

Pyraclostrobin can mitigate salinity stress in tomato crop

Francesca Boari ^a, Vito Cantore ^{a, *}, Donato Di Venere ^a, Lucrezia Sergio ^{a, *}, Vincenzo Candido ^b, Maria Immacolata Schiattone ^b

^a Institute of Sciences of Food Production, National Research Council (CNR-ISPA), Via Amendola, 122/O, 70125, Bari, Italy

^b School of Agriculture, Forest, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano, 10, 85100, Potenza, Italy

ARTICLE INFO

Keywords:

Solanum lycopersicum
Salinity
Strobilurin
Gas exchange
Chlorophyll
Antioxidative enzymes

ABSTRACT

Processing tomato is a widespread crop in the Mediterranean area where often there are problems of high salinity of irrigation water with considerable harmful effects on yield. Pyraclostrobin is a strobilurin based fungicide (PBF) having a broad range of applications. Strobilurins are reported to have biostimulant effect on plant mitigating abiotic stress. Therefore, the objective of this work was to investigate the ability of PBF to improve gas exchange parameters, chlorophyll, activity of antioxidative enzymes as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), yield characteristics, and water use efficiency (WUE) of tomato under salinity.

A two-year research was carried out in Southern Italy, on tomato cv Coronel grown in pots under plastic greenhouse, to compare two soil salinity levels (electrical conductivity, $EC_e = 1.1$ and 5.4 dS m^{-1}) in combination with or without PBF. As expected, salinity had harmful effects on physiological and biochemical parameters of tomato that in turn affected yield and WUE, but improved fruit quality. In fact, salinity reduced fruit mean weight (19.0%) and yield (21.4%), and increased fruit blossom-end rot (55.7%), total soluble solids (18.2%) and dry matter (18.5%) content. Overall, PBF increased leaf chlorophyll content (6.1%) and the activity of SOD, POD, CAT and APX, mainly under salinity. Moreover, it reduced stomatal conductance (12.5%) and transpiration (11.7%), but improved assimilation rate (7.8%) and intrinsic WUE (23.3%), that in turn increased yield (8.1%) and yield WUE (6.5%). The improvement in yield produced by PBF was highest in salt stressed plants. In view of the positive effects of PBF, its use should be promoted in defence programs of tomato crop, above all in areas with salinity problems.

1. Introduction

High salinity is one of the main abiotic stresses causing agricultural production decline. This problem is of particular importance in light of the growing irrigated agriculture. Worldwide, it is reported that about 20% of agricultural land and 33% of irrigated areas are affected by salinity and these areas are growing at a rate of 10% per year. Fifty per cent of agricultural land can be affected by salinity by 2050 (Jamil et al., 2011). In particular, the risk of salinization impends on the soils of coastal agricultural areas where the water used for irrigation can have very high salt content due to the salinization of the aquifers by the marine intrusion (Polemio, 2016). The phenomenon is widespread in several Mediterranean countries such as Italy (Scheidleger et al., 2004; Boari et al., 2012).

Salinity is detrimental to crops as a consequence of its negative physiological, biochemical and morphological effects which results in reduced biomass production and yield (Flagella et al., 2002; Munns, 2002). The reduction of plant growth due to salinity is mainly determined by the following factors that contribute to the decline of the photosynthetic activity (Munns et al., 2006; Munns and Tester, 2008): i) the increase of the osmotic pressure of the medium which reduces the plant ability to absorb water (similar to water stress); ii) ionic excess (e.g., Na^+ and Cl^-) up to a toxic level for plant cells; iii) ionic imbalance that affects the nutritional status of the plant and acts on the biochemical and metabolic components related to plant growth. Salt stress, as well as other environmental stresses, increases production of reactive oxygen species (ROS), to the origin of oxidative stress in plant cells (AbdElgawad et al., 2016; Asadi Karam and Keramat, 2017). ROS are highly cytotoxic and can react with different biomolecules (eg.: nu-

* Corresponding authors.

Email addresses: vito.cantore@ispa.cnr.it (V. Cantore); lucrezia.sergio@ispa.cnr.it (L. Sergio)

cleic acids, proteins, lipids) causing DNA mutation, protein denaturation, lipid peroxidation, until cell death (Kaur and Zhawar, 2016; Nxele et al., 2017). The elimination of ROS can be achieved by the activation of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) (Caverzan et al., 2016; Kaur and Zhawar, 2016).

Among the strategies that can be implemented to reduce the harmful effects of salinity we can mention some concerning the use of biostimulants, that improve physiological and/or biochemical processes of plants under stress (Boari et al., 2014, 2016; Arya et al., 2018). Due to the similarity, in many aspects, of physiological response of plants to water and salt stress, as well as the interesting results reported in literature concerning the biostimulant action of strobilurins in attenuating water stress, it has been hypothesized that these latter compounds can alleviate also the detrimental effects of salinity. Strobilurins are a class of broad-spectrum fungicides of natural origin from which various structural variants have been obtained (Wu and von Tiedemann, 2001). These natural molecules are produced by a group of fungi belonging to the Basidiomycetes [e.g.: *Oudemansiella mucida* (Schrader ex Fr) Hoehn and *Strobilurus tenacellus* (Pers ex Fr) Singer] agents of wood rot of some tree species (Bartlett et al., 2002). The fungicidal action is carried out through the inhibition of mitochondrial respiration of some pathogenic fungi of agricultural crops by blocking the electron transport at the outer side of the cytochrome-bc1 complex (Balba, 2007). In recent years, several studies proved the complementary biostimulant properties of strobilurin-based crop protection products, to reduce water requirements and mitigate abiotic stress. In fact, after the application of these fungicides, a change in the plant metabolism has been observed, often with positive physiological effects, including the increase in the production of abscisic acid (ABA) and the activation of some enzymes involved in oxidative stress (SOD, POD, CAT, APX) (Köehle et al., 2003; Liang et al., 2018) which may improve gas exchange and water use efficiency (WUE), especially under water stress conditions (Venancio et al., 2003; Joshi et al., 2014). Experimental findings on tomato under water stress and treated with strobilurins showed an improvement in the water status of the plant in terms of osmotic potential, relative water content and water stress index, gas exchange, yield and WUE (Giuliani et al., 2011; Cantore et al., 2016; Boari et al., 2017; Giuliani et al., 2018). In addition, a positive effect on nitrate reductase activity, which results in improved nitrogen metabolism, was observed (Joshi et al., 2014; Amaro et al., 2018). It has also been shown that strobilurins promote the increase of chlorophyll (Chl) and slow down its degradation (Baranyiova et al., 2014).

