



# 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 (restoration of 100%, 50% and 0% of plant consumption: WR<sub>100</sub>, WR<sub>50</sub> and WR<sub>0</sub>, 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<sub>100</sub> to WR<sub>50</sub> 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<sub>100</sub> to WR<sub>50</sub> 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<sub>0</sub>. 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 over-expressing 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  $\mu\text{mol m}^{-2}\text{s}^{-1}$  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  $\times$  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  $\text{g m}^{-2}$  of monoammonium phosphate (12-61-0) and 2.63  $\text{g m}^{-2}$  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<sub>100</sub>, considered as control, in which plants were watered at 100% of plant transpiration; WR<sub>50</sub>, in which 50% of the amount of water given to the control plants was supplied; and WR<sub>0</sub>, 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<sub>0</sub>, where no agrochemical was added, as the control, and ii) ST<sub>az</sub>, 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<sub>2</sub> concentration of 400  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ , 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<sub>1</sub>, 30 DAT for both genotypes); ii) one week after the first azoxystrobin treatment (T<sub>2</sub>, 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<sub>3</sub>, 79 DAT and 86 DAT for IT-22/025 and Ikram, respectively-fruit ripening stage). The stomatal conductance ( $g_s$ ) was registered in  $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ , the transpiration rate ( $E$ ) in  $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  and the CO<sub>2</sub> assimilation rate ( $A$ ) in  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ . Intrinsic water use efficiency (WUE<sub>i</sub>) 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 ( $\text{g plant}^{-1}$ ) 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,  $\text{g plant}^{-1}$ ) and plant water used ( $\text{l plant}^{-1}$ ) (DMWUE,  $\text{g l}^{-1}$ ). Water use efficiency was also calculated as the ratio between total fruit yield and plant water used (TYWUE,  $\text{g l}^{-1}$ ). 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<sub>1</sub> and T<sub>2</sub>, 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  $\beta$ -mercaptoethanol and a high concentration of guanidine thiocyanate (Qiagen). The c-DNA samples for real-time RT-PCR experiments were synthesized from 1  $\mu\text{g}$  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  $\beta$ -actin and ERD-15 c-DNA-specific primers, to determine the quality of c-DNA obtained and to evaluate the

size of amplicons. The primers efficiency was estimated with the amplification of five serial dilutions 1:2 of Ikram samples c-DNA. c-DNA was diluted 1:5 and used for qRT-PCR in a CFX96 Touch™ Real-Time PCR Detection System (BioRad). In each well, 1 µl of diluted c-DNA was mixed with 5 µl of SYBR Green, 1 µl of each forward and reverse primers and 3 µl of ddH<sub>2</sub>O. Each biological sample was measured twice, and three biological samples were measured per treatment and gene. The PCR program comprised the following steps: pre-incubation for 2 min at 50 °C and 2 min at 95 °C and 45 cycles of amplification for 15 s at 95 °C, 1 min at 59 °C and 5 s at 65 °C.

## 2.6. Statistical analysis

To quantify the relative changes in gene expression obtained by quantitative Real Time PCR, the  $C_t$  method (also referred as the  $2^{-\Delta\Delta C_t}$  method) was adopted (O'Rourke and Ness, 2008). The whole dataset was tested according to the basic assumptions for the analysis of variance (ANOVA). The normal distribution of the experimental error and the common variance of the experimental error were verified through Shapiro-Wilk and Bartlett's tests, respectively. When required, Box-Cox transformations (Box and Cox, 1964) were applied prior to analysis.

The ANOVA procedure was adopted according to a randomized complete design with four replicates. A three-way (G, WR and ST) factorial ANOVA was performed. The ANOVA results and significance level relative to all the parameters considered are reported in table 1. The differences in the means were determined using Tukey's honest significance difference post hoc tests at the 5% probability level. Pearson's correlation coefficients between parameters were also analysed. Finally, regression analysis was used to identify the relationship between A and  $g_s$ . Statistical analyses were performed using the JMP software package, version 8.1 (SAS Institute Inc., Cary, NC, USA), and

graphs were constructed using SigmaPlot software (Systat Software, Chicago, USA).

## 3. Results

### 3.1. Water regimes

#### 3.1.1. Physiological parameters

IT-22/025 and Ikram both showed significantly lower  $g_s$  values under WR<sub>50</sub> relative to WR<sub>100</sub> at T<sub>1</sub> and T<sub>2</sub>, though the decrease was not significant at T<sub>3</sub> (Fig. 1A–C). However, the two genotypes showed different  $g_s$  decreases from WR<sub>100</sub> to WR<sub>50</sub>, with 26% and 29% at T<sub>1</sub> and T<sub>2</sub> for IT-22/025 and 50% and 39% at T<sub>1</sub> and T<sub>2</sub> for Ikram. The transpiration rate (E) values significantly decreased with increasing water stress for both genotypes (Fig. 1D–F). Also in this case, the decrease from the optimal regime to WR<sub>50</sub> was different for the two genotypes, with IT-22/025 at 25% and 16% and Ikram at 41% and 35%, at T<sub>1</sub> and T<sub>2</sub>, respectively. At T<sub>3</sub>, the two genotypes showed similar decreases under the WR<sub>50</sub> regime (27% for IT-22/025 and 30% for Ikram). Under optimal water conditions, IT-22/025 showed assimilation rates (A) significantly higher than Ikram at T<sub>2</sub> and T<sub>3</sub>. Moreover, at these two stages only for IT-22/025, A decreased significantly with the increase in water stress; consequently the A decrease observed from WR<sub>100</sub> to WR<sub>50</sub> was higher for IT-22/025 than for Ikram (Fig. 1G–I). Finally, as for intrinsic water use efficiency (WUE<sub>i</sub>) (Fig. 1L–N), defined as the ratio A/ $g_s$ , water stress caused an increase of this parameter in T<sub>1</sub> and T<sub>2</sub>. This was more evident for Ikram in T<sub>1</sub>, while the genotype IT-22/025 was less influenced by the water regime for this parameter. In T<sub>3</sub> the two genotypes showed a similar behaviour.

**Table 1**

Results (Fisher's test values) of ANOVA and significance level relative to all the parameters considered.

