On-farm reduced irrigation and fertilizer doses, and arbuscular mycorrhizal fungal inoculation improve water productivity in tomato production

Carmen Biel, Amélia Camprubí, Paulo E. Lovato, Cinta Calvet

Abstract

Viability of horticultural production requires high yields of high-value products, coupled with optimization of inputs. Rational fertilizer and water use are vital for the economic and environmental sustainability of vegetable production. Microbial resources are also used to increase yield, reduce inputs, and improve product quality. That includes arbuscular mycorrhizal fungi (AMF), symbionts associated with 80% of plant species, including many vegetable crops, such as tomato. To evaluate how AMF inoculation and reduced water and fertilizer affect tomato yield and quality, we carried out three independent experiments in commercial-production medium-technology (growth in soil, plastic cover, and no temperature control) greenhouses in Catalonia, Northern Spain. In the first experiment, AMF inoculation increased fruit number, total yield and improved fruit quality (soluble solids concentration or color) using lower fertilizer doses than those usually adopted on the farm. In a second assay, plants grew under three irrigation regimes: the farm usual regime, a managed-deficit regime (75% of the former), and an optimized dose using weather and plant-growth data. Deficit and optimized irrigation increased tomato yield and fruit size, but not fruit soluble solids concentration or color, and there was no effect of AMF inoculation. In the third experiment, fertigation was reduced by regulating irrigation doses according to soil moisture data. AM-inoculated plants sustained yield levels with 13 % lower water and fertilizer rates. Reduced fertigation associated with AMF inoculation resulted in a 1.6 % decrease in costs, which corresponds to at least six times the price of available commercial AMF inoculants. In all experiments, AMF inoculation was associated with increased water productivity, the ratio between product yield and the volume of irrigation water. Gains in input use efficiency, not only simple yield increase, should define commercial AMF inoculant use in vegetable production. We argue that researchers should estimate the financial incentive that AMF inoculation incurs so that farmers and inoculant producers have a base to test and adopt AMF inoculation technology.

Keywords: On-farm experiment, *Rhizoglomus irregulare*, water productivity, value/cost ratio.

1. Introduction

Search for satisfactory yields of high-quality produce is evident in horticulture, and recent efforts have focused on production sustainability (Roberts and Matoo, 2019). Optimization of fertilizer doses has been tested (e. g., Abdul-Baki et al., 1997), and it has been shown that yield can be maintained, and quality may improve with lower fertilizer rates (Hernandez et al., 2020). Water is another resource increasingly scarce and is therefore becoming more expensive. Limitations to plant growth and yield by low water availability is overcome by irrigation. However, as water availability decreases, its cost increases, requiring optimization of water use or increases in water use efficiency.

Water availability is an increased concern due to climate change, and studies on the subject have coined the concepts of water productivity and water footprint. Water productivity corresponds to the quantity of product obtained using a given water volume (Molden et al., 2010). Water Footprint (WF) is an indicator recently developed to quantify the virtual content of water in products or services (Lovarelli et al., 2016), and it is generally expressed as a ratio of water volume used by the crop (evapotranspiration) per mass of the agricultural product. This approach classifies water into three categories, called green, blue, and grey. Green water is the amount made available by rainfall, blue water refers to water taken from an aquifer or other drainage basins, and grey water corresponds to the volume needed to dilute eventual pollutants generated in a system.

Plants can tolerate water stress only up to a specific limit (threshold level), and, beyond that limit, there is a severe decline in yield (Parkash and Singh, 2020). Grafting can improve water use efficiency

(WUE), especially in vegetable production (Schwarz et al., 2010). This technique increases WUE by providing a more extensive root system and inducing changes in shoot physiological traits, such as stomatal conductance (Kumar et al., 2017). The reduction of irrigation doses is another growing trend in vegetable production. Chai et al. (2016) define managed-deficit irrigation as reducing irrigation levels with gains in resource use efficiency that outweighs yield or quality reduction. Evans and Sadler (2008) state that managed-deficit irrigation is the most efficient method to guarantee yields while saving water, and deficit irrigation has been used successfully in tomato production (see review by Khapte et al., 2019). Different studies have placed the water footprint of tomatoes in Spain in the range from 50 to 114 $m^3.t^{-1}$ (Sørensen et al., 2009), and such a wide range indicates that irrigation doses may be reduced in many instances. That should be associated with other techniques that optimize water uses, such as grafting, mentioned above, or the use of plant-microbial associations.

Mycorrhizas are associations between soil fungi and roots that occur in about 90% of plant species, and the most frequent type is the arbuscular mycorrhiza (AM), which involves fungi of the phylum Glomeromycota and about 80% of plant families (Smith and Read, 2002). Plants provide energy for the fungus growth and reproduction, and the fungal partner enhances water and nutrient uptake by the root system. AM fungi also enhance soil particle aggregation, increase plant resistance to root pathogens, contribute to soil carbon stocks and microbial biomass, and enhance plant resistance to soil heavy metal contamination and nutrient depletion (Gianinazzi et al., 2010).

In greenhouse and field experiments testing AMF inoculant in tomato, yield differences were found in the field, except for 100% of the P dose, with a yield in field conditions around 50 tons ha^{-1} (Ziane et al., 2017). Besides yield, mycorrhizas may affect product quality. AM fungal inoculation can improve tomato fruit quality, by increasing nutrient concentration (particularly N, P, and Cu), antioxidant compounds, and carotenoid contents (Hart et al., 2015). Bona et al. (2017) carried out on-farm experiments testing AMF and plant-growth-promoting bacteria; both types of microorganisms positively affected flower and fruit production and concentrations of sugars and vitamins in tomato fruits.. Another on-farm experiment with tomato showed that the joint inoculation of AM fungi and plant-growth-promoting bacteria in tomato allowed a 30% reduction in chemical fertilizers, with an increase in fruit quality, specifically size, sweetness, and concentration of molecules such as citrate, ascorbate, and carotenoids (Bona et al., 2018). Other works have reported gains in yield and quality in horticultural crops, including strawberries (Castellanos-Morales et al., 2010), onions (Mollavali et al., 2016), grapes (Torres et al., 2018), and aromatic herbs (Geneva et al., 2010; Zayova et al., 2018).

Since AM improve water uptake (Smith and Read, 2002), mycorrhizal plants would be more efficient in water use. Ronga et al. (2019) showed that AMF inoculation improved water use by tomato plantlets, and Fracasso et al. (2020) observed that mycorrhizal tomato kept yield levels even when plants were subjected to water stress. Although the authors did not explicitly use the expression water footprint, an on-farm experiment demonstrated that mycorrhizal fungal inoculation increased water productivity in tomato plants (Candido et al., 2015).

There has been a growing interest in arbuscular mycorrhizal (AM) symbiosis for several decades, and field application has long been a goal (Howeler et al., 1987), but only recently consistent inoculant production and field tests with such products have been reported (Abbott et al., 2018; Rocha et al., 2019). There are relatively few studies on mycorrhiza's application involving field tests, and on-farm experiments are even scarcer. On-farm experiments, or conditions close to real-life situations, are needed to obtain data in realistic conditions, preferably closer to the conditions in which farmers work. The use of inoculants is justified in adverse conditions, but it is also useful to reduce inputs. In an on-farm experiment, inoculation increased maize yield only in soils with low phosphorus availability (Bender et al., 2019). A commercial inoculant increased the yield of maize (Stoffel et al., 2020a) and soybeans (Stoffel et al., 2020b) in all fertilization situations, but the frequency of increases was higher with a 50% reduction in phosphate fertilizer.