Tomato, worldwide the second most valuable vegetable crop next to potato (Battilani et al., 2012), is common among open field crops in Southern Italy often in soils affected by salinity problems and/or irrigated with saline water. Tomato is indicated as moderately sensitive to salinity (Flagella et al., 2002) according to the Mass and Hoffman (1977) model. As known, several factors, by different extent, contribute to yield reduction due to salinity, e.g. the number and mean weight of fruits and the percentage of waste, mainly determined by the number of fruits affected by blossom-end rot, in relation to the salinity level, the type of salts and the phenological phase impacted by salt stress (Cucci et al., 2000; Zhang et al., 2017). The adverse effects of salinity on yield are offset by a significant improvement in the quality of fruits due to the increase in dry matter content, total soluble solids and titratable acidity (Cantore et al., 2008a, 2012), which results in improved taste, as occurred also under water deficit conditions (Colella et al., 2014; Candido et al., 2015).

Because of the economic importance of tomato crop, the spreading of salinity problem in many areas occupied by this crop and the economic damage that may result, there is a growing interest in the search of new solutions that can mitigate the negative effects of salinity. We hypothesized that a strobilurin-based agrochemical (Pyraclostrobin), in addition to fungicidal action, may have a biostimulant action able to mitigate the harmful effects of salt stress on tomato plant, as already

noticed for water stress. As far as we know, there are no experimental findings in literature that have studied the interactive effect between salt stress and strobilurins. Therefore, the aim of this work was to evaluate the single and combined effect of salinity and a Pyraclostrobin based fungicide (PBF), on gas exchanges and related parameters, some biochemical characteristics, biomass, yield, quality and WUE of tomato.

2. Material and methods

2.1. Experimental site characteristics

The research was carried out for two years (July-October 2010 and April-July 2011) at experimental farm 'E. Pantanelli' of the University 'Aldo Moro' of Bari, Policoro (MT), Southern Italy (40°10' N, 16°39' E, altitude 15 m a.s.l.). The site is characterized by sub-humid climate according to the De Martonne classification (Cantore et al., 1987). The experimental trials were performed under unheated plastic greenhouse (covered by EVA 200 μm thick film), using cylindrical pots (0.34 m diameter and 0.50 m height), equipped with flowerpot saucers, filled with 40 dm^3 of soil, collected in the same site. The soil was a fine, mixed, sub-active, thermic Chromic Haploxererts (Cassi and Viviano, 2006), with the following physical and chemical characteristics (average of the two years): sand ($2 > \phi > 0.02 \text{ mm}$) 28.6%, silt 38.0%, clay ($\phi < 2 \mu$) 33.4%; pH 7.71; organic matter (Walkley-Black method) 31.2 g kg^{-1} , total N (Kjeldahl method) 1.39 g kg^{-1} , exchangeable K_2O (ammonium acetate method) 265 mg kg^{-1} , available P_2O_5 (Olsen method) 26.7 mg kg^{-1} , active limestone 4.7 g kg^{-1} , total limestone 13.2 g kg^{-1} ; ESP 1.9%; electrical conductivity of soil saturated paste extract (ECe) 0.94 dS m^{-1} ; bulk density 1.23 kg dm^{-3} ; soil moisture at i) wilting point (-1.5 MPa, Richard Pressure Plate Extractor) 15.1% (w/w), and ii) field capacity (FC) 31.6% (w/w) of soil dry weight.

The climatic parameters (temperature and relative humidity) were measured by utilizing a thermistor (model E001, Tecno.El, Rome, Italy) and a hygrometer (C-83_N Rotronic, Zurich, Switzerland). The acquisition and storage of data was carried out by a data-logger (model Kampus, Tecno.El, Rome, Italy) connected to a PC.

2.2. Experimental design and crop management

Two soil salinity levels, in combination with two PBF treatments, were compared. The soil salinity levels (ECe), at transplanting time, were 1.1 (S_0) and 5.4 dS m^{-1} (S_1). The PBF treatments consist of plants sprayed with water (control, Py_0) or PBF water solution (Py_1) (2.5 mL L^{-1} , Cabrio® Duo, by BASF Italia S.p.A.). PBF application were performed every 7–10 days during flowering and fruit enlargement stages for a total of three applications. The PBF was sprayed by a backpack power sprayer (mod. MS073D, Maruyama Mfg. Co, Inc., Japan). Treatments were arranged in a split plot design with seven replicates. Salinity levels were the main plot factor while PBF treatments were the subplot factor. Each subplot consisted of two pots each containing a plant.