	DF	Plant Dry matter	Total yield	DMWUE	TYWUE	Total Soluble Solids	Colour Index
G	1	56.54**	364.22**	20.52**	133.17**	3.01ns	19.99**
WR	2	356.2**	3633**	22.52**	67.54**	26.93**	0.93ns
ST	1	1.25ns	1.21ns	2.28ns	23.34**	0.005ns	0.84ns
G × WR	2	15.46**	91.63**	0.08ns	24.79**	6.81**	1.42ns
G × ST	1	2.85ns	0.08ns	2.67ns	0.54ns	0.55ns	0.0008ns
WR × ST	2	3.37ns	1.81ns	11.3**	21.87**	0.93ns	1.89ns
G × WR × ST	2	0.42ns	10.34**	0.97ns	11.05**	0.06ns	2.22ns
		$g_s$	E	A	WUE <sub>i</sub>	ERD15	
T <sub>1</sub>							
G	1	1.19ns	0.0008ns	9.33**	20.87**	0.08ns	
WR	2	156.13**	116.77**	86.07**	3.63*	1.58ns	
G × WR	2	8.57**	6.9**	7.38**	4.86**	11.12**	
T <sub>2</sub>							
G	1	2.79ns	8.16**	3.23ns	2.87ns	61.63**	
WR	2	238.01**	349.05**	238.02**	1.69ns	21.04**	
ST	1	7.44**	5.3*	5.76*	5.72*	1.74ns	
G × WR	2	4.85*	13.91**	27.31**	3.67*	21.65**	
G × ST	1	0.19ns	0.019ns	0.44ns	0.1ns	18.44**	
WR × ST	2	0.79ns	0.26ns	1.33ns	0.31ns	27.46**	
G × WR × ST	2	0.02ns	0.67ns	1.16ns	0.16ns	13.36**	
T <sub>3</sub>							
G	1	0.33ns	0.69ns	60.42**	23.31**		
WR	2	166.85**	463.68**	446.59**	4.34*		
ST	1	5.66*	5.39*	0.18ns	4.24*		
G × WR	2	2.41ns	2.14ns	3.82*	9.84**		
G × ST	1	0.23ns	7.70**	2.65ns	0.22ns		
WR × ST	2	0.04ns	0.54ns	0.21ns	1.12ns		
G × WR × ST	2	0.21ns	0.75ns	3.18ns	3.86*		

DF, degree of freedom; G, Genotype; WR, Water Regime; ST, Strobilurine treatment; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> sampling dates; DMWUE, water use efficiency calculated on total aerial plant dry matter basis; TYWUE, water use efficiency calculated on total yield basis;  $g_s$ , stomatal conductance; E, transpiration rate; A, assimilation rate; WUE<sub>i</sub>, intrinsic water use efficiency. n.s. = not significant. \* P ≤ 0.05. \*\* P ≤ 0.01.

