.g., N, S, Ca, Mg, K, Zn and Cu) may be enhanced in plants by mycorrhizal fungi (Clark, 1997; Marschner and Dell, 1994; Persad-Chinnery and Chinnery, 1996).

In conclusion, water stress has been shown to adversely affect growth, nutrition and fruit yield of watermelon cultivar 'Crimson Sweet F1'. This study confirms that mycorrhizal colonisation can mitigate the adverse effects of water stress on field-grown watermelon restoring most of the key growth and yield parameters to levels similar or close to those in unstressed plants.

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