The different soil salinity levels were obtained, before filling the pots, by accurately mixing 0.0 (S_0) and 2.0 g dm^{-3} (S_1) of $\text{NaCl} + \text{CaCl}_2$ 1:1 (w/w) to the soil fertilised with 0.05 g dm^{-3} of diammonium phosphate and 0.04 g dm^{-3} of urea.

Tomato (*Solanum lycopersicum* L.) seedlings cv Coronel (ISI sementi S.p.A., Fidenza, PR, Italy) were transplanted at three true leaves stage in the center of each pot on 20th July 2010 and 2th April 2011, respectively. After transplanting, the soil surface was covered with a layer of about 1 cm of wheat straw with the function of mulching, in order to limit evaporation and the rising of the salt on the soil surface. In this way the compaction of the soil during the watering was avoided also. Weeding was carried out manually, disease and pest control by applying the integrated pest management strategies of the Basilicata region.

Evapotranspiration (ET) was estimated by the water balance method by weighing every day the pots, and utilizing the following equation (Ünlükara et al., 2010; Schiattone et al., 2017):

$$ET = \frac{(PW_n - PW_{n+1}) + (I - D)}{\rho_w}$$

where,

ET = daily evapotranspiration (L)

PW_n and PW_{n+1} = pot weights (kg) in two consecutive days

I = applied water (kg)

D = drainage water (kg)

ρ_w = water bulk density (1 kg L⁻¹)

In order to restore the water lost by evapotranspiration, fresh water with an electrical conductivity of 0.5 dS m⁻¹ was supplied manually from the top of the pots. The watering was performed when the allowable water depletion (p) in the S_0 treatment was reached. During the whole growing cycle, the threshold was assumed to be 0.40 of total available water ($p = 0.40$) (Allen et al., 1998). The whole amount of water lost by evapotranspiration has been restored at each watering event in all treatments. To avoid significant variations in soil salinity during the crop cycle, the water that eventually leached in the flowerpot saucers, was collected, measured and used for the subsequent watering in the same pot. On the whole, about 1.4 and 1.6 L pot⁻¹ (average of all pots) of drainage water, respectively in 2010 and 2011, was collected. Irrigation was stopped one week before harvesting.

2.3. Soil salinity

Four soil samples were collected to measure ECe after mixing the fertilizers and the different quantities of salts (in relation to the salinity treatments) to the soil. The same analysis was performed at the end of the growing cycle for the soil of each pot by taking the soil samples along the whole profile of the pot by a cylindrical probe (\varnothing 2.5 cm).

2.4. Gas exchange, chlorophyll, canopy temperature and leaf water potential

Gas exchange (CO₂ assimilation rate - A , transpiration - T , stomatal conductance - g_s), total chlorophyll (Chl), canopy temperature (T_c) and midday leaf water potential (ψ_1), were assessed in three days of each year (August 25th, September 6th and 14th 2010; May 5th, 16th and 26th 2011), between flowering and fruits growth stages.

Leaf gas exchange was measured by a portable photosynthetic open-system ADC-LCA3 (Analytical Development Co., Hoddesdon, UK) equipped with a 6.2 cm² large assimilation leaf chamber. The measurements were performed at noon in three clear sky days (12:00 - 14:00 h), on two upper fully-expanded, healthy, terminal, and sun well-exposed leaves per plant. The measuring order for different treatments was random.

The leaf Chl content was measured by a non-destructive method, using a chlorophyll meter SPAD-502 (Minolta, Ramsey, NJ), which provides the Chl content in arbitrary units (SPAD units). The measurements were performed on five young fully expanded leaves randomly selected for each plant, attributing to each replication the average value of the five leaves.

The T_c was assessed using an IR thermometer (model 112 C; Everest Interscience, Tustin, Calif.). Three measurements for each plant were carried out and the order followed for the measurements of the different plots was random. The ψ_1 was measured by the pressure bomb (Model 3005, Ecosearch, Città di Castello-PG, Italy) on two fully expanded, healthy, young leaves of each plant.

2.5. Antioxidant enzyme assay

On 14th September 2010 and 26th May 2011 an homogeneous sample of mature and healthy leaves (about 50 g) and roots (about 25 g) was taken from each plot, immediately closed in a black plastic bag and placed in a portable refrigerator at a temperature of 4 °C. The samples were then transported to the laboratory and analysed within two hours. For the enzyme extraction, 1 g of plant material was selected randomly from the main sample and homogenized in a cold mortar with 1 mL of buffer. The enzymatic activities of superoxide dismutase (SOD; EC 1.15.1.1), peroxidases (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) were assessed in both leaves and roots as previously described by Sergio et al. (2012).

2.6. Biomass, yield and fruit quality

The crop cycle ended on 27th October 2010 and on 7rd July 2011. The fruits and shoots of all plants were collected to assess the above-ground dry biomass, yield (total, marketable and unmarketable) and mean weight of marketable fruit. The fruits that were green, cracked, with symptoms of blossom-end rot, sun burn, or damaged by tomato fruit-worm were considered unmarketable.

In the same day of harvesting, Hunter chromatic parameters L* (brightness), a* (redness) and b* (yellowness) (CIE, 1986) on ten randomly selected marketable fruits of each plot were analysed. Three measurements per fruit were assessed on the equatorial area of the skin and flesh with a Minolta colorimeter (model CR-400, Konica Minolta, Osaka, Japan) and by Spectra Magic NX software.

On the same fruits, shape index was assessed as length/diameter ratio, and pulp firmness (Fi) was measured by a penetrometer (model 53205 TR, Turoni, Forlì, Italy), fitted with a 3 mm diameter round-head probe on three discs of the skin surface from the fruit equatorial area.