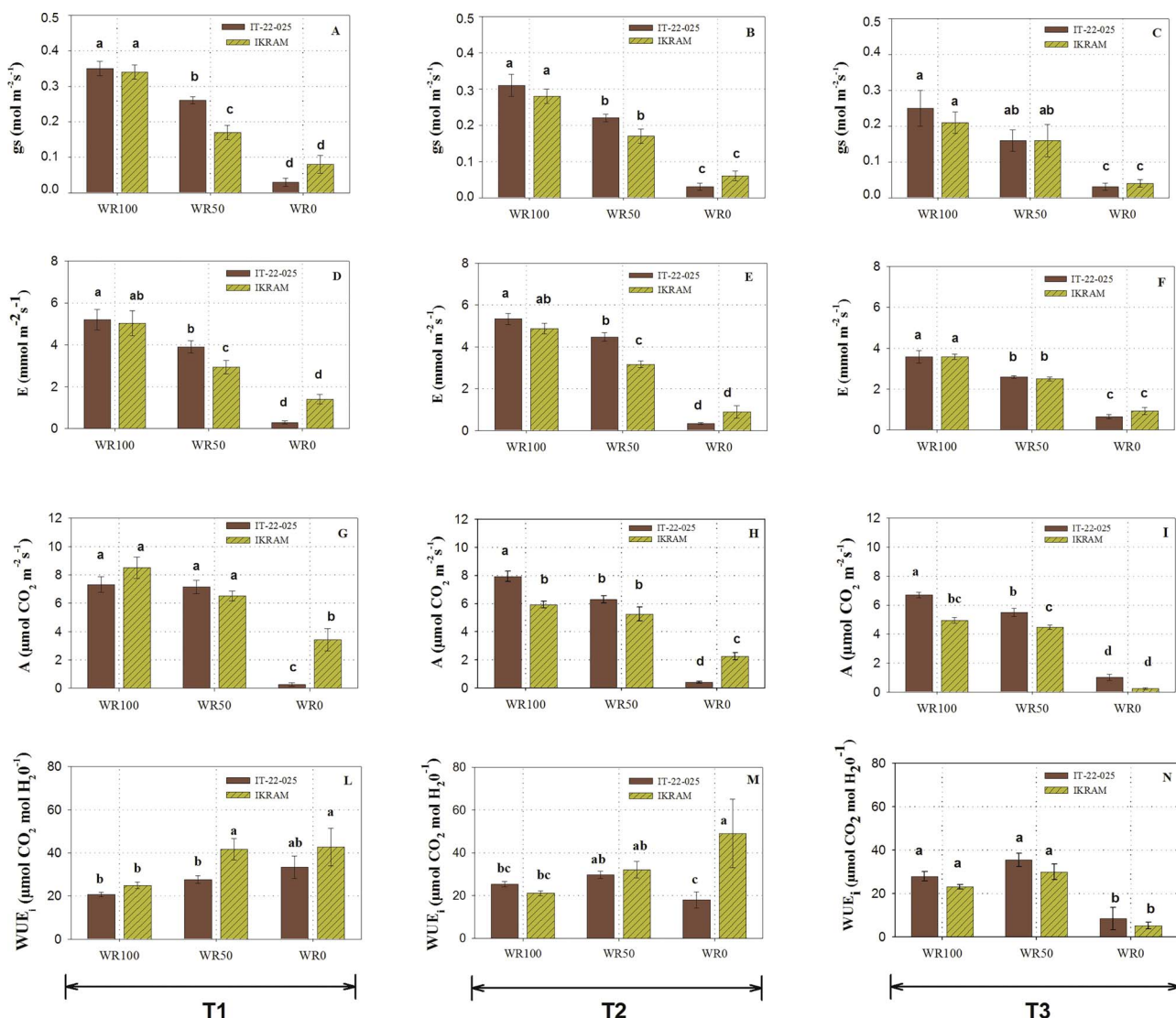


Fig. 1. Effects of the genotype x water regimes interaction on stomatal conductance ( $g_s$ ; A–C), transpiration rate ( $E$ ; D–F), assimilation rate ( $A$ ; G–I) and intrinsic water use efficiency ( $WUE_i$ ; L–N) measured at three sampling dates ( $T_1$ ,  $T_2$  and  $T_3$ ).  $WR_{100}$  plant watered at 100% of plant transpiration;  $WR_{50}$ , 50% of the amount of water given to the control plants;  $WR_0$ , watering only at transplanting, during fertigation and as supplementary irrigation. Different letters indicate values significantly different at  $P < 0.05$  according to a Tukey test. Data reported are means  $\pm$  standard errors ( $n = 8$ ).

### 3.1.2. Biomass, yield and quality parameters

Both genotypes showed a significant decrease in plant dry matter with the increase in water stress applied (Table 2). The genotype IT-22/025 showed values significantly higher than Ikram both under  $WR_{100}$  and  $WR_{50}$ . The decrease percentage from  $WR_{100}$  to  $WR_{50}$  was similar for the two genotypes and equal to 38% for IT-22/025 and 35% for Ikram.

For the total fruit yield, the genotype IT-22/025 showed higher values than Ikram under both  $WR_{100}$  and  $WR_{50}$  regimes, while under  $WR_0$ , the two genotypes showed similar values (Table 2). Moreover, the two genotypes showed different yield decreases from the  $WR_{100}$  to the  $WR_{50}$ , with a 43% decrease for IT-22/025 and a 51% decrease for Ikram. Considering the water regime average, the genotype IT-22/025

Table 2

Effects of the interaction between genotype and water regime on yield, water use efficiency and quality parameters.

	IT-22/025			IKRAM		
	$WR_{100}$	$WR_{50}$	$WR_0$	$WR_{100}$	$WR_{50}$	$WR_0$
Plant water use ( $l \text{ plant}^{-1}$ )	33.2	18.6	5	28.8	16.4	5.3
Plant dry matter ( $g \text{ plant}^{-1}$ )	$162.2 \pm 7.2^a$	$100.9 \pm 3.8^b$	$31.3 \pm 1.7^d$	$115.2 \pm 3.7^b$	$75.1 \pm 5.5^c$	$29.5 \pm 2.1^d$
Total fruit yield ( $g \text{ plant}^{-1}$ )	$814.8 \pm 13.8^a$	$463.711.2^c$	$98.7 \pm 5.6^e$	$629.6 \pm 8.5^b$	$307.3 \pm 9.6^d$	$97.8 \pm 4.6^e$
DMWUE ( $g \text{ l}^{-1}$ )	$4.9 \pm 0.2^a$	$5.4 \pm 0.2^a$	$6.3 \pm 0.3^a$	$4.0 \pm 0.1^a$	$4.6 \pm 0.3^a$	$5.5 \pm 0.4^a$
TYWUE ( $g \text{ l}^{-1}$ )	$24.5 \pm 0.4^a$	$24.9 \pm 0.6^a$	$19.7 \pm 1.1^c$	$21.8 \pm 0.3^b$	$18.7 \pm 0.6^c$	$18.5 \pm 0.8^c$
Total soluble solids (°Brix)	$5.4 \pm 0.2^{bc}$	$5.6 \pm 0.5^{bc}$	$7.1 \pm 0.5^{ab}$	$4.4 \pm 0.7^c$	$7.1 \pm 0.6^{ab}$	$8.3 \pm 0.7^a$
Color index	$42 \pm 3.2^{ab}$	$46.5 \pm 0.8^a$	$44 \pm 1.4^{ab}$	$37.7 \pm 1.2^b$	$37.2 \pm 1.3^b$	$39.4 \pm 2.5^{ab}$

DMWUE: water use efficiency calculated on total aerial plant dry matter basis; TYWUE water use efficiency calculated on total yield basis.  $WR_{100}$ , plant watered at 100% of plant transpiration;  $WR_{50}$ , 50% of the amount of water given to the control plants;  $WR_0$ , watering only at transplanting, during fertigation and as supplementary irrigation. Different letters in each row indicate values significantly different at  $P < 0.05$  according to a Tukey test. Data reported are means  $\pm$  standard errors ( $n = 8$ ).



**Table 3**

Effect of the strobilurin treatment on stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), assimilation rate ( $A$ ) and intrinsic water use efficiency ( $WUE_i$ ) measured at  $T_2$  and  $T_3$  sampling dates.

	$g_s$ ( $\text{mol m}^{-2}\text{s}^{-1}$ )	$E$ ( $\text{mmol m}^{-2}\text{s}^{-1}$ )	$A$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	$WUE_i$ ( $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ )
$T_2$				
ST <sub>0</sub>	0.19 ± 0.02 <sup>a</sup>	3.01 ± 0.4 <sup>b</sup>	4.42 ± 0.5 <sup>b</sup>	23.4 ± 1.2 <sup>b</sup>
ST <sub>az</sub>	0.16 ± 0.02 <sup>b</sup>	3.34 ± 0.42 <sup>a</sup>	5.0 ± 0.6 <sup>a</sup>	30.9 ± 5.75 <sup>a</sup>
$T_3$				
ST <sub>0</sub>	0.15 ± 0.02 <sup>a</sup>	2.21 ± 0.23 <sup>b</sup>	3.83 ± 0.51 <sup>a</sup>	25.5 ± 2.58 <sup>b</sup>
ST <sub>az</sub>	0.13 ± 0.02 <sup>b</sup>	2.39 ± 0.25 <sup>a</sup>	3.77 ± 0.49 <sup>a</sup>	29.0 ± 3.54 <sup>a</sup>

