Scientia Horticulturae 187 (2015) 35–43

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Growth and yield promoting effect of artificial mycorrhization on field tomato at different irrigation regimes

Vincenzo Candido[a], Gabriele Campanelli[b], Trifone D'Addabbo[c,*], Donato Castronuovo[a], Michele Perniola[a], Ippolito Camele[a]

[a] School of Agricultural, Forest, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy
[b] Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca per l'Orticoltura (CRA-ORA), Via Salaria 1, 63077 Monsampolo del Tronto (AP), Italy
[c] Institute for Sustainable Plant Protection (IPSP), National Research Council, Via G. Amendola 122/D 70126 Bari, Italy

ARTICLE INFO

Article history:
Received 20 October 2014
Received in revised form 24 February 2015
Accepted 25 February 2015

Keywords:
Artificial mycorrhization
Irrigation regimes
Tomato
Water use efficiency
Yield

ABSTRACT

Plant inoculation with formulations of vesicular–arbuscular mycorrhiza (VAM) can be a sustainable technique for the improvement of tomato yield and plant resistance to biotic and abiotic stresses. Combination of artificial plant mycorrhization with water deficit irrigation could be an effective agronomical technique for the optimization of water use efficiency of tomato in the areas with a limited water availability. A 2-year research on field tomato was undertaken in Southern Italy (40°24′N; 16°48′E; 10 m a.s.l.) to evaluate the effects on crop growth, yield, and fruit quality of the combination of seedling inoculation with two VAM formulations, alone or integrated with plant growth promoting rhizobacteria (PGPR), with different irrigation regimes (restoration of 0%, 50%, and 100% of maximum crop evapotranspiration). A split-plot experimental design with three reps was followed, with irrigation regimes in the main plots and mycorrhizal treatments in the subplots. Both VAM treatments, either with or without PGPR, demonstrated to be highly and rapidly effective on plant growth, as significantly increasing growth of tomato seedlings and plant biomass at mid and end of both crops compared to the non-inoculated control. Positive effects of mycorrhizal inoculation were extended also to marketable yield, mainly as a result of an increased number and weight of fruits. Both VAM inocula did not significantly affect fruit quality parameters, though increased water use efficiency of marketable yield. Both irrigation regimes positively affected tomato growth and marketable yield, whereas the fruit quality was better in less- and non-watered plants. Adversely to expectations, no synergism was found between artificial mycorrhization and irrigation regimes.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is classified as a crop with a high water demand and sensitive or moderately tolerant to water stress (Zheng et al., 2013; Karlberg et al., 2007). Tomato tolerance to water deficit was generally reported as depending on the cultivar, the phenological stage of deficit occurrence and the severity of the stress (Patanè et al., 2011; Candido et al., 2000; Perniola et al., 1994).

Under conditions of water scarcity, the large water demand of tomato can be a limit to crop productivity and, therefore, all agronomical practices enhancing drought resistance, plant water-use efficiency (WUE), and plant growth can be highly beneficial (Hardeman et al., 1999; Egilla et al., 2001; Kirnak et al., 2001; Sangakkara et al., 2000). Deficit irrigation is an agronomical practice aimed to maximize WUE and to stabilize yields by limiting water applications to the drought-sensitive growth stages of the crop (Fereres and Soriano, 2007). Deficit irrigation has been widely investigated on many crops, among which also tomato, and generally reported as a valuable and sustainable production strategy for dry regions (Geerts and Raes, 2009; Kirda et al., 2004). Under an average reference crop evapotranspiration (ETc), ranging from 3.3 to 7 mm d^{-1} during initial growing stage and full crop growth, respectively. Zairi et al. (2003) reported the application of 65% of ETc as economically acceptable to tomato crop. Moreover, Baselga Yrisarry et al. (1993) documented an enhancement of fruit quality as consequent to a moderate water stress, mainly due to the increase of soluble solids content. Adversely, Topcu et al. (2007)

* Corresponding author at: Institute for Sustainable Plant Protection (IPSP), National Research Council, Via G. Amendola 122/D, 70126 Bari, Italy.
Tel.: +39 0805929233; fax: +39 0805929230.
E-mail address: trifone.daddabbo@ipsp.cnr.it (T. D'Addabbo).

http://dx.doi.org/10.1016/j.scienta.2015.02.033
0304-4238/

reported a significant reduction of tomato yield after the application of 50% of ETc.

Vesicular–arbuscular mycorrhiza (VAM) are mainly known to supply plants with additional nutrients, mainly nitrogen and phosphorus (Hodge et al., 2001; Harrison and van Buuren, 1995). An improvement of plant photosynthetic activity, absorption of microelements, and resistance to root pathogens, as well as of soil properties, were also reported as further effects of VAM symbiosis (Turk et al., 2006; van der Heijden et al., 2006). Moreover, the endotrophic symbiotic association of VAM with plant roots can also induce a better plant WUE and resistance to drought stress-saline (Augè, 2001; Lee et al., 2012).

Plant-growth-promoting rhizobacteria (PGPR), such as nitrogen fixers, fluorescent *Pseudomonas* and sporulating *Bacillus* can also play a relevant role in plant growth and soil pathogen suppression, mainly due to their synergistic interaction with VAM (Elshafie et al., 2013; Compant et al., 2005).

The positive effects of mycorrhizal symbiosis on plant health and growth are raising the interest in the use of these fungi as bio-fertilizers, bio-regulators, and bio-protectors, thus reducing the input of synthetic fertilizers and pesticides (Akhtar and Siddiqui, 2008; Gera Hol and Cook, 2005; Tilman et al., 2002). Seedling pre-inoculation with commercial preparations of VAM in nursery containers is the most promising method for the implementation of mycorrhization in horticultural crops (Candido et al., 2013; Douds et al., 2007; Larsen et al., 2007). A reduced seedling mortality, as well as a greater uniformity of crop growth and an increased crop yield were reported as effects of the pre-inoculation of seedlings with VAM, though largely depending on the specificity of the association between the fungus and the species/cultivars of host plant (Sorensen et al., 2008; Sensoy et al., 2007). Commercial inocula consists mostly of a mixture of VAM (*Glomus mosseae*, *G. intraradices*, *G. viscosum*) either pure or in a mixture with PGPR and ectomycorrhizal fungi (Dalpé and Monreal, 2004).