A sample of ten marketable fruits was dried in a ventilated oven at 55 °C, until a constant weight was reached (about 48 h) to determine the fruit dry matter (DM). In addition, a sample (about 0,5 kg) of marketable fruits was analysed for total soluble solid (TSS), titratable acidity and pH as follow: from the liquid extract, obtained by liquefying the fruit sample in a blender (1 min; 14,000 rpm) and then filtering all mesocarp, the following were determined i) TSS content in juice using a refractometer (model DBR35, XS instruments, Poncarale-BS, Italy) and expressed as °Brix at 20 °C; ii) titratable acidity by titrating juice with NaOH 0.1 M in the presence of phenolphthalein with an automatic titrating machine (Technotrate, Kartell, Noviglio-MI, Italy) until colour change, with the result expressed in terms of monohydrate acid; iii) fruit juice pH using a pH meter (Acorn pH 6 M, Oakton Instruments, Vernon Hills, IL).

2.7. Water use efficiency

WUE was calculated as: i) the ratio between A and g_s , i.e. intrinsic water use efficiency (WUE_i), ii) the ratio between the dry aboveground biomass and evapotranspiration, i.e. biomass WUE (WUE_b) and ii) the ratio between the marketable yield and evapotranspiration, i.e. yield WUE (WUE_y) (Schiattone et al., 2018).

2.8. Statistical analysis

Collected data were analysed according to a split plot design and elaborated by analysis of variance (ANOVA) procedure; mean values were separated by Student-Newman-Keuls (SNK) test at $P = 0.05$. The SPSS 17 software was used for the analysis.

3. Results

3.1. Climatic trend

Due to the different transplant date of the two years, the two tomato crop cycles have been characterized by a different trend in air temperature. In the first year, when the transplant was performed on 20th July, the daily minimum (T_{\min}) and maximum (T_{\max}) temperatures showed a stable trend until the end of August and then decreased thereafter. In the second year, on the other hand, with transplantation on 2nd April, the temperature trend was increasing from transplanting to harvesting (Fig. 1). T_{\min} ranged between 10 and 25.5 °C while T_{\max} between 17.5 and 38 °C, in 2010. In 2011, T_{\min} ranged between 7 and 22 °C, while T_{\max} between 15 and 34 °C (Fig. 1).

Daily maximum relative air humidity in both years ranged between 90 and 100%, except in rare cases in which lower values were recorded. The minimum daily relative humidity, however, was much more variable with values ranging between 20 and 90% in the first year and between 25 and 95% in the second one (Fig. 1).

3.2. Soil salinity, evapotranspiration and water supply

Soil E_c, during the tomato growing cycles, underwent a slight increase (about 1.2 dS m⁻¹ as average of the two years), as a consequence of salts supplied with irrigation water. In fact, from the transplant to the harvest time, in S₀ E_c passed from about 1.1 to about 2.4 dS m⁻¹, while in S₁ these values ranged from about 5.4 to 6.5 dS m⁻¹ (Table 1).

E_{Tc}, as average of the treatments, was about 81 and 72 L pot⁻¹, respectively the first and second year. On average, this parameter underwent a 12.9% reduction between S₀ and S₁ and a slight increase (on average 1.5%) with the application of PBF (Table 2).

Since the irrigation was cut a few days before the harvest, the total water supply (TWS) was lower than E_{Tc}. In particular, it ranged from 81 and 70.7 L pot⁻¹ of S₀ to 70.5 and 62.1 L pot⁻¹ of S₁, in the first and second year, respectively (Table 2).

3.3. Gas exchange, chlorophyll, canopy temperature and leaf water potential

Salinity influenced all the examined physiological parameters of tomato plants (Table 3). In particular, in saline treatment, as the average of the two years, A, T, gs and SPAD index decreased respectively by 19.0, 27.7, 28.3 and 2.2%, while WUE_i, ψ_1 and T_c increased respectively by 13.1, 30.3 and 4.7%. The decline of A, T, gs and SPAD showed a growing trend during the crop cycle.

The application of PBF affected some of the examined physiological parameters (Table 3). In particular, in the measures performed after spraying PBF, in Py₁ in respect to Py₀, A, WUE_i and Chl on average increased respectively of 7.8, 23.3 and 6.1%, while T and gs decreased on average by 11.7 and 12.5%, respectively. In both years, in the last measuring date, the positive effect of PBF on A and Chl was higher in the saline treatment (interaction salinity x PBF).

3.4. Antioxidant enzymes

The activity of antioxidant enzymes was affected differently by salinity both in leaves and roots, depending on the type of enzyme (Fig. 2). In leaves, the activity of SOD, CAT, and APX in the S₁ treatment increased (average of PBF treatments and years) by 14.6, 145.5, and 95.2%, respectively, compared to the control; while, in roots, it increased by 4.9, 29.5, and 37.6%, respectively. Instead, in the same conditions, POD decreased by 17.1 and 11.1% in leaves and roots, respectively.

PBF increased the antioxidant enzyme activity to a large extent in the salt treatment. Indeed, the activity of all the evaluated enzymes was enhanced by the application of PBF, both in leaves and roots, on average (salinity treatments and years) by 41.2 and 28.9% (SOD), 73.0 and 51.2% (POD), 120.2 and 53.5% (CAT), 83.1 and 118.8% (APX), respectively (Fig. 2).