ST<sub>0</sub>, no agrochemical added; ST<sub>az</sub>, azoxystrobin treatment. Different letters in the column within each T indicate values significantly different at  $P < 0.05$  according to a Tukey test. Data reported are means ± standard errors ( $n = 24$ )

showed WUE values calculated on plant dry biomass (DMWUE) significantly higher ( $P < 0.01$ ) than Ikram (5.52 vs 4.7  $\text{g l}^{-1}$ , respectively). For both genotypes, the DMWUE increase from the optimal to the stressed condition was not significant (Table 2). Also for TYWUE, as calculated on a total fruit yield basis, considering the water regime average, genotype IT-22/025 showed values significantly higher ( $P < 0.01$ ) than Ikram (23 vs 19.7  $\text{g l}^{-1}$ , respectively). Under WR<sub>50</sub>, the genotype IT-22/025 maintained a constant TYWUE value, while a significant decrease was reported for Ikram. Moreover, a decrease in TYWUE was evident for both genotypes under WR<sub>0</sub> (Table 2). For quality parameters, only Ikram showed a significant increase in total soluble solids from the control to WR<sub>50</sub>, showing for this water regime a value higher than IT-22/025 (Table 2). Finally, the genotype IT-22/025 showed higher colour index values than Ikram. No significant differences in colour index values were observed among the water regimes in both genotypes.

### 3.2. Strobilurin effects

All the physiological parameters were significantly affected by strobilurin treatment (Table 3). Stomatal conductance was significantly reduced by strobilurin treatment at both  $T_2$  and  $T_3$ . In contrast, transpiration rate ( $E$ ) was increased by strobilurin at both  $T_2$  and at  $T_3$ . At  $T_3$ , the effect of the strobilurin on the transpiration rate was different for the two genotypes: only Ikram had a higher value under ST<sub>az</sub> than under ST<sub>0</sub> (Fig. 2). The plants treated with strobilurin showed  $A$  values significantly higher than those without the treatment only in  $T_2$  (Table 3). Finally, the strobilurin treatment showed  $WUE_i$  values

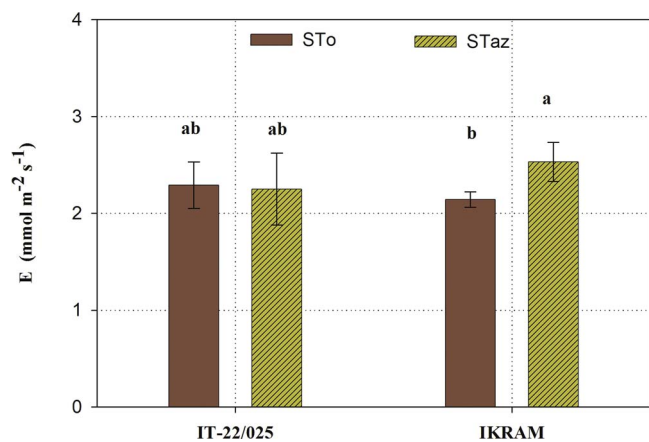


Fig. 2. Effects of the genotype x strobilurin treatment interaction on transpiration rate ( $E$ ) at  $T_3$  sampling date. ST<sub>0</sub>, no agrochemical added; ST<sub>az</sub>, azoxystrobin treatment. Different letters indicate values significantly different at  $P < 0.05$  according to a Tukey test. Data reported are means ± standard errors ( $n = 12$ ).

significantly higher than those without the treatment at both  $T_2$  and at  $T_3$  (Table 3).

For the yield, water use efficiency and quality parameters (Table 4), the effect of strobilurin was significant only for the water use efficiency (both DMWUE and TYWUE) under the extreme water stress level, with ST<sub>az</sub> showing a higher value than ST<sub>0</sub>.

### 3.3. ERD15 gene expression

The relative expression of ERD15 was evaluated for the two genotypes and the three water regimes at  $T_1$  (in absence of strobilurin treatment) and at  $T_2$  (after the first strobilurin treatment) (Fig. 3A and B). At  $T_1$ , 15 days after the beginning of the water stress application, the two genotypes showed similar mean values; however, no significant differences were found among the three water regimes in IT-22/025, while Ikram showed increasing values with increasing water stress applied and showed a significant difference between WR<sub>100</sub> and WR<sub>0</sub> (Fig. 3A). At  $T_2$ , one week after the first strobilurin treatment and 30 days after the beginning of the water stress application, IT-22/025 showed higher values than Ikram. Moreover, IT-22/025 showed a significant decrease in ERD15 relative expression in response to water stress conditions, while for Ikram the relative gene expression was steady among the different water regimes. Finally, the strobilurin treatment significantly increased the ERD15 relative expression in IT-22/025, while no significant effect was observed in Ikram (Fig. 4).