The association of artificial plant mycorrhization with water deficit irrigation can be an interesting technique for the optimization of water use in tomato, due to the better resistance to drought of mycorrhized plants (Davies et al., 2002; Augè, 2001). An increased root length and density or an altered root system morphology, as enhancing soil exploration and water extraction, have been hypothesized as potential mechanisms for the improved drought resistance of mycorrhized plants (Bryla and Duniway, 1997; Davies et al., 1996). Enhancement of plant stomatal control or root water uptake by mycorrhizal hyphae, as well as turgor maintenance by osmotic adjustment, have been also documented (Auge et al., 1986; Allen, 1982). The exploitation of beneficial effects of combining artificial plant mycorrhization with water deficit irrigation can be particularly useful to tomato crop in the areas where the availability of irrigation water is generally limited, such as Southern Italy and, in general, Mediterranean regions. This paper reports the results of a 2-year research on open field tomato aimed to investigate the agronomical effects of the combination of two different VAM inocula (with or without PGPR and saprophytic fungi) with different irrigation regimes (0%, 50%, and 100% restoration of ETc).

2. Materials and methods

The experiment was carried out at the experimental farm "Pantanello" (40°24′N; 16°48′E; 10 m a.s.l.), situated in the Metapontum plain (Basilicata Region, Southern Italy) in the years 2008 and 2009, on a silty-loam soil, with pH 7.68 ± 0.11, a low total nitrogen content (0.80 ± 0.12 g kg^{-1}) and a good level of exchangeable phosphorus (21.2 ± 1.2 mg kg^{-1}) and potassium (215 ± 9 mg kg^{-1}).

Soil was ploughed to a depth of 30 cm and then rotavated and leveled at the time of basal dressing (50 kg ha^{-1} N, 123 kg ha^{-1} P_2O_5, and 245 kg ha^{-1} K_2O).

2.1. Plant material

Tomato hybrid (F_1) cultivar 'Faino' (Syngenta Seeds Co.; Wilmington, DE, USA) was sown on 21 April 2008 and 28 April 2009, respectively, within a 62 × 35 cm box containing a turf-based substrate (COMPO SANA®, COMPO Italia Co., Cesano Maderno, Italy; 60% OM, 6.0–6.5 pH) and reared in a metal-plastic (PE 200 μm) greenhouse provided with lateral openings and anti-insect net. At the full extension of cotyledon leaves, i.e., on 5 May 2008 and 13 May 2009, respectively, the bare root seedlings were singly transferred to 60-cell polystyrene alveolate containers, filled with 63 ml of the same turf-based substrate per each cell. Seedlings at the 4–5 true leaf development stage were transplanted into the field on 26 May 2008 and 28 May 2009, respectively, 30 cm spacing between plants and 150 cm between twin rows (4.44 plants per m^2).

Tomato seedlings were inoculated with VAM formulations at their transfer into the alveolate containers (20 days before transplanting in field), by pipetting 1.15 ml of mycorrhizal formulations into each alveolus, corresponding to 0.1 g plant^{-1} of biologically active ingredient.

2.2. Experimental design

A total of nine experimental treatments were provided, according to a split-plot block design, with three replicates of each treatment. Three irrigation treatments were placed in the main plots, and three mycorrhizal treatments in the 24.3 m^2 (4.5 × 5.4 m) subplots. Each subplot (experimental unit) consisted of three twin rows including 108 tomato plants. The three irrigation regimes were:

(1) *V100*, full restoration (100%) of ETc.
(2) *V50*, 50% restoration of ETc.
(3) *V0*, no restoration of ETc, i.e., a single irrigation at transplanting time.

The mycorrhizal treatments were:

(1) *M0*, control without any inoculation.
(2) *M1*, seedling inoculation with a commercial mycorrhizal formulation (Micosat F®, CCS Aosta S.r.l. Company; Quart, Italy) containing VAM (*G. mosseae* GP 11, *G. intraradices* GB 67, and *G. viscosum* GC 41), rhizosphere bacteria (*Agrobacterium radiobacter* AR 39, *Bacillus subtilis* BA 41, and *Streptomyces* spp. SB 14), and saprophytic fungi (*Beauveria* spp., *Trichoderma harzianum* TH 01, *Pichia pastoris* PP 59). In particular, 100 g of this formulation contained 25 g of ground mycorrhizal roots together with spores and hyphae of *Glomus* (crude inoculum). The percentage of biologically active ingredients was 6.2%.
(3) *M2*, treatment with the single *Glomus* spp. commercial inoculum described above, without bacteria and saprophytic fungi.

A drip irrigation system (water flow 2.5 L h^{-1}/dripper) was used. Hoses, pierced with holes every 30 cm, were placed in the middle of the twin-rows.

Irrigation scheduling of V100 and V50 was based on simplified soil water balance method (Evapotranspirometric Criteria) (Doorenbons and Pruitt, 1977); ETc was calculated according to the evapotranspiration approach of (ETc = ET0 × Kc), where ET0 is the reference evapotranspiration, calculated according to Hargreaves and Samani (1985), and the crop coefficient (Kc) as reported by Tarantino and Onofri (1991) for tomato. Water was applied on a weekly basis.

A 300 m^3 ha^{-1} water volume was applied to V0, V50, and V100 at the transplant of seedlings, as to allow a uniform plant establishment. Two applications of ammonium nitrate, corresponding to 80 kg ha^{-1} total nitrogen, were provided to all plots as a post-transplant mineral fertilization. Treatments for the control of insects and phytopathogens were applied as needed. Only non-systemic formulations were used, in order to avoid any interference with VAM colonization of tomato roots. In particular, Methomyl (Lannate® 25, Du Pont) was used against insects, whereas sulfur (TIOVIT JET®, Syngenta Crop Protection, Basel, Switzerland) and copper (Cupravit 35 WG, Bayer CropScience, Leverkusen, Germany) products were applied against fungal phytopathogens. Hoeing was used for weed control.

2.3. Data recorded

At the transplant of each crop, stem height and diameter, number and total area (Area Meter LI-Cor, Inc., Lincoln, NE, Model 3100) of leaves per plant, and dry weight of epigeal part and root biomass were recorded on 10 inoculated and non-inoculated seedlings. At mid growing season (full flowering–fruit setting), i.e., on 30 June 2008 and 9 July 2009, respectively, plant height, number of stems, stem diameter, above ground dry biomass (stem and leaves) and leaf area index (LAI) were recorded on five plants from each plot.

Tomato fruits were harvested from the central twin row of each plot on 12 August and 8 September 2008 and on 7 and 27 August 2009, respectively. At each harvest, total and marketable yield and number and weight of marketable and unmarketable fruits were recorded in each plot. Fruit diameter, soluble solid content (°Brix), and dry matter content were recorded on ten fruits randomly taken from the marketable yield of each subplot.

At the end of each harvest, fresh shoot weight (stem and leaves) was recorded on the plants from the sampling area of each plot. Samples of these plants were oven dried and weight of total above ground biomass (stem, leaves, and fruits) was determined. Harvest index (HI), i.e., the ratio between fruit and total dry weight, and irrigation water use efficiency (IWUE, kg m^3), corresponding to the ratio between dry weight of marketable yield and total volume of irrigation water (Tarantino et al., 1997; Steduto, 1996), were also calculated.