3.5. Biomass, yield characteristics and water use efficiency

Salinity caused a decline in dry biomass of shoots and fruits, respectively by 12.4 and 18.0%, and an increase in root/shoot ratio dry bio-

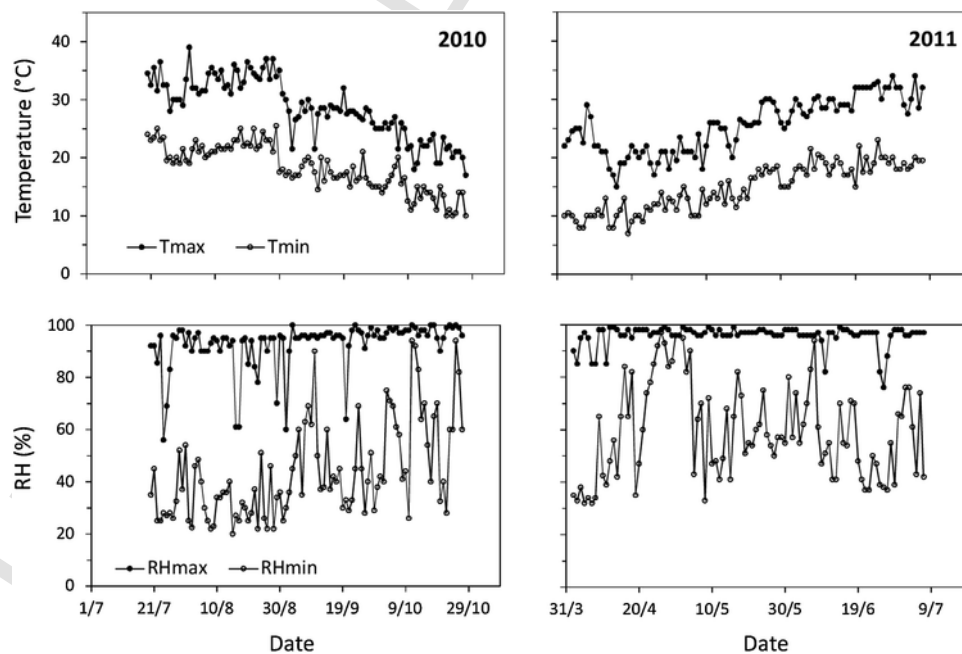


Fig. 1. Trend of maximum (T_{\max}) and minimum (T_{\min}) air temperature, and of maximum (RH_{\max}) and minimum (RH_{\min}) relative humidity (RH) during tomato crop cycles in 2010 and 2011.

Table 1

Mean values \pm SD of soil saturated paste extract (dS m^{-1}) at planting ($n = 4$) and at harvesting ($n = 7$) related to the different treatments and years. S_0 and S_1 are the soil salinity levels; Py_0 and Py_1 are the Pyraclostrobin based fungicide (PBF) treatments.

Treatments	2010		2011	
	Planting	Harvesting	Planting	Harvesting
$S_0 Py_0$	1.11 ± 0.11	2.48 ± 0.15	1.12 ± 0.17	2.29 ± 0.19
$S_0 Py_1$	1.07 ± 0.08	2.50 ± 0.13	1.14 ± 0.08	2.34 ± 0.14
$S_1 Py_0$	5.35 ± 0.33	6.53 ± 0.59	5.48 ± 0.45	6.57 ± 0.47
$S_1 Py_1$	5.41 ± 0.26	6.61 ± 0.46	5.40 ± 0.53	6.48 ± 0.56

Table 2

Mean values \pm SD ($n = 7$) of total crop evapotranspiration (ETc) and total water supply (TWS) related to the different treatments and years. S_0 and S_1 are the soil salinity levels; Py_0 and Py_1 are the Pyraclostrobin based fungicide (PBF) treatments.

Treatments	ETc (L pot^{-1})		TWS (L pot^{-1})	
	2010	2011	2010	2011
$S_0 Py_0$	86.6 ± 2.32	75.8 ± 2.11	81.6 ± 1.39	69.9 ± 1.46
$S_0 Py_1$	87.5 ± 1.95	77.3 ± 1.83	82.4 ± 2.45	71.5 ± 1.67
$S_1 Py_0$	74.2 ± 1.63	67.3 ± 1.34	69.8 ± 1.27	61.8 ± 1.02
$S_1 Py_1$	75.6 ± 1.71	67.9 ± 1.10	71.3 ± 1.48	62.4 ± 1.27

mass by 18.7%, on average (Table 4). Moreover, in S_1 , total and marketable yield decreased on average by 24.7 and 21.4%, respectively. The decline in fruit size, whose average weight decreased by 19%, mainly contributed to the yield reduction due to salinity. The salinity has also led to a 55.7% increase in the number of fruits with blossom-end rot (Table 5). Fruit DM, TSS, TA, pH, and Fi were not influenced by salinity in the first year. However, in the second year, DM, TSS, and TA increased respectively by 18.5, 18.2, and 26.3%, while the other three parameters did not change (Table 6). The chromatic characteristics of fruits showed some changes under salinity conditions. In particular, with reference to fruit surface, L^* underwent an increase which resulted significant only in the first year (7.0%), while a^* and b^* increased in both years (on average 6% and 12.3%, respectively). On the fruit pulp, salinity led to an increase in a^* and b^* , on average by 5.8 and 12.7% (Table 7). WUE_b and WUE_y were not influenced by salinity in the first year, while WUE_y decreased by 20.1% in the second one (Table 8).

PBF affected biomass accumulation and yield, while had no effect on fruit quality. In particular, the dry biomass of shoots and fruits in Py_1 increased by 5.1 and 9.0%, respectively (Table 4).

PBF resulted in an average increase by 7.9 and 8.1% in total and marketable yield, respectively, as well as by 5.9% in fruit mean weight. The positive effect of PBF on yield was greater in the salt treatment (interaction salinity \times PBF levels) (Table 5, Fig. 3). In addition, PBF led to a significant increase (on average 6.5%) in WUE_y (Table 8).