Although field and on-farm experiments have become more frequent, work with economic assessments of arbuscular mycorrhizal inoculants is still scarce. Hijri (2016) tested a commercial inoculant in 231 trials in the field with potato (*Solanum tuberosum*) for four years on farms and a realistic scale (4,000-m^2 plots). He found an average increase of 9.5% in yield, and in almost 80% of the cases, the increase in revenue was higher than the input cost (US$ 135 per hectare). In two on-farm experiments with mycorrhizal inoculation of cassava plants, Ceballos et al. (2013) showed that inoculation increased root yield, especially in low-P additions, and economic analysis showed no gain with inoculation. However, they used twice the recommended dose and assumed European prices in a Latin American country, concluding that using the regular fertilizer dose or reducing it would be profitable.

Higher productivity may be obtained by increased yields or by savings in input use. Farmers use a rule-of-thumb to adopt a new input: the value/cost ratio, the financial gain by the product cost, should be at least three times higher than the product cost (Thuita et al., 2018), or even two in smallholders' farms (Van Vugt et al., 2017). That concept could be useful in applying AM inoculants, but we did not find papers comparing these value/cost ratios in tests with this technology.

As for fertilizers, water is increasingly valued due to uncertainty regarding its availability with the ongoing climate changes. Therefore, gains in productivity with a reduction in water and fertilizers are relevant. We aimed to evaluate productivity gains in a high-value crop under real-life conditions. We tested AMF inoculation's effect combined with reduced fertilizer or irrigation doses and with a combined reduction in both water and fertilizer supply. We also sought to estimate the economic gains of inoculation with arbuscular mycorrhizal fungi by comparing the inoculant cost with gains in terms of higher yield or lower input costs.

2. Materials and Methods

2.1. General growing conditions

All experiments were carried out in production greenhouses near Mataró, a vegetable-production, coast-line region 30 km from Barcelona, Spain. Mean annual values for temperature, rainfall and evapotranspiration are 16.4ºC, 619 mm, and 997mm, respectivel. The soil in all sites is a sandy clay loam. The production system used is classified as med-tech (Page et al., 2012), as the greenhouses have plastic cover and no temperature control, with production is restricted to the warmer portions of the year, and plants grow in soil beds covered with plastic mulching. Site choice was determined by absence of former use of arbuscular mycorrhizal inoculant and according to availability as defined by farm owners. Two sites were about 100 m apart, and a third site was around 1100 meters from them.

2.2. Mycorrhizal inoculation under reduced fertilizer application

This experiment was carried out at Agrícola Maresme Vives (41.549940 N, 2.473938 E), in an open-air, plastic-cover greenhouse with a total surface of 0.11 ha. The soil had an initial nitrate concentration of 38.5+ 10.5 mg L^{-1}, and final concentration was 86.5 + 21,4 mg L^{-1}. Irrigation water was supplied from a well, and water composition is in Table S 1. Factors were fertilization (commercial fertilization and reduced fertilization) and mycorrhizal inoculation (inoculated and non-inoculated), arranged in a 2 x 2 factorial design. There were four replicates, with sixteen tomato plants of Egara cultivar for each treatment. Fertilization treatments were farm fertilization (Farm), the farm's usual nutrient solution, and reduced fertilization (Red), which consisted of nitrogen, phosphorous, and potassium needed to equilibrate the nutrient solution during the initial (30 days, from transplant to blooming), middle (46 days, from blooming until harvest start), and final (56 days from the first until the last harvest) phenological stages. Nitrate-N levels were 2.0, 2.5, and 1.0 mMol L^{-1} in the initial, middle, and final stages, respectively. Phosphorus concentration was 0.25 mMol L^{-1}, and potassium levels were 5.0, 5.8, and 4.25 mMol L^{-1} in the initial, middle, and final stages respectively. Mycorrhizal plants were inoculated at transplant with 40 g of bulk inoculum from mycorrhizal leek (*Allium porrum* L.) cultures, applied under the root system. The arbuscular mycorrhizal fungus was *Rhizoglomus irregulare* (Blaszk, Wubet, Renker and Buscot) Sieverd, Silva and Oehl comb. nov., isolated from a citrus nursery (Estaun et al., 1994), and its effectiveness in promoting plant growth has been demonstrated in agricultural production and land restoration (Calvet et al., 2001; Camprubí et al., 2007). A soil sample was taken from each plot at the beginning of the assay, before inoculation, to estimate the mycorrhizal inoculum potential, using the MPN bioassay (Porter, 1979). The bioassay, using leeks, was performed in a greenhouse at IRTA Cabrils Center (41°30'58.6"N, 2°22'36.7"E)

Plantlets (inoculated or non-inoculated) were transplanted in mid-May, and harvest started mid-July from four groups of four plants in each plot. Fruits were collected twice a week, taken in consideration 8-9 nodes. Fruit quality (fruit weight, diameter, sugar concentration) was evaluated four times along the experiment in five fruits per replicate. Total plant biomass was measured in four plants per plot at the end of the experiment. Soil nitrate content was measured at the beginning and the end of the assay at two depths (0-30 and 30-60 cm). At the end of the experiment, mixed root samples were taken from four plants per plot, to check the presence of mycorrhizal colonization (Giovannetti and Mosse, 1980) after root clearing and staining (Koske and Gemma, 1989).

2.3. Reduced irrigation doses

The second assay was established at the Agrícola Maresme Floriach (41.545343 N 2.459898 E) in a greenhouse with a 6.6-m width and 2.4-m height, covered with Duraterminc 720 GG plastic cover, no climate control, and a total surface of 0.13 ha. No information on soil attributes was provided by the farm management. The experiment had a 3 x 2 factorial design; the irrigation factor had three levels: optimal irrigation with 100% of the crop daily evapotranspiration (Etc), deficit irrigation with 75% of the optimal treatment (Def), and the usual irrigation adopted at the farm (Farm), which was calculated twice a month using Etc data from Cabrils meteorological station. The other factor was arbuscular mycorrhizal inoculation, with two levels: non-inoculated and inoculated at plantlet transplant. Each inoculated plant received 40 g of *R. irregulare* inoculum, as described in section 2.2. There were three replicates, with two rows of eight plants in each replicate. Plant lines were 2.0 meters apart, and plant rootstocks were 0.45 m apart within each line, resulting in1.11 plants m-2. As each rootstock received two scions, the stems were trained in opposite directions from the line (Figure S1), resulting in 2.22

production stems m^{-2}. Mycorrhizal inoculum potential (MPN) was estimated at the beginning of the experiment, as described in section 2.2.