Mycorrhizal root colonization was estimated on thirty fragments, each 2-cm length, randomly taken from each root sample of inoculated and non-inoculated plants after clearing and staining (Brundrett et al., 1984). Frequency of colonization, intensity of colonization and presence of arbuscules and vesicles were determined according to Trouvelot et al. (1986) using Mycocalc software (INRA, Dijon, France). Analyses were carried out in three stages, i.e., at transplant and at mid and end crop-growing season.

Weather data were collected during both tomato crops. Air temperature and relative humidity were measured by 50Y probes (CS500-L mod., Campbell Scientific Inc., Utah, USA), whereas an electronic tipping bucket rain gauge (TB4MM-L mod., Campbell Scientific Inc., Utah, USA – 0.2 mm resolution) was used for rainfall measurement. All data were recorded by a CR 10× data-logger (Campbell Scientific Inc., Utah, USA).

Crop data were subjected to factorial analysis of variance, considering years and irrigation and mycorrhizal treatments as sources of variation, and means were separated by the Student–Newman–Keuls (SNK) test at $P \leq 0.05$. Statistical package SAS (ver. 17 9.1., 2005) (SAS Institute Inc., Cary, NC, USA) was used.

3. Results and discussion

3.1. Weather data

Crop season was less rainy in the first than in the second crop, as 38 and 113 mm total rainfall were recorded during the first and second crop, respectively, vs. a 88 mm multi-year average (Fig. 1A). In the first crop, rainfalls were concentrated from 10 to 20 May, i.e., before tomato transplanting, and from 20 to 31 July, 19 and 16 mm, respectively, whereas in the second crop most of rainfall occurred from 20 to 31 May (17 mm) and from 20 June to 31 July (87 mm), i.e., during flowering, setting, and early growth of tomato.

In the first crop, maximum air temperatures were significantly lower than the multi-year average in the period 20–31 May, but higher from 20 June to the end of the crop (Fig. 1B). Peak temperatures were recorded in the interval 20–30 June (35.6 °C) and from 1 to 10 July (34.8 °C) and August (35.3 °C). In the second crop, maximum temperatures were above the multi-year average at start growing season, i.e., from 20 to 31 May (30 °C). The highest values were reached from 20 to 31 July (36.1 °C) and from 10 to 20 August (34.3 °C), whereas the lowest values occurred in the period 20–30 June. Trend of minimum and maximum temperatures was almost similar to that of maximum values. Ten-day mean minimum values maintained above 20 °C more frequently in the first than in the second crop.

Ten-day minimum and maximum mean values of relative humidity (RU) were lower in the first than in the second crop (Fig. 1C). Maximum and minimum RU values ranged 70–75% and 20–22%, respectively, in the period from end June to end August 2008. Relative humidity was particularly high from 20 to 30 June and from 1 to 10 July 2009, ranging maximum and minimum values largely above the multi-year average, 95% and 40%, respectively.

3.2. Irrigation data

Eleven and 10 irrigations were provided to the first and the second crop, respectively. In the first crop, 4260 and 2280 m^3 ha^{-1} of total irrigation volumes were applied to V100 and V50, respectively, vs. 3705 and 2003 m^3 ha^{-1}, respectively, applied to the second crop. The higher irrigation volumes of the first crop were mainly due to the longer growing season and the larger evapotranspiration.

3.3. Effects of mycorrhizal treatments on tomato seedlings

Effects of mycorrhization were evident since the early stages of plant growth, as at the transplant of both crops frequency of colonization was significantly higher on the seedling roots from both M1 and M2 treatments than on those from M0 (Table 1). In the first crop, frequency of colonization was significantly higher on seedling roots inoculated with M2 than with M1, whereas no significant difference was found between the two-micorrhizal treatments in the second crop.

In both crops, all the growth parameters of seedlings from both M1 and M2 treatments were significantly larger than those from M0, except for number of leaves and root dry weight of M1 in the first crop. Growth data of seedlings from M2, except for number of leaves and root dry weight, were significantly larger than those from M1 in the first crop, whereas no significant difference between the two mycorrhizal treatments was found in the second crop. Growth effect of mycorrhization on tomato seedlings has been also documented in a study of Conversa et al. (2007).

3.4. Effects of irrigation and mycorrhizal treatments on root mycorrhization

Interaction "year × irrigation" was statistically significant only for the intensity of colonization and presence of arbuscules and vesicles at crop end (Table 2), as differences of these two mycorrhization indices among irrigation regimes were larger in the first than in the second crop (Fig. 2). A significant interaction "year × mycorrhization" was found only for the frequency of colonization at both mid and end growing season, due to the significantly larger variations of this parameter among mycorrhizal