4. Discussion

4.1. Effect of salinity on physiological, biochemical, morphological and yield features of tomato

As expected, salinity negatively affected the gas exchange of tomato plants. The increase in soil salinity initially causes the reduction of g_s by osmotic effect and, if the salinity stress lasts, the toxic effect caused by a gradual Na^+ and Cl^- ions accumulation in plant organs can occur; such effect interferes with the ion balance in the cytoplasm (Munns and Tester, 2008; Maathuis et al., 2014). The decrease in g_s leads to the simultaneous reduction of T and A. Generally, as effect of osmotic stress, T decreases more than A (Hernández and Almansa, 2002; Parida and Das, 2005); but, as the toxic effect appears, A is more inhibited because of the damage suffered at the biochemical level (Munns and Tester,

2008). This would lead to an initial increase in WUE_i and a subsequent decrease (Lovelli et al., 2012). In our trial, the increase in WUE_i led us to hypothesize that salt stress experienced by tomato has been such as to make osmotic stress prevailing, as it is also evident from the considerable reduction of the xylematic potential (Álvarez et al., 2012). T reduction is also confirmed by T_c data, which in S_1 increased as a consequence of the cooling effect reduction due to lower T (Sirault et al., 2009). On the other hand, also leaf Chl content, a toxic stress indicator (Zhang et al., 2018), declined only at the last measurement date. Chl reduction can be attributed to the inhibition of saline-induced Chl biosynthesis (Khan, 2006), which may be caused by nutritional imbalance.

The negative effects of salinity on A have led to the reduction of biomass production and to the variation in its partitioning, also. The production of biomass in salinity conditions is reduced both for decline in A and for the greater allocation of photosynthetates in roots to the detriment of leaves whose expansion can dramatically decrease, further reducing photosynthesis (Maggio et al., 2007). In fact, an increase in the root/shoot ratio was already reported as response to saline stress, mainly due to osmotic effect rather than to a salt-specific one (Hsiao and Xu, 2000). This is confirmed by our trial in which the root dry biomass remained unchanged for the two salinity levels, while root/shoot ratio increased with increase in salinity, as obtained on tomatoes also by other authors (Albacete et al., 2008; Lovelli et al., 2012). The higher proportion of the roots may constitute a typical mechanism of resistance to salt stress (Cassaniti et al., 2012).

Salinity has led to a smaller yield reduction than expected based on the characteristics parameters values of Maas and Hoffman (1977) model for tomato. In fact, using the critical threshold (2.5 dS m^{-1}) and slope ($9.9\% \text{ m dS}^{-1}$) values reported in the literature for tomato (Flagella et al., 2002), and considering the average EC_e recorded in S_1 , a 35% reduction in yield would be expected instead of 20%. Therefore, we can consider that 'Coronel', the cultivar used in our trial, presents a greater tolerance to salinity, confirming the genotypic variability of tomato plant for this character, as already observed by several authors (Cucci et al., 2000; Cantore et al., 2008a).

Yield reduction, determined by salinity, was mainly caused by the decline of fruit size. Other authors have shown that in the early phases of stress and with mild or moderate salt stress the reduction of mean weight of fruits prevails, while with a stronger stress there is also a decrease in number of fruits due to both the reduction in number of flowers and fruit set (Cuartero and Fernández-Munöz, 1999).

Plants affected by salinity showed a high incidence of fruits with blossom-end rot. Saline stress is considered among the predisposing factors of this disease, due to the $\text{Na}^+/\text{Ca}^{2+}$ antagonism, at the origin of the nutritional imbalance that leads to the reduction of calcium in fruits (Grattan and Grieve, 1999). In the second year, the salinity improved some quality traits of tomato, in particular DM, TSS and TA, as already demonstrated under salt (Cucci et al., 2000; Santamaria et al., 2004; Boari et al., 2016) and water stress (Giuliani et al., 2017; Lovelli et al., 2017). However, in the first year no effect was observed, probably due to the different cultivation time, then ripening of fruits coincided with lower temperatures. The very high DM and TSS values recorded in the first year can be attributed to favourable environmental factors. Salinity did not have any effect on the fruit Fi as noticed by Cuartero et al. (1996). Instead, with higher salinity levels, other authors reported a decline in tomato fruit Fi (Cantore et al., 2012).

Salinity caused the increase in fruit red index (a^*) and yellow index (b^*), which are positively correlated to the lycopene and β -carotene content, respectively (Dumas et al., 2003; Cantore et al., 2008b). There are numerous experimental findings showing a positive effect of salt stress on the content of these important antioxidants improving tomato nutritional value (Ehret et al., 2013).

The WUE_b and WUE_y were not affected by salinity in the first year, while WUE_y was negatively affected in the second one. Similar results for WUE_b have been reported by Romero-Aranda et al. (2001) and

Table 3

Physiological measures carried out in 2010 (a) and 2011 (b) before (25/8/2010 and 5/5/2011) and after (6 and 14/9/2010, 16 and 26/5/2011) Pyraclostrobin based fungicide (PBF) application. Effect of salinity level and PBF application on net assimilation (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration (T, $\text{mmol m}^{-2} \text{s}^{-1}$), stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), intrinsic water use efficiency (WUE_i , $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mol m}^{-2} \text{s}^{-1}$), leaf water potential (ψ_l , MPa), canopy temperature (T_c , °C) and leaf chlorophyll content (Chl, SPAD unit).