## 4. Discussion

### 4.1. Water regimes

#### 4.1.1. Physiological parameters

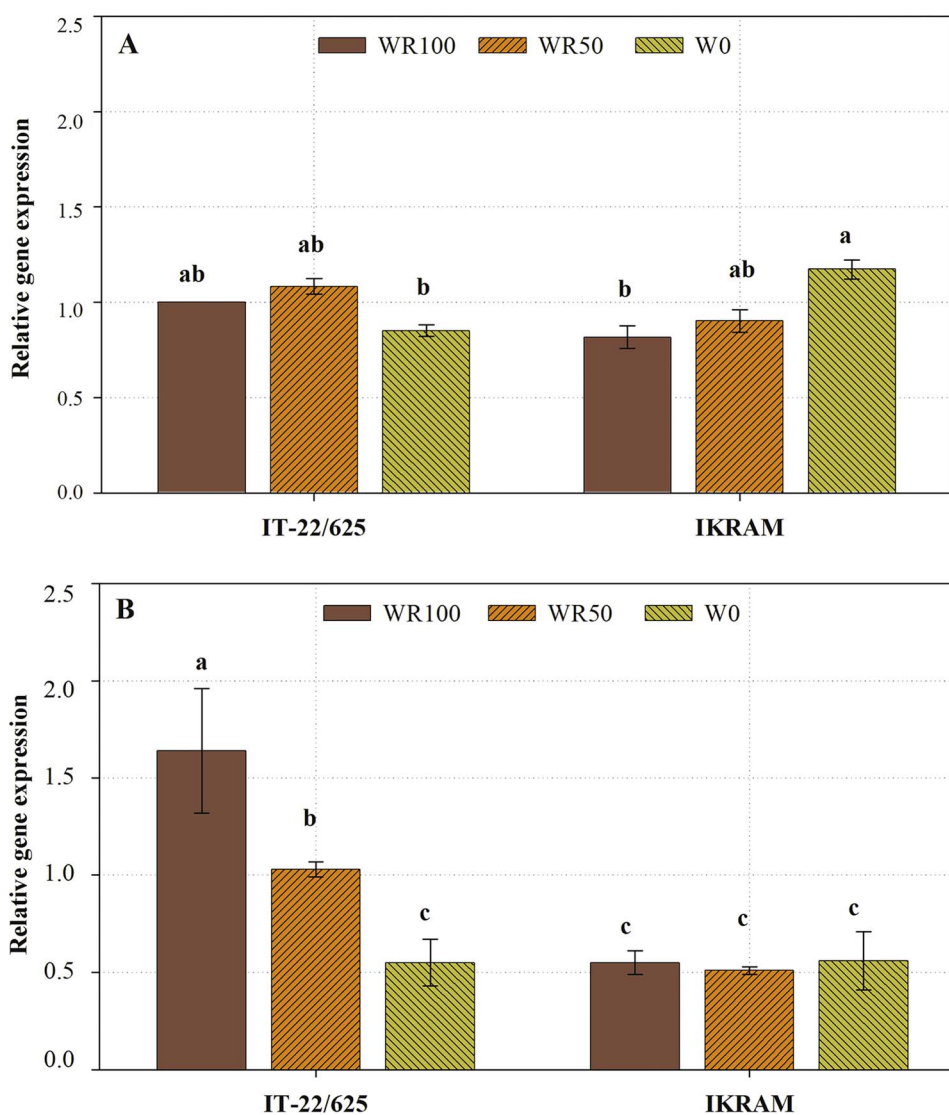
The lower  $g_s$  values obtained under water stress conditions in both genotypes at the three data samples, are in agreement with Savić et al. (2009), Nardella et al. (2012) and Giuliani et al. (2016). A decrease in stomatal conductance from 33 to 44% was reported in tomatoes grown in a greenhouse under water deficit conditions (Davies et al., 2000; Campos et al., 2009). These values are similar to those observed at  $T_1$  and  $T_2$  for Ikram, while the  $g_s$  decrement observed for IT-22/025 was lower, showing that under water stress conditions, this genotype closed its stomata less. The  $E$  trend was very similar to that described for stomatal conductance. On the other hand, as expected, the correlation between these two parameters was significantly higher (Table 5). The decrease in both  $E$  and  $A$  observed at the three data sampling under WR<sub>50</sub> compared to WR<sub>100</sub> is in agreement with the literature (Li et al., 2007; Yang et al., 2012; Valerio et al., 2017). The minimal decrease in transpiration observed for IT-22/025 at both  $T_1$  and  $T_2$  showed that this genotype maintained the transpiration process by reducing the stomatal closure relative to Ikram. To the contrary, at  $T_2$ , the  $A$  decrease observed under water stress conditions relative to the control was higher for IT-22/025 than for Ikram, which maintained a CO<sub>2</sub> assimilation rate at a level comparable to that of the optimal irrigation. Therefore, in Ikram, water stress decreased  $E$  more than  $A$ , as was also reported by Li et al. (2007) and Yang et al. (2012); the opposite trend occurred in IT-22/025 in which the water stress reduced more  $A$  than  $E$ . The  $WUE_i$  increase with increasing water stress, observed especially in Ikram at  $T_1$  and  $T_2$ , was due to a different sensitivity of  $A$  and  $g_s$  to water deficit conditions. Indeed, at  $T_2$ , in Ikram,  $A$  decreased less than  $g_s$  between the control and the WR<sub>50</sub> regime (12% and 39%, respectively), while IT-22/025 showed a similar decrease of  $A$  and  $g_s$  (20% and 29%, respectively). The different behaviour of the two genotypes is also shown in Fig. 5. The different results obtained for IT-22/025 and Ikram in terms of  $E$ ,  $A$ ,  $g_s$  and  $WUE_i$ , especially at  $T_2$  corresponding to flowering stage, is of particular interest because this period is considered the most sensitive stage to water stress in tomato growth (Giuliani et al., 2017) and showed the existence of differences between tomato genotypes in the physiological responses to drought stress.

**Table 4**

Effects of the interaction between water regime and strobilurin treatment on yield, water use efficiency and quality parameters.

	WR <sub>100</sub>		WR <sub>50</sub>		WR <sub>0</sub>	
	ST <sub>0</sub>	ST <sub>az</sub>	ST <sub>0</sub>	ST <sub>az</sub>	ST <sub>0</sub>	ST <sub>az</sub>
Plant dry matter (g plant <sup>-1</sup> )	145.3 ± 13.4 <sup>a</sup>	132.1 ± 9.5 <sup>a</sup>	90.7 ± 8.9 <sup>b</sup>	85.3 ± 5.3 <sup>b</sup>	26.6 ± 0.9 <sup>c</sup>	34.2 ± 1.1 <sup>c</sup>
Total yield (g plant <sup>-1</sup> )	725.3 ± 36.5 <sup>a</sup>	719.0 ± 48.5 <sup>a</sup>	383.2 ± 43.6 <sup>b</sup>	387.8 ± 27.6 <sup>b</sup>	87.5 ± 1.2 <sup>c</sup>	108.9 ± 2.3 <sup>c</sup>
DMWUE (g l <sup>-1</sup> )	4.6 ± 0.3 <sup>b</sup>	4.2 ± 0.2 <sup>b</sup>	5.1 ± 0.4 <sup>b</sup>	4.8 ± 0.2 <sup>b</sup>	5.2 ± 0.24 <sup>b</sup>	6.6 ± 0.24 <sup>a</sup>
TYWUE (g l <sup>-1</sup> )	23.3 ± 0.5 <sup>a</sup>	23.1 ± 0.8 <sup>ab</sup>	21.6 ± 1.9 <sup>bc</sup>	22.0 ± 1.1 <sup>abc</sup>	17.0 ± 0.3 <sup>d</sup>	21.2 ± 0.6 <sup>c</sup>
Total soluble solids(°Brix)	5.2 ± 0.4 <sup>c</sup>	4.6 ± 0.2 <sup>c</sup>	6.1 ± 0.3 <sup>b</sup>	6.4 ± 0.7 <sup>b</sup>	7.5 ± 0.3 <sup>a</sup>	7.9 ± 0.6 <sup>a</sup>
Colour index	42.3 ± 2.3 <sup>a</sup>	37.4 ± 3 <sup>a</sup>	42 ± 1.7 <sup>a</sup>	41.8 ± 3 <sup>a</sup>	41 ± 0.8 <sup>a</sup>	42.4 ± 2 <sup>a</sup>