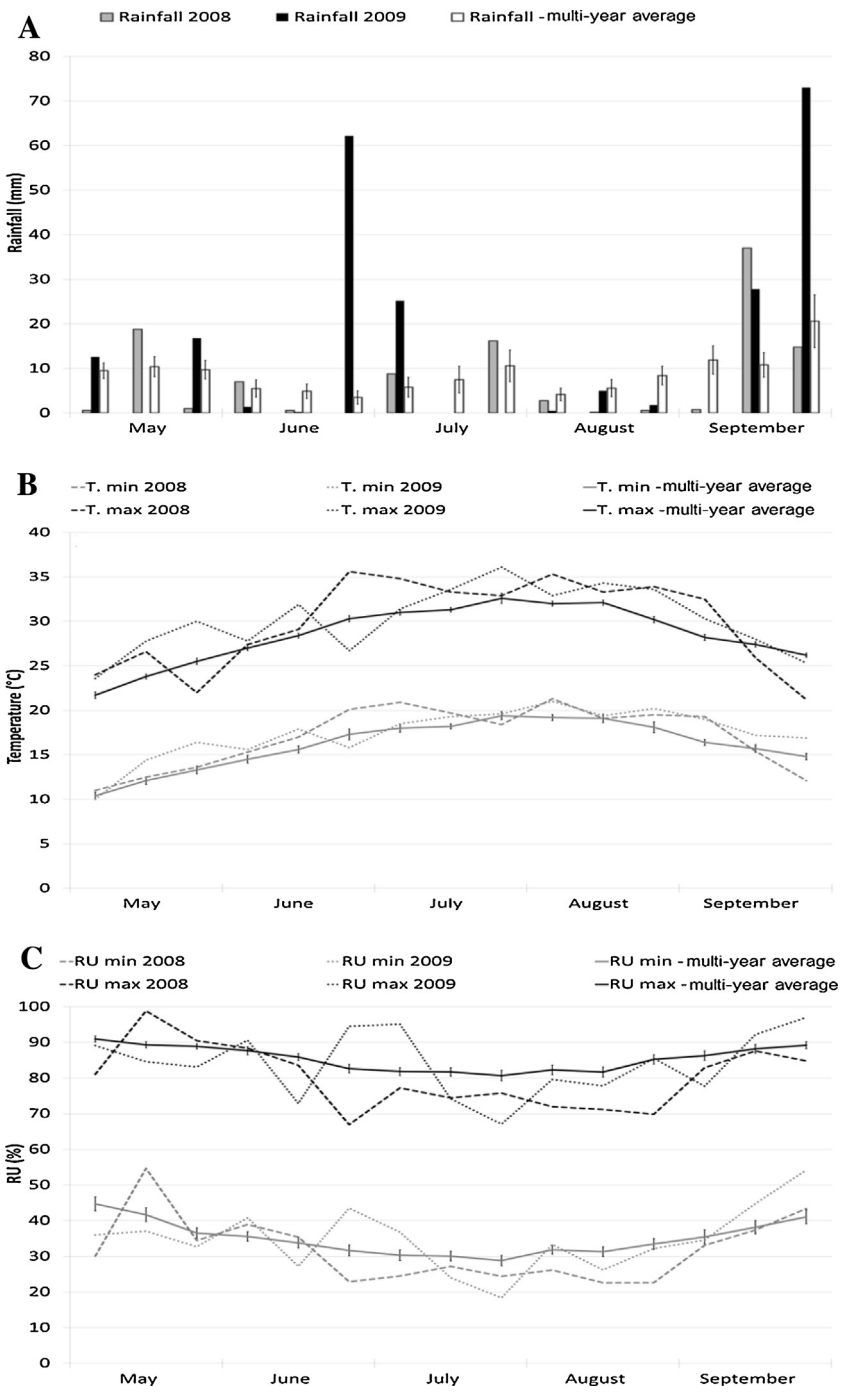

Fig. 1. Trend of 10-day minimum and maximum temperatures (A), rainfall (B), and minimum and maximum relative humidity (RU) (C) recorded during the two tomato growing seasons, in comparison to multi-year average. Standard error is reported for each 10-day multi-year average.

treatments in the first than in the second crop (Fig. 3). Interaction "irrigation × mycorrhization" was never significant for any mycorrhizal parameter at both mid and end crop.

At mid growing season, frequency of colonization and presence of arbuscules and vesicles of both M1 and M2 were significantly higher than M0 (Table 2), whereas intensity of colonization did not statistically differ among the three mycorrhizal treatments. At crop end, all mycorrhizal indexes were significantly lower for M0 than for M1 and M2, and intensity of colonization of M2 was significantly higher than M1. Low values of mycorrhization indices of M0 seem to indicate that natural mycorrhizal symbiosis of tomato in the pedoclimatic conditions of Southern Italy occurs more difficultly and later than on artificially mycorrhized plants.

Frequency of colonization and presence of arbuscules and vesicles at mid growing season were significantly higher on the roots from V50 and V100 than from V0, without any significant difference between the two irrigation regimes. At crop end, V0, V50, and V100 significantly differed among them for all mycorrhization indices but frequency of colonization.

3.5. *Effects of irrigation and mycorrhizal treatments on plant growth*

At mid growing season, the interaction "year × irrigation" was statistically significant only for shoot dry biomass and LAI (Table 3), due to the significantly higher values of these two parameters

Table 1
Effects of mycorrhizal treatments on morphological traits of seedlings at the transplant of the two tomato crops.

Mycorrhizal treatments[1]	Stems		Leaves		Seedling dry weight (mg)			Frequency of colonization
	Height (cm)	Diameter (mm)	Number	Area (cm^2)	Shoot	Root	Shoot/root ratio	
2008								
M0	7.5c	2.0c	3.3b	16.7c	67.3c	54.3b	1.2b	21c
M1	9.0b	2.2b	3.3b	23.2b	94.3b	53.3b	1.8a	79b
M2	11.3a	2.5a	4.0a	35.7a	144.2a	78.8a	1.8a	94a
Significance[2]	*	*	**	**	**	**	**	**
2009								
M0	7.1b	3.0b	4.3b	20.1b	144.9b	67.8b	1.9b	29b
M1	11.2a	3.5a	5.0a	43.8a	301.5a	121.6a	2.5a	100a
M2	11.3a	3.4a	5.1a	42.2a	301.9a	124.0a	2.4a	100a
Significance[2]	**	*	**	**	**	**	*	**

[1] Means followed by the same letters in the same column and within each experiment are not significantly ($P \leq 0.05$) different according to the SNK test.
[2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; *ns*, no significant difference.

Table 2
Effects of irrigation and mycorrhizal treatments on frequency of colonization (F), intensity of colonization (m), and presence of arbuscules and vesicles (a) at mid and end growing season of the two tomato crops.

Treatments	Mid growing season			End growing season		
	F	m	a	F	m	a
Years (Y)[1]						
2008	60.6	0.3	9.9	95.6	7.6	63.4
2009	85.5	0.9	37.1	99.5	1.0	80.3
Significance[2]	**	*ns*	**	*	**	**
Irrigation regimes (IR)[1]						
V0	55.5b	0.8	8.6b	95.9	1.0c	58.2c
V50	82.0a	0.5	29.5a	97.6	3.9b	73.6b
V100	81.7a	0.5	32.4a	99.2	8.0a	83.7a
Significance[2]	**	*ns*	*	*ns*	*	**
Mycorrhizal treatments (M)[1]						
M0	51.0b	0.7	14.2b	92.6b	1.1c	50.0c
M1	79.4a	0.4	26.3a	100.0a	4.6b	84.6a
M2	88.8a	0.7	30.0a	100.0a	7.2a	80.9a
Significance[2]	**	*ns*	*	**	*	**
Interactions[2]						
Y × IR	*ns*	*ns*	*ns*	*ns*	*	**
Y × M	**	*	*ns*	**	*ns*	*ns*
IR × M	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Y × IR × M	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[1] Means followed by the same letters in the same column and within each experiment are not significantly ($P \leq 0.05$) different according to the SNK test.
[2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; *ns*, no significant difference.

at V100 and at both V50 and V100 than at V0 in the first and second crop, respectively (data not shown). Plant height and number and diameter of stems showed a significant interaction "irrigation × mychorrhization," as at V100 height and diameter of stems were significantly lower for M1 and M2 than for M0, whereas number of stems was significantly higher in non-mycorrhized plants (data not shown). Interaction "year × mychorrhization" was not significant for all growth parameters but the stem diameter.