(a)							
Treatments	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	T ($\text{mmol m}^{-2} \text{s}^{-1}$)	gs ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$)	ψ_l (MPa)	T_c (°C)	Chl (SPAD unit)
25/8/2010							
Salinity (S)	**	**	**	*	**	*	ns
S ₀	20.7	4.7	0.51	40.6	-0.95	30.2	53.8
S ₁	17.7	3.5	0.39	45.4	-1.15	32.0	53.5
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns	ns	ns
Py ₀	19.3	4.1	0.46	42.0	-1.06	31.3	54.3
Py ₁	19.1	4.1	0.44	43.4	-1.04	30.9	53.0
S x PBF	ns	ns	ns	ns	ns	ns	ns
6/9/2010							
Salinity (S)	**	**	**	ns	**	ns	ns
S ₀	18.7	4.2	0.41	45.6	-0.91	28.7	54.5
S ₁	15.4	3.1	0.32	48.1	-1.20	29.2	53.4
Pyraclostrobin (PBF)	*	*	*	**	ns	ns	*
Py ₀	16.3	3.8	0.39	41.8	-1.06	28.8	52.3
Py ₁	17.8	3.5	0.34	52.4	-1.05	29.1	55.6
S x PBF	ns	ns	ns	ns	ns	ns	ns
14/9/2010							
Salinity (S)	**	**	**	*	**	*	*
S ₀	16.2	3.5	0.37	43.8	-0.90	27.5	53.7
S ₁	11.4	2.5	0.23	49.6	-1.12	29.6	51.4
Pyraclostrobin (PBF)	*	*	*	**	ns	ns	*
Py ₀	13.4	3.2	0.32	41.9	-0.99	28.6	50.7
Py ₁	14.2	2.8	0.28	50.7	-1.03	28.5	54.5
S x PBF	*	ns	ns	ns	ns	ns	**
(b)							
Treatments	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	T ($\text{mmol m}^{-2} \text{s}^{-1}$)	gs ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$)	ψ_l (MPa)	T_c (°C)	Chl (SPAD unit)
5/5/2011							
Salinity (S)	**	**	**	*	**	*	ns
S ₀	21.3	5.2	0.55	38.7	-0.90	27.6	53.4
S ₁	18.1	3.7	0.40	45.3	-1.19	29.2	53.7
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns	ns	ns
Py ₀	19.6	4.4	0.47	41.7	-1.05	28.1	53.2
Py ₁	19.8	4.5	0.48	41.3	-1.04	28.7	53.9
S x PBF	ns	ns	ns	ns	ns	ns	ns
16/5/2011							
Salinity (S)	**	**	**	*	**	*	ns
S ₀	20.3	5.1	0.48	42.3	-0.88	30.3	54.0
S ₁	17.5	3.9	0.36	48.6	-1.15	31.2	53.1
Pyraclostrobin (PBF)	ns	ns	ns	*	ns	ns	ns
Py ₀	18.6	4.6	0.44	42.3	-1.01	30.5	53.0
Py ₁	19.2	4.4	0.40	48.0	-1.03	31.1	54.1
S x PBF	ns	ns	ns	ns	ns	ns	ns
26/5/2011							
Salinity (S)	**	**	**	*	**	*	*
S ₀	21.7	5.4	0.50	43.4	-0.92	31.6	55.2
S ₁	16.6	3.6	0.33	50.3	-1.30	32.9	52.2
Pyraclostrobin (PBF)	*	*	*	**	ns	ns	**
Py ₀	18.0	4.9	0.45	40.0	-1.02	32.5	51.5
Py ₁	20.3	4.1	0.38	53.4	-1.00	32.0	55.9
S x PBF	*	ns	ns	ns	ns	ns	**

ns, *, ** indicate *F* test not significant or significant at $P < 0.05$ and $P < 0.01$, respectively.

Reina-Sánchez et al. (2005), while Al-Karaki (2000) reported a decline of this parameter in saline conditions. This discrepancy might be attributed to the range of salinity tested by Al-Karaki (2000), which was much higher than that used in our trial as well as by Reina-Sánchez et al. (2005) and Romero-Aranda et al. (2001). WUE_i reduction caused by salinity in the second year of our trial is in accordance with findings of Romero-Aranda et al. (2001) and Reina-Sánchez et al. (2005). Actually, the water used by the plant decreased with the increase in salinity, to which a proportional reduction of total biomass was related, while the fruit production decreased more drastically. Probably, water uptake by plants under salinity conditions was primarily used to meet transpira-

tion and vegetative growing demands and secondarily allocated to fruit growth (Ehret and Ho, 1986; Johnson et al., 1992). The different results obtained in the two years for the WUE_i were probably attributable to the different cultivation time and, therefore, to the different climatic conditions during the crop cycle.

4.2. Effect of Pyraclostrobin on physiological, biochemical, morphological and yield features of tomato

According to previous literature, PBF influenced physiological and biochemical parameters of tomato with consequent effects on morpho-