DMWUE, water use efficiency calculated on total aerial plant dry matter basis; TYWUE, water use efficiency calculated on total yield basis. WR<sub>100</sub>, plant watered at 100% of plant transpiration; WR<sub>50</sub>, 50% of the amount of water given to the control plants; WR<sub>0</sub>, watering only at transplanting, during fertigation and as supplementary irrigation. ST<sub>0</sub>, no agrochemical added; ST<sub>az</sub>, azoxystrobin treatment. Different letters in each row indicate values significantly different at P < 0.05 according to a Tukey test. Data reported are means ± standard errors (n = 8).



**Fig. 3.** Effects of the genotype x water regime interaction on relative ERD15 expression at T<sub>1</sub> (A) and T<sub>2</sub> (B) sampling dates. WR<sub>100</sub> plant watered at 100% of plant transpiration; WR<sub>50</sub>, 50% of the amount of water given to the control plants; WR<sub>0</sub>, watering only at transplanting, during fertigation and as supplementary irrigation. Different letters indicate values significantly different at P < 0.05 according to a Tukey test. Data reported are means ± standard errors (n = 8).

#### 4.1.2. Yield, water use efficiency and quality parameters

The plant dry biomass decrease observed under water stress conditions for the two genotypes was similar to those found in the literature for tomato grown under controlled conditions (Tahi et al., 2007; Campos et al., 2009). However, Patanè et al. (2011) found, in tomato grown in open field conditions, that total dry biomass accumulation was significantly depressed by soil water deficit only when a very early cut of irrigation was applied (0% ETc), while irrigation at a reduced

rate (50% ETc) from initial stages did not induce any losses in final dry biomass. The total fruit yield trend was similar to that of the total dry biomass and these two parameters were highly correlated (Table 5), demonstrating that the crop regulates its fruit biomass to total biomass cumulated, as also reported by Patanè et al. (2011). The genotype IT-22/025 also showed higher yield value than Ikram in the optimal regime.

On the other hand, IT-22/025 showed a higher A than Ikram under

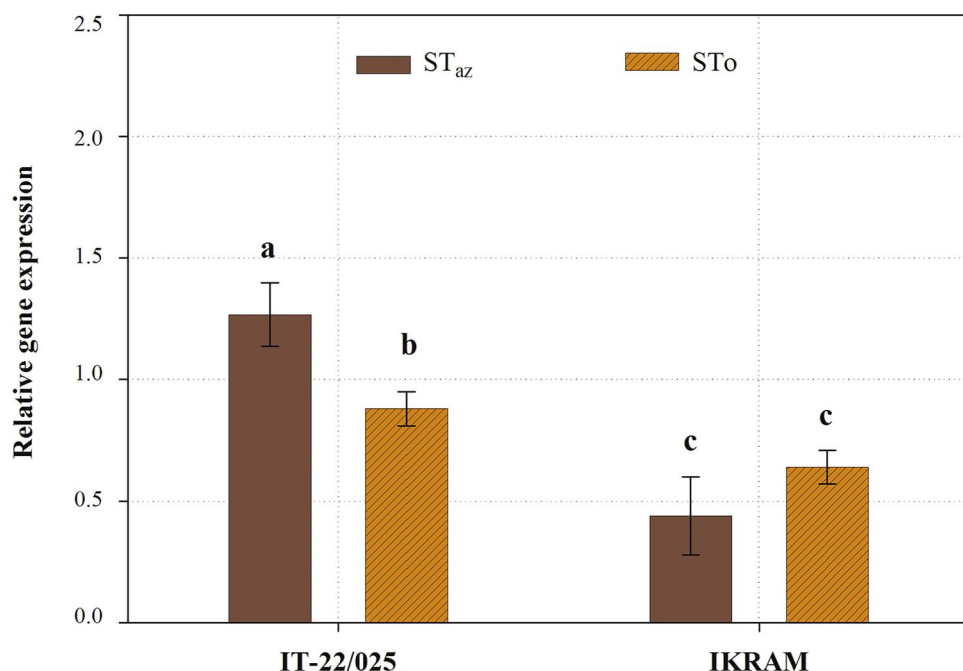


Fig. 4. Effects of the genotype x strobilurin treatment interaction at T<sub>2</sub> sampling date. ST<sub>0</sub>, no agrochemical added; ST<sub>az</sub>, azoxystrobin treatment. Different letters indicate values significantly different at P < 0.05 according to Tukey test. Data reported are means ± standard errors (n = 12).

WR<sub>100</sub> at T2 and T3; indeed, the two parameters were highly correlated at these sampling dates (Table 5). Moreover, the different yield decreases of the two genotypes under water stress conditions is interesting. Under WR<sub>50</sub> the yield decreased 43% for IT-22/025 and 51% for Ikram. Several authors reported a yield decrease under water stress conditions from 50% to 60% in tomato cultivated both in open field and in greenhouse (Topcu et al., 2007; Favati et al., 2009; Patanè and Cosentino, 2010; Patanè et al., 2011; Giuliani et al., 2016); IT-22/025 showed a slightly lower yield reduction than those widely reported in the literature. While the two genotypes showed a slight increase in WUE

calculated on plant dry biomass (DMWUE), under water stress, they showed a different behaviour in TYWUE. Under WR<sub>50</sub>, a significant decrease was observed for Ikram, while IT-22/025 maintained similar values between the two water regimes. In the literature, it is widely reported that water use efficiency increases with decreases in the amount of irrigation water (Wang et al., 2007; Li et al., 2010b). However, our results are in agreement with Kuşçu et al. (2014), who reported that WUE is also dependent on the genotypes and their degree of water stress tolerance. Additionally, Farooq et al. (2009) reported that drought-tolerant species maintain water-use efficiency by reducing the

Table 5  
Pearson's correlation coefficients values between all the parameters evaluated at three sampling dates (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>).