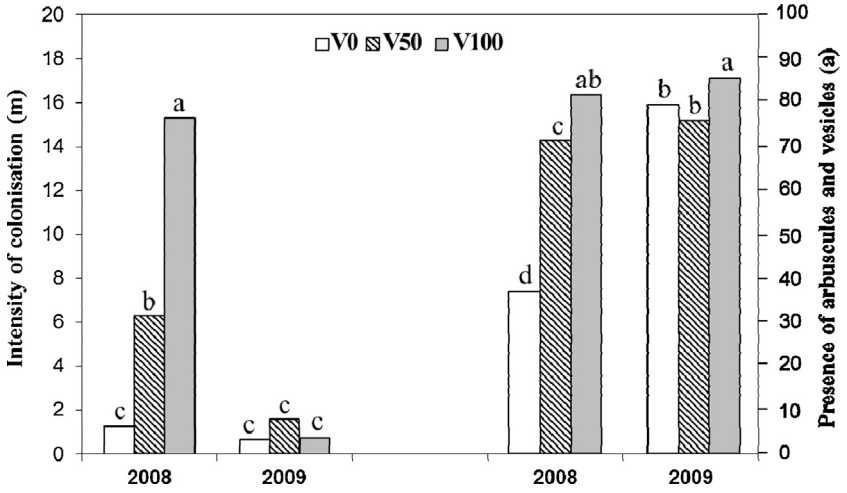

Fig. 2. Interactive effect of "year × irrigation" on intensity of colonization (m) and presence of arbuscules and vesicles (a) at crop end. Histograms with the same letters within each trait are not significantly ($P \leq 0.05$) different according to the SNK test.

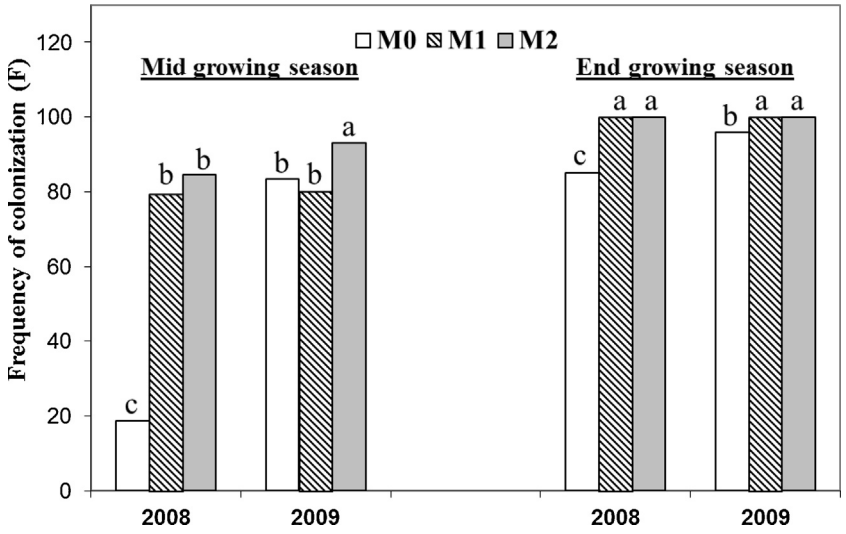

Fig. 3. Interactive effect of "year × mycorrhization" on the frequency of colonization (F) at mid and end growing season of tomato. Histograms with the same letters within each trait are not significantly ($P \leq 0.05$) different according to the SNK test.

Tomato growth parameters at mid growing season were significantly higher in the second than in the first crop, as well as significantly higher for M1 and M2 than for M0 (Table 3).

At end growing season, weight of dry epigeal biomass, HI and IWUE showed a significant "year × irrigation" interaction, as differences among the values of these parameters at V0, V50, and V100 were lower in the first than in the second crop (data not shown). No other significant interaction was found, except for "year × mycorrhization" on shoot weight. Dry weight of shoots and total epigeal biomass resulted significantly higher in the second than in the first crop, which, adversely, resulted in higher values of HI (+0.2) and IWUE (+0.5 kg m^{-3}). All growth indices at crop end were significantly higher for M1 and M2 than for M0, except for HI. Final weight of dry fruits and epigeal biomass and HI values were significantly larger at both V50 and V100 than at V0 and at V100 than at V0, whereas IWUE was significantly higher in non-irrigated soil.

3.6. Effects of irrigation and mycorrhizal treatments on tomato yield

All the yield parameters but the fruit diameter showed a statistically significant "year × irrigation" interaction (Table 4). As previously observed for plant growth parameters, this interaction can be attributed to the significantly larger differences among irrigation regimes in the second than in the first crop (data not shown). Interactions "year × mycorrhization" and "irrigation × mycorrhization" were found statistically significant only for the yield at first harvest and the fruit mean weight, respectively. The absence of any other significant interaction "mycorrhization × irrigation on crop yield parameters is in contrast to initial hypotheses and expectations of our research, as indicating no synergism of artificial mycorrhization with deficit irrigation. However, this is in good agree with the poor and not significant effects of the interaction "irrigation × mycorrhization" on

Table 3
Effects of irrigation and mycorrhizal treatments on tomato plant growth at mid and end crop-growing season.

Treatments	Mid growing season					End growing season				
	Height (cm)	Stem number	Stem diameter (mm)	Shoot dry biomass (T ha^{-1})	LAI	Epigeal dry biomass (T ha^{-1})			HI	IWUE (kg m^{-3})
						Fruits	Shoots	Total		
Years (Y)[1]										
2008	55.5	6.2	11.7	1.7	1.7	5.4	2.5	7.9	0.68	5.3
2009	68.3	9.5	14.9	4.7	4.7	5.3	4.7	10.0	0.52	4.9
Significance[2]	**	**	**	**	**	*ns*	**	**	**	**
Irrigation regimes (IR)[1]										
V0	60.9a	7.7a	12.7a	2.8c	2.9c	3.9c	3.0c	6.9c	0.57a	11.6a
V50	62.7a	8.0a	13.4a	3.2b	3.3b	5.5b	3.5b	9.0b	0.61b	2.3b
V100	62.1a	7.9a	13.9a	3.5a	3.5a	6.7a	4.2a	10.9a	0.63b	1.5c
Significance[2]	*ns*	*ns*	*ns*	**	**	**	**	**	**	**
Mycorrhizal treatments (M)[1]										
M0	58.8b	7.8	12.8b	2.9b	2.9b	4.9b	3.4b	8.3b	0.59	4.7b
M1	62.2a	7.9	13.8a	3.3a	3.4a	5.6a	3.6a	9.2a	0.61	5.2a
M2	64.7a	7.8	13.4a	3.3a	3.3a	5.5a	3.7a	9.3a	0.60	5.3a
Significance[2]	**	*ns*	*	**	**	**	**	**	*ns*	**
Interactions[2]										
Y × IR	*ns*	*ns*	*ns*	**	**	**	**	**	**	**
Y × M	*ns*	*ns*	**	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*
IR × M	*	**	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Y × IR × M	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[1] Means followed by the same letters in the same column and within each experiment are not significantly ($P \leq 0.05$) different according to the SNK test.
[2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; *ns*, no significant difference.

