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Combined effects of deficit irrigation and strobilurin application on gas exchange, yield and water use efficiency in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Water is the major factor limiting plant productivity in many regions of the world. The aim of this study was to evaluate the combined effect of deficit irrigation (restitution of 100%, 50% and 0% of plant consumption: WR_{100}, WR_{50} and WR_0, respectively) and strobilurin treatment (no agrochemical added *vs* azoxystrobin treatment) in two tomato genotypes, IT-22/025, a wild-type plant, and Ikram, a commercial hybrid. Water use efficiency (WUE), physiological, yield and quality parameters and the expression of ERD15, a gene involved in abiotic stress response were evaluated. The two genotypes showed a different behaviour in response to water stress. Stomatal conductance decrease from WR_{100} to WR_{50} was in mean 27.5% for IT-22/025 and 44.5% for Ikram. Moreover, in Ikram, water stress decreased transpiration more than assimilation rate, while the opposite occurred in IT-22/025. The ERD15 expression decrease from WR_{100} to WR_{50} was higher for IT-22/025. These effects corresponded to higher total fresh fruit yield and WUE for IT-22/025. Strobilurin determined lower stomata conductance, maintaining higher assimilation rate, leading to an increase in WUE in WR_0. Finally, strobilurin caused an increase in ERD15 expression only in IT-22/025. This study underlines the possibility to reduce the water used in tomato crop, maintaining acceptable yield and quality, by using agronomic and genetic strategy.

1. Introduction

Water is the major factor limiting plant productivity in agriculture in many regions of the world, especially in arid and semi-arid zones (Tahi et al., 2007). Worldwide, water is a progressively scarce resource due to increasing demand, climate changes and qualitative degradation. Thus, there is an increasing necessity to reduce the amount of water used during irrigation practices (Zegbe-Domínguez et al., 2003) and to improve the drought tolerance and water use efficiency of food crops. Tomato is a high-water-demand vegetable crop and is generally cultivated under irrigation. Moreover, tomato has the highest acreage of any vegetable crop in the world (Jensen et al., 2010). Therefore, the adoption of deficit irrigation (DI) could save a substantial amount of water (Zegbe-Domínguez et al., 2003; Cantore et al., 2016). It is reported that DI, where only a portion of evapotranspiration is given to plants during the crop cycle, may improve the water use efficiency (WUE) of crops without subsequent yield reduction (Senyigit et al., 2011; Nardella et al., 2012). DI has been assessed for tomato with contrasting results (Zegbe-Domínguez et al., 2003; Kirda et al., 2004; Patanè et al., 2011; Giuliani et al., 2016).

The main plant response to drought stress, in the short term, is stomata closure to reduce leaf transpiration and to prevent excessive water deficit in its tissues (Cochard et al., 2002). Abscisic acid (ABA) is the chemical signal used by the plant during water stress to reduce stomatal conductance before leaf hydration decreases (Liu et al., 2003). ABA accumulation starts within 2 h after dehydration and induces the expression of a large number of genes (Kiyosue et al., 1994). However, some genes, such as Early Responsive to Dehydration (ERD) genes, are induced prior to the accumulation of ABA. ERD are rapidly activated during water stress (Kiyosue et al., 1994). In particular, the ERD15 response to different environmental stressors has been studied in *Arabidopsis* and wheat (Dunaeva and Adamska, 2001; Park et al., 2009; Li et al., 2010a), showing high variability in its induction and function (Kariola et al., 2006; Ziaf et al., 2011). In *Arabidopsis*, the plants showing increased tolerance to salt stress also showed higher transcription levels of ERD15 than control plants (Park et al., 2009). In contrast, Kariola et al. (2006) reported that *Arabidopsis* plants overexpressing ERD15 manifested susceptibility to drought and freezing

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stress. In *Solanum pennellii*, gradual increase in the ERD15 mRNA accumulation enhanced by drought, salinity, cold and ABA treatments has been reported (Ziaf et al., 2011). In some crop species, an increase in ABA concentration linked to the stomata opening was observed after strobilurin treatment, improving plant water storage under water stress conditions (Venancio et al., 2003). Strobilurins are important active ingredients in agricultural fungicides, but in addition to their fungicide effect, physiological effects have also been reported in treated plants by several authors (Swoboda and Pedersen, 2009; Fagan et al., 2010; Joshi et al., 2014). Studies on the effect of strobilurins on physiological, yield and quality parameters have been focused on wheat, barley and soybean, with fewer studies on tomato available. Giuliani et al. (2011) and Cantore et al. (2016) observed an improvement in plant water status, water use efficiency and yield in tomato plants treated with strobilurin in water-limited conditions.

The general purpose of this research was to evaluate strategies that allow the reduction of the amount of water used during the tomato crop cycle while maintaining the yield and quality response. Although the effect of deficit irrigation on tomato crop was already widely investigated, to the best of the authors' knowledge very few information are available, in the scientific literature, on the combined effect of deficit irrigation and strobilurin treatment on physiological, yield and quality parameters on tomato. To this aim, the combined effects of DI and strobilurin treatment on physiological, yield and quality response of two tomato genotypes (IT-22/025 a wild-type plant, and Ikram, a commercial hybrid) have been studied. Moreover, the expression of ERD15, a gene involved in abiotic stress response, was evaluated in the two genotypes also in relation to strobilurin application for the first time.