T <sub>1</sub>	ERD15 expression	A	g <sub>s</sub>	E	WUE <sub>i</sub>	Total fruit yield	Plant dry matter	DMWUE
A	-0.03ns							
g <sub>s</sub>	-0.12ns	0.92**						
E	-0.1ns	0.95**	0.99**					
WUE <sub>i</sub>	0.6**	0.31*	-0.03ns	0.06ns				
Total fruit yield	-0.16ns	0.82**	0.97**	0.96**	-0.18ns			
Plant dry matter	-0.13ns	0.81**	0.94**	0.94**	-0.15ns	0.98**		
DMWUE	0.27ns	-0.74**	-0.64**	-0.67**	-0.3*	-0.54**	-0.49**	
TYWUE	0.1ns	0.5**	0.67**	0.63**	-0.32*	0.72**	0.72**	0.08ns
T <sub>2</sub>								
A	0.6**							
g <sub>s</sub>	0.5**	0.92**						
E	0.57**	0.97**	0.96**					
WUE <sub>i</sub>	-0.15ns	-0.24ns	-0.54**	-0.38*				
Total fruit yield	0.65**	0.92**	0.96**	0.95**	-0.46*			
Plant dry matter	0.61**	-0.94**	0.96**	0.94**	-0.41*	0.98**		
DMWUE	-0.12ns	-0.56**	-0.65**	-0.62**	0.55**	-0.54**	-0.49*	
TYWUE	0.63**	0.69**	0.61**	0.7**	-0.06ns	0.72**	0.72**	0.08ns
T <sub>3</sub>								
A	-							
g <sub>s</sub>	-	0.94**						
E	-	0.93**	0.97**					
WUE <sub>i</sub>	-	0.84**	0.63**	0.66**				
Total fruit yield	-	0.91**	0.93**	0.94**	0.63**			
Plant dry matter	-	0.94**	0.94**	0.93**	0.69**	0.98**		
DMWUE	-	-0.55**	-0.72**	-0.69**	-0.23ns	-0.54**	-0.49*	
TYWUE	-	0.69**	0.51**	0.58**	0.74**	0.72**	0.72**	0.08ns

DMWUE, water use efficiency calculated on total aerial plant dry matter basis; TYWUE, water use efficiency calculated on total yield basis; g<sub>s</sub>, stomatal conductance; E, transpiration rate; A, assimilation rate; WUE<sub>i</sub>, intrinsic water use efficiency. ns: not significant; \* significant at P < 0.05; \*\* significant at P < 0.01.

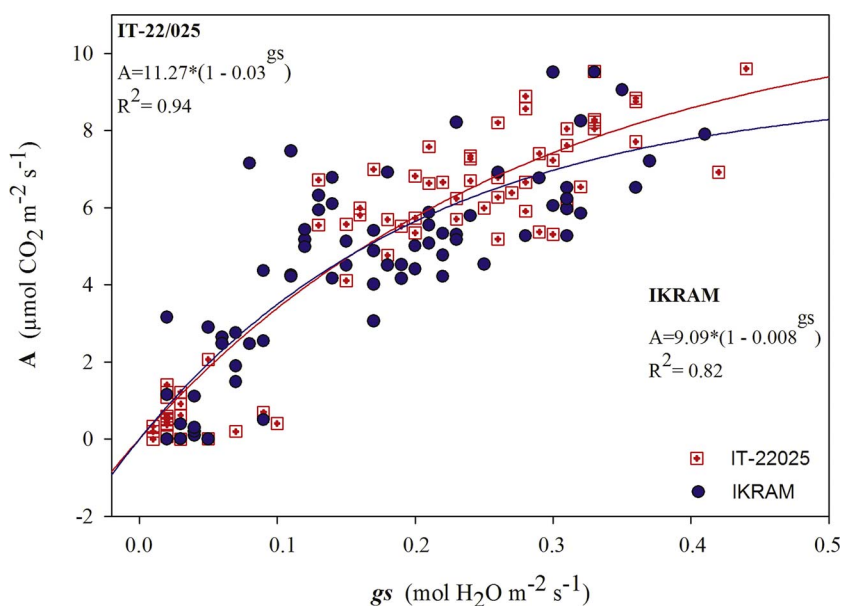


Fig. 5. Assimilation rate ( $A$ ) expressed as a function of stomatal conductance ( $g_s$ ) of the two tomato genotypes.

water loss. In particular, when slight stomatal closure occurs, WUE related to fruit yield can increase or remain steady as reported for IT-22/025; however, it is more evident that stomatal closure led to a decrease in the yield and WUE, as shown in Ikram.

The increase in total soluble solids with decreasing water amount also reported in the literature (Zegbe-Domínguez et al., 2003; Patané et al., 2011; Giuliani et al., 2016) can be explained by a decrease in water accumulation by the fruit without any significant modification in the quantity of accumulated sugars (Guichard et al., 1999; Zheng et al., 2013). In this parameter, the two genotypes showed a different effect of the water regime being Ikram more reactive than IT-22/025 under water stress condition, let us suppose that the last one limited the decrease in water accumulation by the fruit.

#### 4.2. Strobilurin effects

Strobilurin is an important class of agricultural fungicides (Bartlett et al., 2002) considered to also have positive physiological effects on plants (Venancio et al., 2003; Kanungo and Joshi, 2014). Studies on the effect of strobilurin on physiological, yield and quality parameters have been focused on wheat, barley and soybean, with fewer studies on tomato available.