The irrigation system had an integrated non-self-compensating dripper (Netafilm) every 30 cm with a nominal flow of 1.5 L.h^{-1} in the Deficit and Farm irrigation, and 2.0 L.h^{-1} in the Etc irrigation. The optimal treatment irrigation dose (ETc) was calculated each week using agroclimatic data collected at the IRTA Cabrils Center, the nearest agroclimatic meteorological station, located at a 7.7-km distance. Different Kc values were applied according to plant phenology (FAO56), with a 20% reduction due to plastic mulching (Moreno and Moreno, 2008). Each irrigation line had a water meter, and data were manually recorded. The total amounts of water supplied were 620 liters m^{-2} in the Etc irrigation, 510 liters m^{-2} in the Deficit irrigation, and 486 liters m^{-2} in the Farm irrigation (Figure S2).

Tomato cv. Otello variety plants were grafted on Silex cultivar rootstocks and transplanted in mid-June. Harvest started at the end of August and was done once a week in eight plants (9-10 nodes per plant) in each plot, for three months. Fruit quality (fruit weight, diameter, sugar) was evaluated three times in ten fruits per replicate. Mixed root samples were taken from each plot at the end of the experiment, to check mycorrhizal colonization. We adopted water productivity as the criterion for water use efficiency since it is the ratio used by the farmers. Water productivity (*WP*), the ratio between fruit yield and applied (blue) water was calculated as:

$WP = Y/W$

where Y is marketable (commercial-grade fruit) fruit yield (kg ha^{-}1) and W is the amount of applied irrigation water (m^{3} ha^{-1}). At the end of the experiment root samples were taken from four plants per plot, to evaluate mycorrhizal colonization as described in section 2.2.

2.4. Reduced irrigation and fertilizer doses

The third assay was carried out at Agrícola Maresme Rodon (41.548536 N, 2.474378 E), in a multi-tunnel greenhouse (8 m wide x 3 m height), covered with Diamante 800 GG Trilayer plastic cover, and a total surface of 0.18 ha. Temperature control was done using vertical fans in summer, with no heating system for low ambient temperatures. Soil attributes before and after the essay are described in Table S2. The experiment had a factorial design, with mycorrhizal inoculation and irrigation doses as the main factors. The mycorrhizal inoculation factor had two levels: mycorrhizal plants (Myc) and non-mycorrhizal plants (NM). Each inoculated plant received–*R. irregulare* inoculum, as described in section 2.2. The irrigation factor also had two levels: irrigation according to information provided by soil moisture sensors (Sens) and the usual irrigation adopted by the farm manager (Farm). In the Sens treatment, sensors were installed in the row of replicate 2 of the mycorrhizal and non-mycorrhizal treatments. There were two soil moisture sensors (Meter Group Devices, Pullman, USA), one installed at 20 cm (Teros 12) and the other at 40-cm depth (Teros 10). The 20-cm depth sensor also measured soil temperature and electrical conductivity. There was a sensor for water conductivity and temperature (ES-2, Meter Group Devices, Pullman, USA), as well as a flowmeter (Zenner, Spain). All sensors were connected to a datalogger (Modpow, Spain) that sent the data to a gateway with GPRS connection for data download. The data were downloaded weekly and the irrigation dose calculated from the evapotranspiration data was modulated according to the soil moisture in each of the Sens treatment (one mycorrhizal and one non-mycorrhizal) treatments, which had independent irrigation valves. Sensors were also installed in the Farm treatment, at the same depths, in a mycorrhizal and other in the in a non-mycorrrhizal treatment. A flowmeter (Zenner, Spain) was installed for the Farm treatment. All the Farm sensors were connected to a datalogger (Em50, Meter Group Devices, Pullman, USA).

Mycorrhizal inoculum potential (MPN) was estimated before AMF inoculation, as described in section 2.2. Tomato cv Riesling (a cherry tomato) plants were transplanted mid-February. Harvest started in June and was done once a week in six plants in each replicate, taken in consideration 15 nodes per plant. Fruit quality (fruit weight, diameter, sugar contents) was evaluated two times along the experiment in ten fruits per replicate. At the end of the experiment, mixed root samples were taken from each plot to check mycorrhizal colonization, as described at the end of section 2.2.

3. **Results**

3.1. Mycorrhizal inoculation under reduced fertilizer application

The soil had no AMF propagules, according to the MPN test. Root mycorrhizal colonization was higher in the inoculated treatments than in the non-inoculated ones, which had marginal (10% or less) colonization rates. Mycorrhizal inoculation resulted in a 17% increase in fruit mass and a 21% increase in fruit number (Table 1). Fertilizer application rates did not affect tomato yield, either as fruit number or total mass. Soil nitrate content at 30-cm depth increased 23% in the regular (Farm) fertilization rate, and 68% and 18% in non-mycorrhizal and inoculated plants with reduced fertilization (Red), respectively (Fig S2). At 30 and 60 cm depths, nitrate contents increased 212% in Farm and 83% in Red fertilizer application.

Table 1. Fresh weight yield and number of commercial-grade fruits from greenhouse-grown tomato plants with regular (Farm) and reduced (Red) fertilization, without (NM) or with (Myc) inoculation of arbuscular mycorrhizal fungi. Values are the mean ± standard error.

Main factor	Treatments	Yield kg plant-1	Number of fruits number plant^{-1}	Water productivity kg m-3
Fertilization (F)	Farm	3.52 a†	19.74 a	5.00 a
	Red	3.76 a	20.19 a	5.35 a
Mycorrhiza (M)	Myc	3.96a	21.86 a	5.63 a
	NM	3.38b	18.12 b	4.81 b
F x M interaction	Farm-Myc	3.81	21.63	5.42
	Farm-NM	3.35	18.54	4.77
	Red-Myc	4.11	22.09	5.85
	Red-NM	3.41	18.28	4.85

† Numbers followed by the same letter in each column within the same variable do no differ according to Tukey test. ($p \leq 0.05$). ‡

3.2. Reduced irrigation doses

In the second experiment, the soil had 0.74 propagules of mycorrhizal fungi per 100 g of soil, and root mycorrhizal colonization was low (under 10 %) in all treatments. The irrigation doses affected the number and total weight of commercial-grade tomatoes, while mycorrhizal inoculation did not affect yield (Table 2), and the interaction between factors was not significant. The usual farm irrigation regime resulted in the lowest yields in commercial-grade fruit number and total mass. The highest mass yield occurred with the deficit irrigation, which also led to an intermediate number of commercial-grade fruits. Irrigation significantly affected individual fruit weight and diameter, but there was no effect of irrigation rate nor inoculation on fruit soluble solids concentration (Brix) or color grading (data non shown).

Table 2. Yield, fruit traits, and water supply and productivity of tomato plants, non-inoculated (NM) or inoculated (Myc) with arbuscular mycorrhizal fungi, under three irrigation regimes: deficit, usual farm rate (Farm), or with 100% of the crop evapotranspiration (Etc).

Factor	Treatment	Yield kg.m^{-2}	Yield number.m^{-2}	Fruit mass g fruit^{-1}	Fruit size mm	Water productivity kg m^{-3}
	Deficit	3.40 a†	26.5 ab	131 a	65 a	6.67 a
Irrigation	Farm	2.65 b	21.3 b	120 b	63 b	5,45 ab
	Etc	3.23 ab	26.7 a	137 a	66 a	5.21 b
Inoculation	Myc	3.26 ns	26.0 ns	132 ns	65 ns	6.05 ns
	NM	2.92 ns	23.7 ns	128 ns	65 ns	5,42

† Numbers followed by the same letter in each line, within each factor, do no differ according to Tukey test ($p \leq 0.05$) test. ns = no significant effect.