Table 4
Effects of irrigation and mycorrhizal treatments on yield and quality traits of the two tomato crops.

Treatments	Yield (T ha^{-1})			Number of fruits per plant		Fruit quality parameters			
	At first harvest	Marketable	Total	Marketable	Total	Mean weight (g)	Diameter (mm)	Soluble solids (°Brix)	Dry matter content (%)
Years (Y)[1]									
2008	43.0a	71.2a	83.5a	56.1b	73.1b	22.2a	33.2a	5.1b	6.8b
2009	38.5b	69.7b	75.5b	73.1a	82.7a	19.1b	31.0b	6.6a	7.2a
Significance[2]	**	*	**	**	**	**	**	**	**
Irrigation (IR)[1]									
V0	28.4b	42.5c	47.6c	52.0b	60.8b	15.2c	29.1c	6.6a	8.1a
V50	46.2a	73.6b	83.7b	68.9a	82.4a	20.8b	32.4b	5.8b	6.7b
V100	47.7a	95.3a	107.2a	73.0a	90.6a	25.9a	34.7a	5.1c	6.2c
Significance[2]	**	**	**	**	**	**	**	**	**
Mycorrhizal treatments (M)[1]									
M0	36.3b	65.7b	74.3b	59.4b	73.1b	20.6a	32.2a	5.9a	7.0a
M1	43.8a	73.2a	82.5a	65.8a	78.8a	21.1a	32.1a	5.8a	7.0a
M2	42.2a	72.5a	81.7a	68.7a	81.9a	20.2a	31.9a	5.9a	7.1a
Significance[2]	**	**	**	**	**	*ns*	*ns*	*ns*	*ns*
Interactions[2]									
Y × IR	**	**	**	**	**	**	*ns*	**	**
Y × M	**	*ns*	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*
IR × M	**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Y × IR × M	**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[1] Means followed by the same letters in the same column and within each experiment are not significantly ($P \leq 0.05$) different according to the SNK test.
[2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; *ns*, no significant difference.

tomato biochemical and agronomical parameters previously documented by other studies (Di Cesare et al., 2012; Kaya et al., 2003).

Total and marketable yield, as well as the yield at first harvest, resulted significantly larger in the first than in the second crop (Table 4). Adversely, second crop resulted in a statistically larger number of total and marketable fruits per plant. Yield at first harvest and total and marketable yield also were significantly larger at both V50 and V100 than at V0, due to a larger number and weight of total and marketable fruits per plant. A positive yield response of tomato to increasing irrigation volumes was reported also by previous studies (Candido et al., 2000; Patanè and Cosentino, 2010). In a study of Di Cesare et al. (2012), total yield of different tomato varieties increased by 41.5–64.4% moving from no irrigation to normal irrigation conditions. Genotype-dependent effects of irrigation on yield and fruit weight were documented also by Pernice et al. (2010). Adversely, some studies reported a reduction of green fruit yield and blossom-end rot as associated to irrigation (Candido et al., 2000; Warner et al., 2007).

Plants from M1 and M2 provided a significantly larger marketable and total yield compared to M0 (Table 4), due to a higher number of total and marketable fruit, whereas no significant difference was found between M1 and M2. An increase of crop yield following VAM inoculation has been already reported either on tomato (Salvioli et al., 2012) and other vegetable crops (Douds et al., 2007; Regvar et al., 2003). Conversa et al. (2012) reported a higher number of flowers and total and marketable fruits in field tomato inoculated with a commercial formulation of *G. intraradices*. Adversely, Bosco et al. (2007) did not find any significant increase of total and marketable yield of organic tomato artificially mycorrhized with M2 formulation applied in our experiment. Absence of significant differences between the effects of M1 and M2 on tomato yield was also reported by Di Cesare et al. (2012). Reasons of such a huge variability of results can be found in the large dependence of the effectiveness of mycorrhization on the specificity of fungus–host plant (species and/or cultivars) relationship more than on environmental factors (Sensoy et al., 2007; Gosling et al., 2006). Results of our experiment seem to confirm a minor importance of environmental factors for mycorrhization effectiveness, as in the presence of two climatically different crop seasons the effects of mychorrizal treatments on tomato yield were quite similar in both crops.

3.7. *Effects of irrigation and mycorrhizal treatments on tomato fruit quality*

Interaction "year × irrigation" was significant for all fruit quality parameters but the diameter (Table 4), due again to the lower differences among V0, V50, and V100 in the first than in the second crop (data not shown). As for crop yield, fruit quality parameters did not show any significant interaction "irrigation × mycorrhization," whereas the interaction "year × mycorrhization" was statistically significant only for the mean fruit weight. Weight and diameter of fruits were significantly larger in the first than in the second crop (Table 4), as well as significantly larger at both V50 and V100 than at V0. Adversely, a significantly higher content of soluble solids and dry matter was recorded in the second growing season and in absence of irrigation (V0). Variable effects of irrigation on tomato fruit quality were previously documented also in other studies (Cahn et al., 2001; Candido et al., 2000; Favati et al., 2009), though full irrigation regimes were generally found to reduce content of total and soluble solids, color and firmness of tomato fruits (Colla et al., 2001; Dumas et al., 1994).

Mycorrhizal treatments M1 and M2 did not significant affect fruit quality, adversely to positive impacts previously documented for root inoculation with beneficial rhizosphere microorganisms (Kaya et al., 2003; Mena-Violante et al., 2006).

4. Conclusions

Inoculation of nursery seedlings with VAM formulations, either alone or combined with PGPR, confirmed to provide considerable growth and yield benefits to tomato crop. Further benefits of plant artificial mycorrhization can be the higher WUE also observed in this study and, more generally, the improved crop sustainability related to a lower application of fertilizers and pesticides. The large variability of plant response to mycorrhizal treatments requires, however, additional multi-year studies on a wider range of tomato genotypes.

A further indication emerging from this study is that, under the climatic conditions of Southern Italy, irrigation is the best agronomical practice for an enhanced yield performance of tomato, regardless of variety and crop system, tough at expense of fruit quality.

The absence of any synergism between artificial mycorrhization and irrigation volumes observed in this study may indicate that combined application of plant mycorrhization and deficit irrigation can be an effective tool for the optimization of crop water demand only if specifically adapted to tomato genotype more than to pedoclimatic and agronomical conditions.

Acknowledgments

This work was carried out under the PROM research project (Progetto di Ricerca per potenziare la competitività di Orticole in aree Meridionali – Contributo finanziario MIPAF con fondi C.I.P.E., Delibera 17/2003). The scientific coordinator of this project was Dr. Agostino Falavigna. The authors thank Mr. Paolo Putignano and Mr. Cosimo Danzi for their valuable help in conducting the experimental trials and in collecting agronomic data.