2. Materials and methods

2.1. Plant material and growth condition

The study was carried out at the Department of Agricultural, Food and Environmental Sciences of the University of Foggia. Two fresh tomato genotypes, Ikram (Syngenta Seeds Spa) and IT-22/025 (selected by Department of Soil Sciences, Plants and Food of University of Bari "A. Moro"), were grown under controlled conditions from April 26 to August 3, 2016. The day/night temperatures were 22–26 °C/18 °C, the relative humidity was 60%, and the photosynthetically active radiation (PAR) was 500 μmol $m^{-2}s^{-1}$ plant height (with a 16 h/8 h photoperiod). Transplanting was carried out on April 26, at three-life stage, in PVC pots (0.4 m diameter × 0.4 m high) that contained 18 kg of clay soil, sand and peat mixture in a 6:3:1 ratio by volume. The location of pots within the growth chamber was rotated frequently to avoid positional effects. Fertilization was performed using throughout 3.14 g m^{-2} of monoammonium phosphate (12-61-0) and 2.63 g m^{-2} of ammonium nitrate (26-0-0). The harvesting was done at different times because of the gradual ripening of the fruits. Harvest of IT-22/025 was performed on July 21 and July 29 and harvest of Ikram on July 29 and August 3.

2.2. Water regimes and strobilurin treatment

From the time of transplanting to 15 DAT (days after transplanting), all plants were well watered to allow root system establishment. After that, three water treatments were applied: WR_{100}, considered as control, in which plants were watered at 100% of plant transpiration; WR_{50}, in which 50% of the amount of water given to the control plants was supplied; and WR_0, in which watering was only at transplanting, during fertigation and as supplementary irrigation. The amounts of water applied (Table 2) were estimated based on plant water use in the control treatment, which was measured by weighing the pots every day. Watering was performed once daily. Throughout the cycle, strobilurin effect was evaluated by comparing two groups: i) ST_0, where no agrochemical was added, as the control, and ii) ST_{az}, the azoxystrobin treatment. The foliar azoxystrobin application was performed at second truss flowering (35 DAT for IT-22/025 and 40 DAT for Ikram) and at fruit ripening of the first truss (72 DAT for IT-22/025 and 79 DAT for Ikram) according to the standard for fungicide application. The experiment was arranged in a complete randomized design with four replicates and three factors (genotype, G; water regime, WR; strobilurin treatment, ST).

2.3. Gas exchange

Gas exchange measurements were done inside the growth chamber using the LI-6400XT portable gas exchange system (LiCor Inc., Lincoln, NE, USA) on fully expanded leaves that were clean, dry and without sign of disease or damage, at a CO_2 concentration of 400 μmol CO_2 mol air^{-1}, relative humidity of 28% and temperature of 26 °C. Three replicate leaves per plant were used. Measurements were performed at three stages of the crop cycle: i) fifteen days after the water stress application (T_1, 30 DAT for both genotypes); ii) one week after the first azoxystrobin treatment (T_2, 43 DAT and 47 DAT for IT-22/025 and Ikram, respectively-full flowering stage); and iii) one week after the second azoxystrobin treatment (T_3, 79 DAT and 86 DAT for IT-22/025 and Ikram, respectively-fruit ripening stage). The stomatal conductance (g_s) was registered in mol $H_2O\,m^{-2}s^{-1}$, the transpiration rate (E) in mmol $H_2O\,m^{-2}s^{-1}$ and the CO_2 assimilation rate (A) in μmol $CO_2\,m^{-2}s^{-1}$. Intrinsic water use efficiency (WUE_i) was calculated as the ratio between assimilation and stomatal conductance (A/g_s) (Yan et al., 2017).

2.4. Biomass, yield and quality parameters

At the end of the experiment (94 DAT for IT-22/025 and 99 DAT for Ikram), the plants were harvested to estimate the biomass. The dry weight of the aerial parts (stems, leaves and fruit) was determined after drying at 70 °C until a constant weight. At each harvest time, for each plant, individual fruit fresh weight was evaluated for yield estimation. The total fruit yield (g $plant^{-1}$) was calculated as the sum of fruit fresh weight at each harvest. Water use efficiency was calculated as the ratio between total aerial plant dry matter at harvest (stems, leaves and fruits, g $plant^{-1}$) and plant water used (l $plant^{-1}$) (DMWUE, g l^{-1}). Water use efficiency was also calculated as the ratio between total fruit yield and plant water used (TYWUE, g l^{-1}). Three fruits per replication were randomly chosen for the quality measurements. Skin colour was measured at harvest three times on two opposite sides of the middle part of each fruit using a CM-700d spectrophotometer (KONICA MINOLTA, Inc., Tokyo, Japan). The colour index was calculated according to Messina et al. (2012). After sampling for colour, fruits were cut into halves and a few drops from each half were used to measure total soluble solids (°Brix) with a hand-held refractometer with automatic temperature compensation (mod. DBR35, XS INSTRUMENTS, Carpi, Italy).

2.5. Analysis of ERD15 gene expression

At T_1 and T_2, simultaneously with the physiological measurements, one leaf for each plant was collected for the ERD15 gene expression evaluation. The leaf samples were kept in RNA Stabilization Solution RNAlater (Invitrogen) in order to stabilize and protect cellular RNA in unfrozen tissue samples for one day at 37 °C. After that, leaf samples were stored at −80 °C. Total RNA was extracted using an extraction buffer contained β-mercaptoethanol and a high concentration of guanidine thiocyanate (Qiagen). The c-DNA samples for real-time RT-PCR experiments were synthesized from 1 μg of total RNA and random nonamer primers, using the kit SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's protocol. A PCR was carried out, using β-actin and ERD-15 c-DNA-specific primers, to determine the quality of c-DNA obtained and to evaluate the