3.3. Reduced irrigation and fertilizer doses

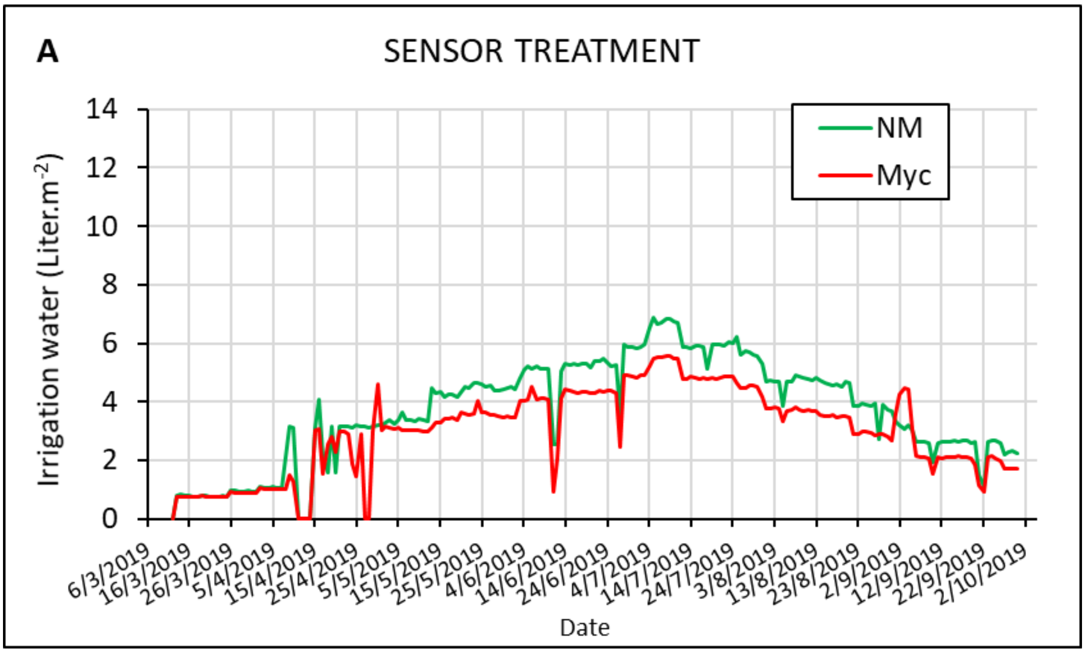
In the third trial, the soil had no detectable arbuscular mycorrhizal fungal propagules, and mycorrhizal inoculation led to increases in root colonization, which ranged between 18 and 26% in inoculated plants and were below 10% in the plots with no inoculation. The colonization rates were almost null in the non-inoculated plants with the regular (Farm) fertigation regime. Mycorrhizal plants had lower water consumption in the sensor-driven irrigation treatment than in the regular farm treatment, with no effect of mycorrhizal inoculation on fruit yield. Water productivity, the fruit mass obtained with a cubic meter of applied water, was 10 to 14% higher in inoculated plants under reduced fertigation than in all other treatments.

Table 3: Fruit yield and water consumption and footprint of tomato plants grown in a greenhouse, non-inoculated (NM) or inoculated with arbuscular mycorrhizal fungi (Myc), and with sensor-optimized (Sens) or standard farm (Farm) water supply.

Treatment	Yield kg m^{-2}	Water supply L m^{-2}	Water productivity kg m^{-3}
Sens – Myc	10.5ns	614†	17.1†
Sens – NM	11.7	754	15.5
Farm – Myc	10.5	704	14,9
Farm – NM	10.5	704	14.9

ns = no significant differences; † no statistical analysis available.

Water supply was more uniform in the sensor-driven irrigation (Sensor) treatment (Figure 1a) than in the standard (Farm) irrigation system (Figure 1b), which was expected due to the use of sensors in the soil. Figure 2a shows that, generally, soil water content was higher with inoculated plants; water use was lower in the mycorrhizal plants than in the non-inoculated ones. At the 40-cm depth, the water content in soil with the mycorrhizal plant was higher than non-inoculated plants (Figure 2a), a trend also shown in the regular Farm irrigation (Figure 2b).


A
SENSOR TREATMENT
NM
Myc
Irrigation water (Liter.m^{-2})
14
12
10
8
6
4
2
0
6/3/2019
16/3/2019
26/3/2019
5/4/2019
15/4/2019
25/4/2019
5/5/2019
15/5/2019
25/5/2019
4/6/2019
14/6/2019
24/6/2019
4/7/2019
14/7/2019
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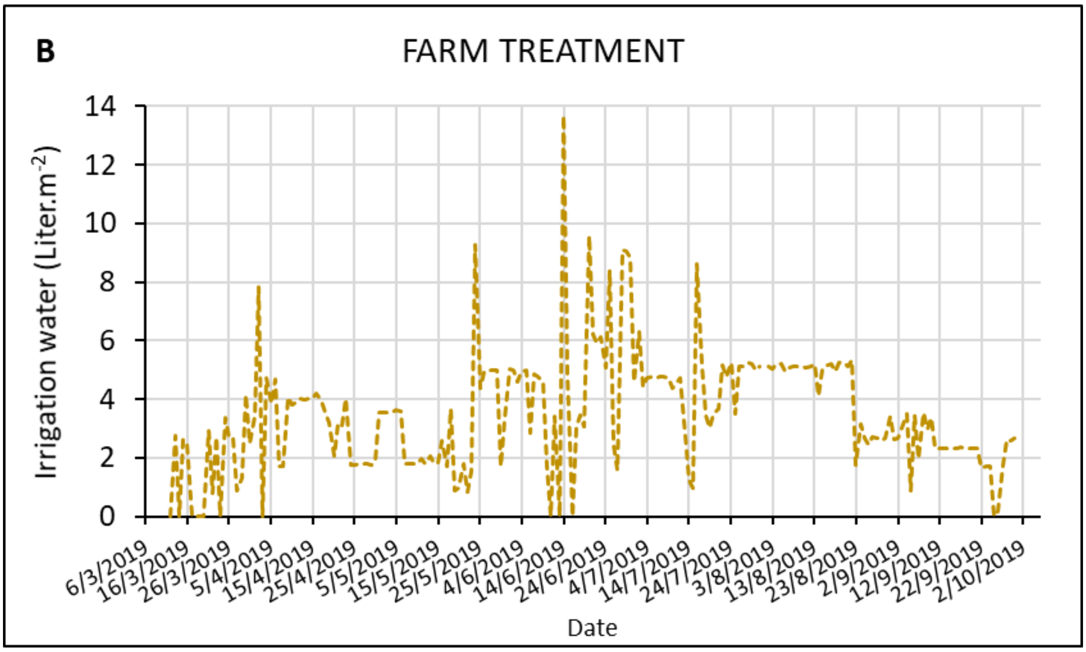
23/8/2019
2/9/2019
12/9/2019
22/9/2019
2/10/2019
Date


B
FARM TREATMENT
Irrigation water (Liter.m^{-2})
14
12
10
8
6
4
2
0
6/3/2019
16/3/2019
26/3/2019
5/4/2019
15/4/2019
25/4/2019
5/5/2019
15/5/2019
25/5/2019
4/6/2019
14/6/2019
24/6/2019
4/7/2019
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24/7/2019
3/8/2019
13/8/2019
23/8/2019
2/9/2019
12/9/2019
22/9/2019
2/10/2019
Date

Figure 1. Irrigation water supplied during the vegetative and production time in sensor-optimized (A, Sens) and standard farm (B, Farm) irrigation rates of non-inoculated (NM) or inoculated with arbuscular mycorrhizal fungi (Myc) tomato plants in Experiment 3.