References

Akhtar, M.S., Siddiqui, Z.A., 2008. Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Siddiqui, Z.A., Akhtar, M.S., Futai, K. (Eds.), Mycorrhizae: Sustainable Agriculture and Forestry. Springer, Netherlands, ISBN 978-1-4020-8769-1, pp. 61–97.

Allen, M.F., 1982. Influence of vesicular–arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.B.K.). Lag ex Steud. New Phytol. 91, 191–196.

Augè, R.M., 2001. Water relations, drought and vesicular arbuscular mycorrhizal symbiosis. Mycorrhiza 11, 3–42.

Auge, R.M., Schekel, K.A., Wample, R.L., 1986. Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. New Phytol. 103, 107–116.

Baselga Yrisarry, J.J., Prieto Losada, M.H., Rodríguez del Rincón, A., 1993. Response of processing tomato to three different levels of water and nitrogen applications. Acta Hort. (ISHS) 335, 149–156.

Bosco, M., Giovannetti, G., Picard, C., Baruffa, E., Brondolo, A., Sabbioni, F., 2007. Commercial plant-probiotic microorganisms for sustainable organic tomato production systems , Proceedings 3rd QLIF Congress, Hohenheim, Germany, pp. 468–471.

Brundrett, M.C., Piché, Y., Peterson, R.L., 1984. A new method for observing the morphology of vesicular arbuscular mycorrhizas. Can. J. Bot. 62, 2128–2134.

Bryla, D.R., Duniway, J.M., 1997. Effects of mycorrhizal infection on drought tolerance and recovery in safflower and wheat. Plant Soil 197, 95–103.

Cahn, M.D., Herrero, E.V., Snyder, R.L., Hanson, B.R., 2001. Water management strategies for improving fruit quality of drip-irrigated processing tomatoes. Acta Hort. (ISHS) 542, 111–116.

Candido, V., Campanelli, G., D'Addabbo, T., Castronuovo, D., Renco, M., Camele, I., 2013. Growth and yield promoting effect of artificial mycorrhization combined with different fertiliser rates on field-grown tomato. Ital. J. Agron. 8 (3), 168–174.

Candido, V., Miccolis, V., Perniola, M., 2000. Effects of irrigation regime on yield and quality of processing tomato (*Lycopersicon esculentum* Mill.) cultivars. Acta Hort. (ISHS) 537 (2), 779–788.

Colla, G., Battistelli, A., Moscatello, S., Proietti, S., Casa, R., Lo Cascio, B., Leoni, C., 2001. Effect of reduced irrigation and nitrogen fertirigation rate on yield, carbohydrate accumulation, and quality of processing tomatoes. Acta Hort. (ISHS) 542, 187–196.

Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951–4959.

Conversa, G., Lazzizera, C., Bonasia, A., Elia, A., 2012. Yield and phosphorus uptake of a processing tomato crop grown at different phosphorus levels in a calcareous soil as affected by mycorrhizal inoculation under field conditions. Biol. Fertil. Soil 49, 1–13.

Conversa, G., Elia, A., La Rotonda, P., 2007. Mycorrhizal inoculation and phosphorus fertilization effect on growth and yield of processing tomato. Acta Hort. (ISHS) 758, 333–338.

Dalpé, Y., Monreal, M., 2004. Arbuscular mycorrhiza inoculum to support sustainable cropping systems. Online Crop Management, http://dx.doi.org/10.1094/CM-2004-0301-09-RV, http://www.plantmanagementnetwork.org/pub/cm/review/2004/amfungi

Davies JrF.T. Olalde-Portugal, V., Aguilera-Gomez, L., Alvarado, M.J., Ferrera-Cerrato, R.C., Boutton, T.W., 2002. Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis) with arbuscolar mycorrhiza indigenous to Mexico. Sci. Hort. 92 (3), 347–359.

Davies, F.T., Svenson, S.E., Henderson, J.C., Phavaphutanon, L., Duray, S.A., Olalde Portugal, V., Meier, C.E., Bo, S.H., 1996. Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. Tree Physiol. 16, 985–993.

Di Cesare, L.F., Migliori, C., Ferrari, V., Parisi, M., Campanelli, G., Candido, V., Perrone, D., 2012. Effects of irrigation–fertilization and irrigation mycorrhization on the alimentary and nutraceutical properties of tomatoes. In: Lee, T.S. (Ed.), Irrigation Systems and Practices in Challenging Environments. InTech, Rijeka (Croatia), pp. 207–232, http://dx.doi.org/10.5772/32580, Available from: http://www.intechopen.com/books/irrigation-systems-and-practices-in-challenging-environments/effects-of-irrigation-fertilization-and-irrigation-mycorrhization-on-the-alimentary-and-nutraceutica

Doorenbons, J., Pruitt, W.O., 1977. Guidelines for Predicting Crop Requirements. FAO Irrigation and Drainage Paper No. 24. Food and Agriculture Organization of United Nations, Rome, Italy.

Douds Jr., D.D., Nagahashi, G., Reider, C., Hepperly, P., 2007. Inoculation with AM fungi increases the yield of potatoes in a high P soil. Biol. Agric. Hortic. 25, 67–78.

Dumas, Y., Leoni, C., Portas, C.A.M., Bièche, B., 1994. Influence of water and nitrogen availability on yield and quality of processing tomato in the European Union Countries. Acta Hort. (ISHS) 376, 185–192.

Egilla, J.N., Davies, F.T., Drew, M.C., 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: plant growth, leaf macro-and micronutrient content and root longevity. Plant Soil 229, 213–224.

Elshafie, H.S., Camele, I., Ventrella, E., Scrano, L., Lovelli, S., Bufo, S.A., Amato, M., 2013. Use of plant growth promoting bacteria (PGDB) for promoting tomato growth and its evaluation as biological control agent. Int. J. Microbiol. Res. 5 (5), 452–457.

Favati, F., Lovelli, S., Galgano, F., Miccolis, V., Di Tommaso, T., Candido, V., 2009. Processing tomato quality as affected by irrigation scheduling. Sci. Hort. 122, 562–571.

Fereres, E., Soriano, M.A., 2007. Deficit irrigation for reducing agricultural water use. J. Exp. Bot. 58 (2), 147–159.

Gera Hol, W.H., Cook, R., 2005. An overview of arbuscular mycorrhizal fungi-nematode interactions. Basic. Appl. Ecol. 6, 489–503.

Geerts, S., Raes, D., 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agric. Water Manage. 96 (9), 1275–1284.

Gosling, P., Hodge, A., Goodlass, G., Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113, 17–35.