In this study, the lower  $g_s$  value observed in  $ST_{az}$  at both sampling dates, indicates a higher tomato stomatal closure under strobilurin treatment. These results are in agreement with Nason et al. (2007), who studied wheat, barley and soybean under greenhouse conditions and with Cantore et al. (2016) who observed, in tomato open field studies, a slight reduction in  $g_s$  due to the application of strobilurin. In several studies conducted on wheat, it has been reported that the stomatal closure associated with strobilurin treatment is due to the increase in endogenous ABA content (Grossmann et al., 1999; Nason et al., 2007). Moreover, the reduced stomatal opening appeared not be limiting for the  $CO_2$  income. In agreement with the literature, our experiment found higher  $A$  values in the plants treated with strobilurin at  $T_2$ . Also in wheat plants, strobilurins are reported to enhance the net rate of photosynthesis in the treated leaves (Oerke and Dehne, 2004), indicating more carbon fixation by after strobilurin application (Kanungo and Joshi, 2014). In contrast, Nason et al. (2007) reported a decrease in net rate of photosynthesis in wheat, barley and soybean plant treated with strobilurin. The reduction in stomatal opening but not in photosynthetic rate resulted in an increase in the  $WUE_i$  with the strobilurin application at both sampling dates. In our experiment, the strobilurin

treatment also caused an increase in  $E$  that was more evident for Ikram than for IT-22/025 in  $T_3$ . This result is not in accordance with the literature where it has been reported that the stomatal closure under strobilurin treatment, in wheat, barley and soybean, causes a decrease in transpiration rate (Grossmann et al., 1999; Nason et al., 2007). Our results clearly demonstrated that there is variability in the physiological responses to the strobilurin treatment not only among the different species but also among genotypes within the same species. Moreover, the significant effect of the strobilurin treatment on both DMWUE and TYWUE under extreme water stress levels confirmed that the application of strobilurin could significantly improve water use efficiency related to fruit yield (Giuliani et al., 2011; Cantore et al., 2016). On the other hand, studies carried out on soybean revealed that fungicides applied in the absence of foliar disease did not produce non-fungicidal physiological effects or associated yield improvements, and it was suggested that environmental conditions and assessment of disease levels should be used as a guide for foliar fungicide application on soybean (Swoboda and Pedersen, 2009). Our results clearly showed effects of strobilurin on water use efficiency and physiological traits in tomato grown in growth chambers, even in the absence of foliar disease.

#### 4.3. ERD15 gene expression

ERD15 has been used as a stress-responsive gene in various stress experiments (Dunaeva and Adamaska, 2001; Park et al., 2009; Li et al., 2010a). To the best of the authors' knowledge no other work has described the ERD15 expression level during tomato flowering and ripening time

The obtained results highlighted the different behaviour of the two genotypes in the two sampling dates ( $T_1$  and  $T_2$ ). At  $T_1$ , 15 days after the application of water stress, the two genotypes showed similar ERD15 mean values but a different response to water stress; only Ikram showed an increase of ERD15 expression with increasing water stress levels. This result is in agreement with Ziaf et al. (2016), who, in a study on a tomato (*Solanum lycopersicum*, L) cultivar, observed a steady-state increase in ERD15 transcript accumulation in response to drought after one-hour, reaching a maximum after six hours of treatment. In contrast, at  $T_2$ , 30 days after the water stress started and 7 days after the strobilurin application, the relative expression of ERD15 appeared to be steady in Ikram and significantly decreased in IT-22/025 from  $WR_{100}$  to  $WR_0$ . As reported by Ziaf et al. (2016), ERD15 has been characterized in different plant species showing differences in amino acids sequence; for



this reason, the same gene from two different genotypes may have variation in its expression patterns. Moreover, from T<sub>1</sub> to T<sub>2</sub>, IT-22/025 showed a slight increase in ERD15 expression in agreement with Ziaf et al. (2016) who reported that in *Lycopersicon esculentum*, ERD15 accumulated more in older leaves which reflects its association with senescence processes. Moreover, IT-22/025 showed a higher gene expression in T<sub>2</sub> in response to strobilurin application. In contrast, Ikram showed a lower ERD15 expression in T<sub>2</sub> relative to T<sub>1</sub>, and a negative effect of the strobilurin on the ERD15 relative expression. In the literature, it is reported that strobilurin increases the concentration of endogenous ABA levels (Venancio et al., 2003), and it is also reported that the ERD15 gene is induced by ABA, even if it is also a negative regulator of ABA (Kariola et al., 2006). Ziaf et al. (2016) observed an increase in ERD15 transcription level in tomato plants treated with ABA. This could explain the significant increase in ERD15 gene expression in IT-22/025 under azoxystrobin treatment.

Finally, in T<sub>2</sub>, ERD15 gene expression was highly, positively correlated with A, g<sub>s</sub>, E, plant dry matter, total fruit yield and TYWUE. Indeed, IT-22/025, together with the higher ERD15 expression in T<sub>2</sub> also showed higher A, g<sub>s</sub> and E values than Ikram. These results clearly suggest that, during flowering stage considered the most sensitive stage to water stress in tomato growth, ERD15 played a role in plant stomatal adjustment leading to stomatal opening even under water stress conditions, with a consequent increase in assimilation rate and therefore in yield, as was evident for IT-22/025. To the best of the authors' knowledge, this is the first report that has analysed the correlation among genetic, physiologic and yield parameter during the flowering and ripening stage in tomato crop.

In conclusion, in our experimental conditions, the two genotypes showed a different behaviour in response to water stress. In particular, under water stress conditions, IT-22/025 showed higher stomatal conductance, transpiration, assimilation rate and ERD15 relative expression than Ikram. The physiological and genetic plant response corresponded to a higher total fresh fruit yield, DMWUE and TYWUE for IT-22/025 relative to Ikram. Strobilurin treatment resulted in a lower stomatal conductance, maintaining higher A relative to the group without treatment. Moreover, strobilurin significantly increased both DMWUE and TYWUE under extreme water stress levels. Finally, strobilurin application caused an increase in ERD15 expression only in IT-22/025. This study emphasizes the possibility of reducing the amount of water used during the tomato crop cycle, while maintaining acceptable yield and quality, by using an agronomic and genetic strategy. Moreover, in this study, the ERD15 gene expression level has been investigated for the first time during flowering and ripening allowing for the identification of correlations to physiological, molecular and yield responses to water reduction. Further studies are needed to build on the physiological and genetic information obtained in the present study to create water-saving strategies in tomato crops.

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