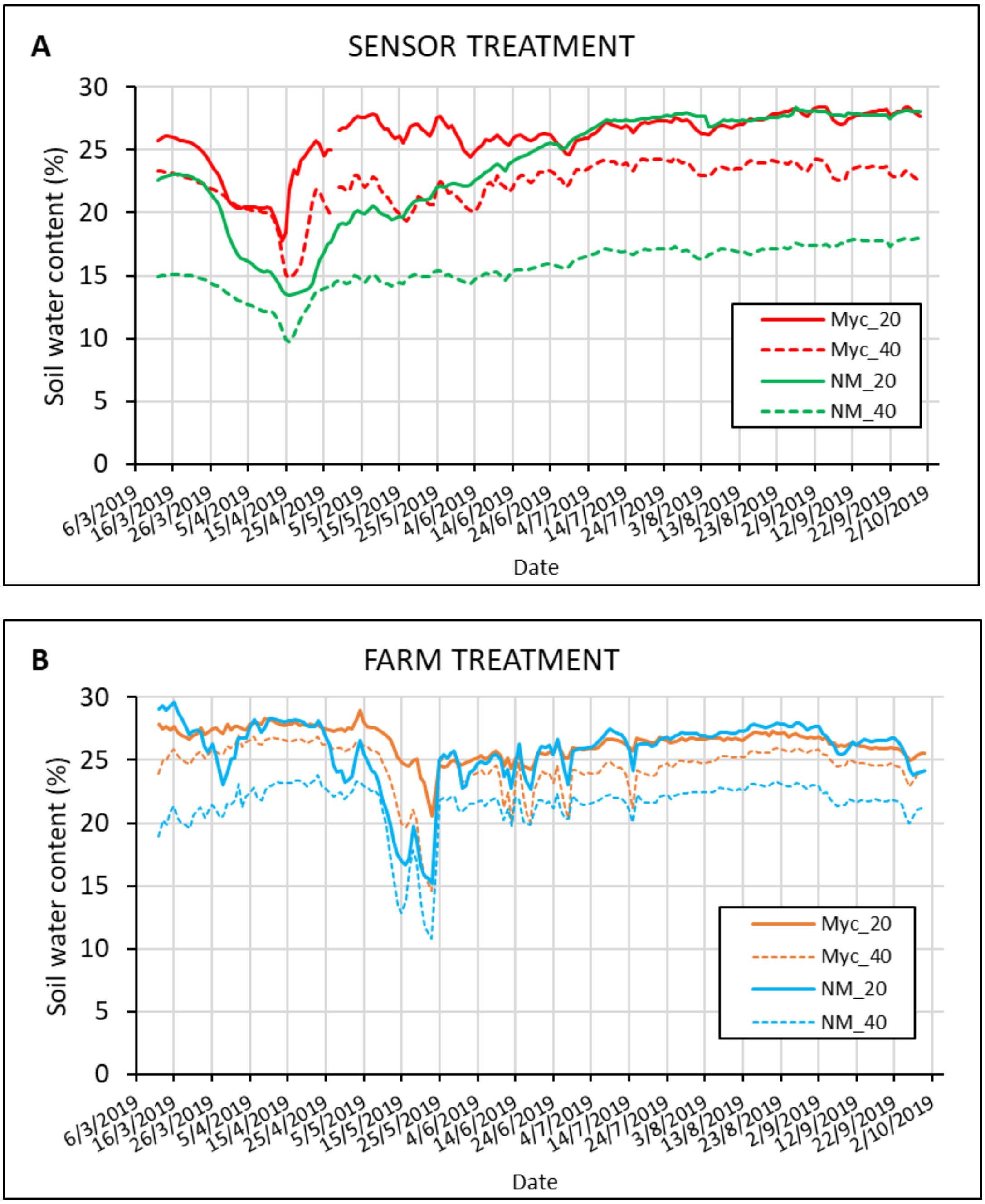

Figure 2. Soil water content at 20 and 40 cm depth during the vegetative and production time in sensor-optimized (A, SENSOR) or standard farm (B, Farm) irrigation rates, and non-inoculated (NM) or inoculated with arbuscular mycorrhizal fungi (Myc) tomato plants in Experiment 3.

4. Discussion

In all experiments, mycorrhizal inoculation or reduced irrigation led to some gain in productivity, resulting from lower fertilizer use and associated costs, besides less environmental impact. In the first experiment, mycorrhizal inoculation resulted in a 17% increase in yield, corresponding to 13% or 4,6 t ha^{-1} with the regular fertilization rate (Farm). That increase was more marked with reduced fertilization (Red), with gains reaching 20% or 7,0 t ha^{-1}. On the other hand, even when yield did not increase with lower fertilizer application, it reduced costs. In the other experiments, gains are linked to reduced costs, besides indirect gains in lowered environmental impacts.

In the second trial, there was no effect of AMF inoculation on tomato yield or quality due to the low levels of root colonization. However, changes in fruit yield and quality were associated with irrigation rates. The deficit-irrigation regime increased total mass yield, fruit diameter, and individual fruit mass compared with the regular (Farm) regime, while the optimized (ETc) regime increased fruit number and fruit size compared with the farm irrigation regime. Those are important qualities for the Otello variety, a salad cultivar, as larger fruits are more attractive for consumers. We could not find gains in quality in terms of color or soluble solids concentration, which estimates sugar concentration. That contradicts previous work showing gains in quality (Bona et al., 2017, 2018). The optimized (ETc) regime received 27% more irrigation water than the regular farm regime, and even the deficit regime (75% of the optimized dose) received 5% more water than the standard farm irrigation rates. That gain in water productivity in the ETc regime corresponds to a 4.6% increase in water productivity, as compared with the farm regime. However, the deficit regime increased water productivity by more than 20% due to the increase in plant yield, a behavior found in other irrigation trials (Chukalla et al., 2017). That corresponds to blue water (Lovarelli et al., 2016), taken from an aquifer, which also demands the use of energy, installation, and equipment maintenance, and such savings are, therefore, substantial. Those cost reductions would correspond to around 1400 € ha^{-1}, assuming the water price at 0,25 € m^{-3} (Cáceres Hernández et al., 2018).