Hardeman, T.L., Taber, H.G., Cox, D.F., 1999. Trickle irrigation of vegetables: water conservation without yield reduction. J. Veg. Crop. Prod. 5, 23–33.

Hargreaves, G.H., Samani, Z.A., 1985. Reference crop evapotranspiration from temperature. Appl. Eng. Agric. 1, 96–99.

Harrison, M.J., van Buuren, M.L., 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378, 626–629.

Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413, 297–299.

Karlberg, L., Rockström, J., Annandale, J.G., Steyn, J.M., 2007. Low-cost drip irrigation a suitable technology for southern Africa? An example with tomatoes using saline irrigation water. Agric. Water Manage. 89, 59–70.

Kaya, C., Higgs, D., Kirnak, H., Tas, I., 2003. Mycorrhizal colonisation improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water stressed conditions. Plant Soil 253, 287–292.

Kirda, C., Cetin, M., Dasgan, S., Topcu, H., Kaman, B., Ekici Derici, M.R., Ozguven, A.I., 2004. Yield response of greenhouse grown tomato to partial root drying and conventional deficit irrigation. Agric. Water Manage. 69 (3), 191–201.

Kirnak, H., Kaya, C., Higgs, D., Gercek, S., 2001. A long-term experiment to study the role of mulches in physiology and macro-nutrition of strawberry grown under water stress. Aust. J. Agric. Res. 52, 937–943.

Larsen, J., Ravnskov, S., Sorensen, J.N., 2007. Capturing the benefits of arbuscular mycorrhizae in horticulture. In: Hamel, C., Plenchette, C. (Eds.), Mycorrhizae in Crop Production. The Haworth Press Inc, New York, USA, pp. 123–149.

Lee, B.R., Muneer, S., Avice, J.C., Jung, W.J., Kim, T.H., 2012. Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation in perennial ryegrass under well-watered and drought-stressed conditions. Mycorrhiza 22, 525–534.

Mena-Violante, H.G., Ocampo-Jiménez, O., Dendooven, L., Martínez-Soto, G., González-Castañeda, J., Davies Jr., F.T., Olalde-Portugal, V., 2006. Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho (*Capsicum annuum* L. cv San Luis) plants exposed to drought. Mycorrhiza 16, 261–267.

Patanè, C., Cosentino, S.L., 2010. Effects of soil water deficit on yield and quality of processing tomato under a Mediterranean climate. Agric. Water Manage. 97 (1), 131–138.

Patanè, C., Tringali, S., Sortino, O., 2011. Effects of deficit irrigation on biomass, yield, water productivity and fruit quality of processing tomato under semi-arid Mediterranean climate conditions. Sci. Hort. 129, 590–596.

Pernice, R., Parisi, M., Giordano, I., Pentangelo, A., Graziani, G., Gallo, M., Fogliano, V., Ritieni, A., 2010. Antioxidant profile of small tomato fruits: effect of water regime and industrial process. Sci. Hort. 126 (2), 156–163.

Perniola, M., Rivelli, A.R., Candido, V., 1994. Yield response to water and stress indexes on tomato. Acta Hort. (ISHS) 376, 215–226.

Regvar, M., Vogel-Mikuš, K., Ševerkar, T., 2003. Effect of AMF inoculum from field isolates on the yield of green pepper, parsley, carrot, and tomato. Folia Geobot. 38, 223–234.

Salvioli, A., Zouari, I., Chalot, M., Bonfante, P., 2012. The arbuscular mycorrhizal status has an impact on the transcriptome profile and aminoacid composition of tomato fruit. BMC Plant Biol. 12, 44.

Sangakkara, U.R., Frehner, M., Nosberger, J., 2000. Effect of soil moisture and potassium fertilizer on shoot water potential, photosynthesis and partitioning of carbon in mung bean and cowpea. J. Agron. Crop Sci. 185, 201–207.

Sensoy, S., Demir, S., Turkmen, O., Erdinc, C., Savur, O.B., 2007. Responses of some different pepper (*Capsicum annuum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. Sci. Hort. 113, 92–95.

Sorensen, J.N., Larsen, J.M., Jakobsen, I., 2008. Pre-inoculation with arbuscular mycorrhizal fungi increases early nutrient concentration and growth of field-grown leeks under high productivity conditions. Plant Soil 307, 135–147.

Steduto, P., 1996. Water use efficiency. In: Sustainability of Irrigated Agriculture. Springer Netherlands, pp. 193–209.

Tarantino, E., Onofri, M., 1991. Determinazione dei coefficienti colturali mediante lisimetri. Bonifica 8, 119–136, in Italian.

Tarantino, E., Rivelli, A.R., Perniola, M., Nardiello, I., 1997. Efficienza nell'uso dell'acqua di alcune colture erbacee sottoposte a differenti regimi irrigui: valutazione a livello di pieno campo. Irrigazione e Drenaggio 44 (1), 8–16, in Italian.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677.

Topcu, S., Kirda, C., Dasgan, Y., Kaman, H., Cetin, M., Yazici, A., Bacon, M.A., 2007. Yield response and N-fertiliser recovery of tomato grown under deficit irrigation. Eur. J. Agron. 26, 64–70.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorrhization VA d'un systeme radiculaire. Recherce de méthodes d'estimation ayant une signification fonctionelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (Eds.), Mycorrhiza: Physiology and Genetics. INRA, Paris, France, pp. 217–221, in French.

Turk, M.A., Assaf, T.A., Hameed, K.M., Al-Tawaha, A.M., 2006. Significance of mycorrhizae. World J. Agric. Sci. 2 (1), 16–20.

van der Heijden, M.G.A., Streitwolf-Engel, R., Siegrist, S., Neudecker, A., Ineichen, K., Boller, T., Wiemken, A., Sanders, I.R., 2006. The micorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytol. 172, 739–752.

Warner, J., Tan, C.S., Zhang, T.Q., 2007. Water management strategies to enhance fruit solids and yield of drip irrigated processing tomato. Can. J. Plant Sci. 87, 345–353.

Zairi, A., El Amami, H., Slatni, A., Pereira, L.S., Rodrigues, P.N., Machado, T., 2003. Coping with drought: deficit irrigation strategies for cereals and field horticultural crops in Central Tunisia. In: Rossi, G., Cancelliere, A., Pereira, L.S., Oweis, T., Shatanawi, M., Zairi, A. (Eds.), Tools for Drought Mitigation in Mediterranean Regions. Kluwer, Dordrecht, pp. 181–201.

Zheng, J., Huang, G., Jia, D., Wang, J., Mota, M., Pereira, L.S., Huang, Q., Xu, X., Liu, H., 2013. Responses of drip irrigated tomato (*Solanum lycopersicum* L.) yield, quality and water productivity to various soil matric potential thresholds in an arid region of Northwest China. Agric. Water Manage. 129, 181–193.