In the third experiment, the combination of reduced fertilizer and irrigation rates, previously tested separately, affected tomato production. Root colonization rates were higher in inoculated plants than in the non-inoculated ones. Mycorrhizal plants had a 20% lower water consumption than non-mycorrhizal plants in the sensor treatment, in which water supply was linked to plant growth and water availability in the soil. However, neither the irrigation regimes nor mycorrhizal inoculation had any effect on fruit yield or quality. The use of sensors resulted in a steadier water supply to the plants and a less variable amount of water in the soil, especially in later stages of the plant cycle, when fruits were accumulating mass. Mycorrhizal inoculation affected soil water content at 40-cm depth (Figure 2); in both fertigation regimes, soils with AMF-inoculated plants had higher water content. That suggests either an improvement in soil water retention, which is promoted by glomalin (Singh et al., 2013), or higher water content in the plants due to water supply provided by the arbuscular mycorrhizal fungal mycelium network. Water productivity increased 10% with mycorrhizal inoculation, and that also means a lower environmental impact production, increasingly considered in agricultural systems (Lovarelli et al., 2016), even farmers are motivated mostly by financial gains in the short-term.

Irrigation costs in med-tech systems, such as the one adopted in all three experiments reported here, are about 4% of variable costs, while fertilizers amount to around 8% of those costs (Cáceres-Hernández et al. 2018). Even not considering eventual savings in energy used for irrigation, a 19% reduction of two inputs (water and fertilizers, which amount to 12% of total cost) corresponds to a 2.3% decrease in total costs. That may seem small, but it amounts to over one thousand euros, considering that tomato production costs are around 60 thousand euros per hectare (Cáceres Hernández et al., 2018). Farmers have a rule of thumb of using a new input if the value/cost ratio, the increase in financial gains (increased yield or cost reduction) divided by the input cost, is three or higher (Thuita et al., 2018). The owners of the farms where our study was carried out informed that the inoculant cost ranges from 100 to 180 euros per hectare. The value/cost ratio would, therefore, range between six and 12. That is higher than the ratio of three given by Thuita et al. (2018), which may even be two for small landholder farmers (Van Vugt et al., 2017), the case of many vegetable producers. In short, savings in water and fertilizers in tomato production would justify the use of mycorrhizal inoculation.

We found a small number of papers discussing the economic viability of arbuscular mycorrhizal inoculants. Our analysis of the large dataset of Hijri (2016) found that 71 and 67% of the tested fields would be profitable if the value/cost ratios of two and three were applied. The work by Stoffel et al. (2020a) with corn estimated a gain of US$ 226 per hectare using a commercial inoculant, which would allow the use of products costing up to US$ 75 per hectare. In another AMF inoculant test with soybeans, Stoffel et al. (2020b) did not calculate value/gain ratios, but we estimated that the observed mean yield increase of 0.90 ton ha^{-1} (ranging from 0.46 to 1.4 ton ha^{-1} increases) would represent a mean gain of US$ 335 ha^{-1} (ranging from 161 to 490), considering a historical soybean price of US$ 350 ton^{-1}. That would make feasible the use of inoculants costing about 100 US$ ha^{-1}. Nevertheless, grains have lower added-value than horticultural crops, like tomato, for which mycorrhizal inoculants would be more promising, even if their prices were higher than those used by Stoffel et al. (2020a, 2020b).

In conclusion, we observed that mycorrhizal inoculation increases fruit yield and quality with reduced fertilizer doses; deficit and optimized irrigation rates increase tomato yield and fruit size, although not affecting fruit quality in terms of color of concentration of soluble solids. Deficit and optimized irrigation increased tomato yield and fruit size, but not quality, and plants inoculated with mycorrhizal fungi sustained yield levels with reduced fertigation, which corresponds to savings well above the inoculant cost. We conclude that in most cases, mycorrhizal inoculation increases water productivity in tomato production. Besides the economic gains, that corresponds to a reduction in agricultural production ecological footprint, a factor of increasing importance. However, even restricting the focus on economic gains for farmers, we observed that in all cases, mycorrhizal inoculation presented a potential for gains in tomato productivity in real-life conditions. In one case, there were yield increases, while in other situations, the presence of mycorrhizas guaranteed yield maintenance, and in one assay, there was quality improvement when input use was lowered. In all cases, the value/cost ratio justified the use of commercial mycorrhizal inoculant in tomato production. There is a positive trend in mycorrhiza research on increasing the frequency of field and on-farm experiments, but economic assessments are still rare. We advocate here that researchers estimate the financial incentive that mycorrhizal inoculation would incur so that farmers and inoculant producers have a firm base to test and adopt mycorrhizal inoculation technology.

References

Abbott, L.K., Macdonald, L.M., Wong, M.T.F., Webb, M.J., Jenkins, S.N., Farrell, M., 2018. Potential roles of biological amendments for profitable grain production – A review. Agric. Ecosyst. Environ. 256, 34–50. https://doi.org/10.1016/j.agee.2017.12.021

Abdul-Baki, A. A., Teasdale, J. R., Korcak, R. F., 1997. Nitrogen requirements of fresh-market tomatoes on hairy vetch and block polyethylene mulch. HortSci. 32, 217–221. https://doi.org/10.21273/HORTSCI.32.2.217

Bender, S.F., Schlaeppi, K., Held, A., Van der Heijden, M.G.A., 2019. Establishment success and crop growth effects of an arbuscular mycorrhizal fungus inoculated into Swiss corn fields. Agric. Ecosyst. Environ. 273, 13–24. https://doi.org/10.1016/j.agee.2018.12.003

Bona, E., Cantamessa, S., Massa, N., Manassero, P., Marsano, F., Copetta, A., Lingua, G., D’Agostino, G., Gamalero, E., Berta, G., 2017. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza 27, 1–11. https://doi.org/10.1007/s00572-016-0727-y

Bona, E., Todeschini, V., Cantamessa, S., Cesaro, P., Copetta, A., Lingua, G., Gamalero, E., Berta, G., Massa, N., 2018. Combined bacterial and mycorrhizal inocula improve tomato quality at reduced fertilization. Sci. Hortic. 234, 160–165. https://doi.org/10.1016/j.scienta.2018.02.026

Cáceres Hernández, J.J., Godenau, D., González Gómez, J.I., Martín Rodríguez, G., Ramos Henríquez, J.M., 2018. Tomate canario de exportación: una evaluación de costes. Inf. Tec. Econ. Agrar. 114, 280–302. https://doi.org/10.12706/itea.2018.017

Calvet, C., Pinochet, J., Hernández-Dorrego, A., Estaún, V., Camprubí, A., 2001. Field microplot performance of the peach-almond hybrid GF-677 after inoculation with arbuscular mycorrhizal fungi in a replant soil infested with root-knot nematodes. Mycorrhiza 10, 295–300. https://doi.org/10.1007/PL00009998

Camprubí, A., Estaún, V., Bakali, M.A., Garcia-Figueres, F., Calvet, C., 2007. Alternative strawberry production using solarization, metham sodium and beneficial soil microbes as plant protection methods. Agron. Sustain. Dev. 27, 179–184. https://doi.org/10.1051/agro:2007007Candido, V., Campanelli, G., D'Addabbo, T., Castronuovo, D., Perniola, M., Camele, I., 2015. Growth and yield promoting effect of artificial mycorrhization on field tomato at different irrigation regimes. Sci. Hortic. 187, 35–43. https://doi.org/10.1016/j.scienta.2015.02.033

Castellanos-Morales, V., Villegas, J., Wendelin, S., Vierheilig, H., Eder, R., Cárdenas-Navarro, R., 2010. Root colonisation by the arbuscular mycorrhizal fungus Glomus intraradices alters the quality of strawberry fruits (Fragaria × ananassa Duch.) at different nitrogen levels. J. Sci. Food Agric. 90, 1774–1782. https://doi.org/10.1002/jsfa.3998

Ceballos, I., Ruiz, M., Fernández, C., Peña, R., Rodríguez, A., Sanders, I.R., 2013. The In Vitro Mass-Produced Model Mycorrhizal Fungus, Rhizophagus irregularis, Significantly Increases Yields of the Globally Important Food Security Crop Cassava. PLoS One 8, e70633. https://doi.org/10.1371/journal.pone.0070633

Chai, Q., Gan, Y., Zhao, C., Xu, H.L., Waskom, R.M., Niu, Y., Siddique, K.H.M., 2016. Regulated deficit irrigation for crop production under drought stress. A review. Agron. Sustain. Dev. 36, 1–21. https://doi.org/10.1007/s13593-015-0338-6

Chukalla, A.D., Krol, M.S., Hoekstra, A.Y., 2017. Marginal cost curves for water footprint reduction in irrigated agriculture: Guiding a cost-effective reduction of crop water consumption to a permit or benchmark level. Hydrol. Earth Syst. Sci. 21, 3507–3524. https://doi.org/10.5194/hess-21-3507-2017

Estaun, V., Calvet, C., Camprubi, A., 1994. Arbuscular mycorrhizae and growth enhancement of micropropagated Prunus rootstock in different soilless potting mixes. Agric. Food Sci. 3, 263–267. https://doi.org/10.23986/afsci.72704

Evans, R.G., Sadler, E.J., 2008. Methods and technologies to improve efficiency of water use. Water Resour. Res. 44, 1–15. https://doi.org/10.1029/2007WR006200

Fracasso, A., Telò, L., Lanfranco, L., Bonfante, P., Amaducci, S., 2020. Physiological Beneficial Effect of Rhizophagus intraradices Inoculation on Tomato Plant Yield under Water Deficit Conditions. Agron. 10, 1-21. https://doi.org/10.3390/agronomy10010071

Geneva, M.P., Stancheva, I. V., Boychinova, M.M., Mincheva, N.H., Yonova, P. A., 2010. Effects of foliar fertilization and arbuscular mycorrhizal colonization on Salvia officinalis L. growth, antioxidant capacity, and essential oil composition. J. Sci. Food Agric. 90, 696–702. https://doi.org/10.1002/jsfa.3871

Gianinazzi, S., Gollotte, A., Binet, M.-N., Van Tuinen, D., Redecker, D., Wipf, D., 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20, 519–530. https://doi.org/10.1007/s00572-010-0333-3

Giovannetti, M., Mosse,B., 1980. An evaluation of techniques for measurign vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Hart, M., Ehret, D.L., Krumbein, A., Leung, C., Murch, S., Turi, C., Franken, P., 2015. Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. Mycorrhiza 25, 359–376. https://doi.org/10.1007/s00572-014-0617-0

Hernández, V., Hellín, P., Fenoll, J., Flores, P., 2020. Impact of nitrogen supply limitation on tomato fruit composition. Sci. Hortic. 264, 109173. https://doi.org/10.1016/j.scienta.2020.109173

Hijri, M., 2016. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. Mycorrhiza 26, 209–214. https://doi.org/10.1007/s00572-015-0661-4

Howeler, R.H., Sieverding, E., Saif, S., 1987. Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant Soil 100, 249–283. https://doi.org/10.1007/BF02370945

Khapte, P.S., Kumar, P., Burman, U., Kumar, P., 2019. Deficit irrigation in tomato: Agronomical and physio-biochemical implications. Sci. Hortic. 248, 256-264. https://doi.org/10.1016/j.scienta.2019.01.006

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486–488. https://doi.org/10.1016/S0953-7562(89)80195-9

Kumar, P., Rouphael, Y., Cardarelli, M., Colla, G., 2017. Vegetable grafting as a tool to improve drought resistance and water use efficiency. Front. Plant Sci. 8, 1130. https://doi.org/10.3389/fpls.2017.01130

Lovarelli, D., Bacenetti, J., Fiala, M., 2016. Water Footprint of crop productions: A review. Sci. Total Environ. 548–549, 236–251. https://doi.org/10.1016/j.scitotenv.2016.01.022

Molden, D., Oweis, T., Steduto, P., Bindraban, P., Hanjra, M.A., Kijne, J., 2010. Improving agricultural water productivity: Between optimism and caution. Agric. Water Manag. 97, 528–535. https://doi.org/10.1016/j.agwat.2009.03.023

Mollavali, M., Bolandnazar, S.A., Schwarz, D., Rohn, S., Riehle, P., Zaare Nahandi, F., 2016. Flavonol Glucoside and Antioxidant Enzyme Biosynthesis Affected by Mycorrhizal Fungi in Various Cultivars of Onion (Allium cepa L.). J. Agric. Food Chem. 64, 71–77. https://doi.org/10.1021/acs.jafc.5b04791

Moreno, M.M., Moreno, A., 2008. Effect of different biodegradable and polyethylene mulches on soil properties and production in a tomato crop. Sci. Hortic. 116, 256–263. https://doi.org/10.1016/j.scienta.2008.01.007

Page, G., Ridoutt, B., Bellotti, B., 2012. Carbon and water footprint tradeoffs in fresh tomato production. J. Clean. Prod. 32, 219–226. https://doi.org/10.1016/j.jclepro.2012.03.036

Parkash, V., Singh, S., 2020. A review on potential plant-basedwater stress indicators for vegetable crops. Sustain. 12, 3945. https://doi.org/10.3390/SU12103945

Porter, W.M., 1979. The 'most probable number' method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res. 17, 515-519. https://doi.org/10.1071/SR9790515

Roberts, D.P., Mattoo, A.K., 2019. Sustainable Crop Production Systems and Human Nutrition. Front. Sustain. Food Syst. 3, 1–14. https://doi.org/10.3389/fsufs.2019.00072

Rocha, I., Ma, Y., Carvalho, M.F., Magalhães, C., Janoušková, M., Vosátka, M., Freitas, H., Oliveira, R.S., 2019. Seed coating with inocula of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for nutritional enhancement of maize under different fertilisation regimes. Arch. Agron. Soil Sci. 65, 31–43. https://doi.org/10.1080/03650340.2018.1479061

Ronga, D., Caradonia, F., Francia, E., Morcia, C., Rizza, F., Badeck, F.W., Ghizzoni, R., Terzi, V., 2019. Interaction of tomato genotypes and arbuscular mycorrhizal fungi under reduced irrigation. Hortic. 5, 1–15. https://doi.org/10.3390/horticulturae5040079

Schwarz, D., Rouphael, Y., Colla, G., Venema, J.H., 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. Sci. Hortic. 127, 162–171. https://doi.org/10.1016/j.scienta.2010.09.016

Singh, P.K., Singh, M., Tripathi, B.N., 2013. Glomalin: An arbuscular mycorrhizal fungal soil protein. Protoplasma 250, 663–669. https://doi.org/10.1007/s00709-012-0453-z

Smith, S.E., Read, D.J., 2002. Introduction, in: Smith, S.E., Read, D.J. (Eds.), Mycorrhizal Symbiosis, second ed. Academic Press, London, pp. 1–8. https://doi.org/10.1016/B978-

012652840-4/50001-2

Sørensen, J., Haubjerg Nicolaisen, M., Ron, E., Simonet, P., 2009. Molecular tools in rhizosphere microbiology—from single-cell to whole-community analysis. Plant Soil 321, 483–512. https://doi.org/10.1007/s11104-009-9946-8

Stoffel, S.C.G., Soares, C.R.F.S., Meyer, E., Lovato, P.E., Giachini, A.J., 2020a. Yield increase of corn inoculated with a commercial arbuscular mycorrhizal inoculant in Brazil. Ciênc. Rural 50, e20200109. https://doi.org/10.1590/0103-8478cr20200109

Stoffel, S.C.G., Soares, C.R.F.S., Meyer, E., Lovato, P.E., Giachini, A.J., 2020b. Yield increase of soybean inoculated with a commercial arbuscular mycorrhizal inoculant in Brazil. Afr. J. Agric. Res. 16, 702-713. https://doi.org/10.5897/AJAR2020.14766

Thuita, M., Vanlauwe, B., Mutegi, E., Masso, C., 2018. Reducing spatial variability of soybean response to rhizobia inoculants in farms of variable soil fertility in Siaya County of western Kenya. Agric. Ecosyst. Environ. 261, 153–160. https://doi.org/10.1016/j.agee.2018.01.007

Torres, N., Goicoechea, N., Carmen Antolín, M., 2018. Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures. Agric. Water Manag. 202, 285–298. https://doi.org/10.1016/j.agwat.2017.12.004

Van Vugt, D., Franke, A.C., Giller, K.E., 2017. Participatory research to close the soybean yield gap on smallholder farms in Malawi. Exp. Agric. 53, 396–415. https://doi.org/10.1017/S0014479716000430

Zayova, E., Stancheva, I., Geneva, M., Hristozkova, M., Dimitrova, L., Petrova, M., Sichanova, M., Salamon, I., Mudroncekova, S., 2018. Arbuscular mycorrhizal fungi enhance antioxidant capacity of in vitro propagated garden thyme (Thymus vulgaris L.). Symbiosis 74, 177–187. https://doi.org/10.1007/s13199-017-0502-7

Ziane, H., Meddad-Hamza, A., Beddiar, A., Gianinazzi, S., 2017. Effects of arbuscular mycorrhizal fungi and fertilization levels on industrial tomato growth and production. Int. J. Agric. Biol. 19, 341–347. https://doi.org/10.17957/IJAB/15.0287

Supplementary material:

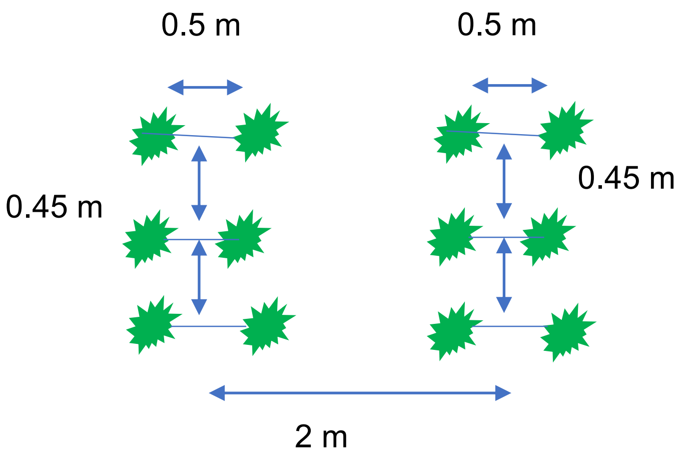

Figure S1. Schematic representation of plant distribution in Experiment 2.

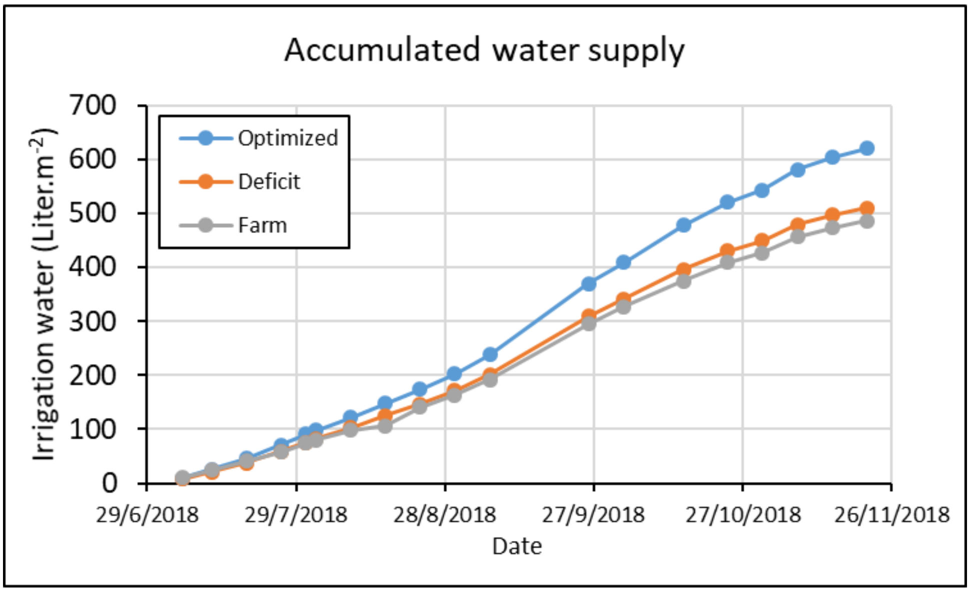

Figure S2. Accumulated water supply to tomato plants in Experiment 2, with optimized (Etc), deficit (Def), or farm standard irrigation (Farm) doses.

Table S1. Chemical characteristics of the well water used for irrigation.

Characteristic	Values
Electrical conductivity (dS.m^{-1})	2.55
pH	7.09
Bicarbonate (mg.L^{-1})	293
Carbonate (mg.L^{-1})	0
Nitrate (mg.L^{-1})	367
Potassium (mg.L^{-1})	6
Calcium (mg.L^{-1})	393
Magnesium (mg.L^{-1})	78
Sodium (mg.L^{-1})	112
Sulfate (mg.L^{-1})	526
Chloride (mg.L^{-1})	345

Table S2. Initial and final soil chemical attributes in the reduced irrigation and fertilizer experiment (Item 2.4). M: with mycorrhizas; NM: non-inoculated.

Attribute	Unit	Initial	Farm M	FARM NM	SENSOR M	SENSOR NM
pH		7.8	7.9	7.8	8.0	7.8
Electrical conductivity	dS.m^{-1}	1.6	0.9	1.1	1.2	0.7
Organic matter	g kg^{-1}	26	22	24	23	21
N-Nitrate	mg L^{-1}	108	107	143	99	94
Phosphorus	mg L^{-1}	171.0	71.1	83.2	52.7	55.6
Potassium	mg L^{-1}	130	260	311	101	170
Magnesium	mg L^{-1}	360	443	456	500	397
Calcium	mg L^{-1}	2580	3396	3553	3797	3218
Sodium	mg L^{-1}	140	164	186	210	134