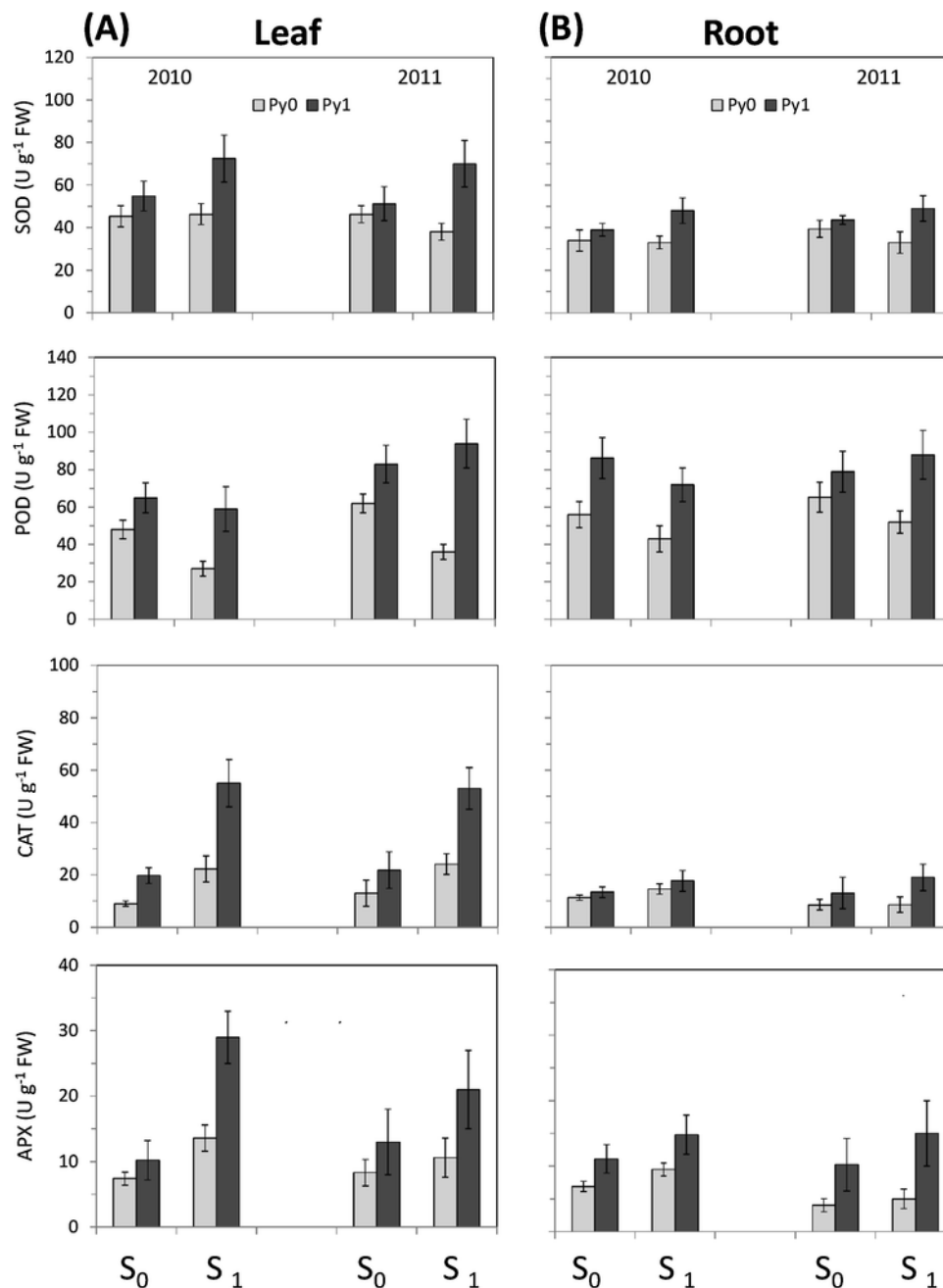


Fig. 2. Interactive effect of salinity level (S_0 and S_1) and Pyraclostrobin based fungicide (PBF) treatment (Py_0 and Py_1) on enzymatic activity (SOD, POD, CAT, APX) in tomato leaves (A) and roots (B) in 2010 and 2011. Vertical bars indicate SD ($n = 7$). U = unit; FW = fresh weight. One unit of SOD was defined as the amount of enzyme which produced a change of 0.01 of absorbance at 560 nm per minute; one unit of CAT was defined as the amount of enzyme needed to halve the concentration of nitrogen peroxide in the reaction mixture at 25 °C, per minute; one unit of POD was defined as the absorbance change at 470 nm per minute, at 25 °C; the APX unit was defined as the amount of enzyme that produced a change of 0.01 in absorbance at 290 nm, per minute.

logical and productive characteristics as well as WUE. Moreover, the interactive effect of PBF with salinity on physiological and productive performances of tomato was demonstrated, thus confirming the hypotheses inspiring our experiment.

PBF reduced g_s , in agreement with previous findings on tomato (Cantore et al., 2016; Giuliani et al., 2018), rice (Debona et al., 2016), barley, soybean, and wheat (Nason et al., 2007). Studies carried out on wheat (Grossmann et al., 1999; Nason et al., 2007) and grapevine (Diaz-Espejo et al., 2012) showed that this would occur due to the increased production of ABA, a hormone notoriously involved in regulation of the stomatal opening (Venancio et al., 2003). Furthermore, according to g_s decline, PBF reduced T, as also previously observed as a

result of the application of strobilurins on tomato (Marek et al., 2018), wheat, barley, and soybean (Nason et al., 2007). By applying Azoxystrobin on tomato plants, Giuliani et al. (2018) obtained genotype-dependent results; actually they found increase or no changes in T values depending on the considered cultivar, thus demonstrating differences related to the genotype in the physiological responses to strobilurins, even within the same species.

Several studies showed the positive effect of strobilurins on the photosynthetic activity of tomato (Giuliani et al., 2018; Marek et al., 2018) and wheat (Baranyiova et al., 2014). According to these results, in our trial PBF promoted tomato A increase, despite the concomitant decline of g_s , that reduced the flow of CO_2 to the photosynthetic sites.

Table 4
Effect of salinity and Pyraclostrobin based fungicide (PBF) treatments on dry biomass (g plant⁻¹) of roots, shoots + leaves and fruits in the two years.

Treatments	2010			2011		
	Roots/Shoots	Shoots	Fruits	Roots/Shoots	Shoots	Fruits
Salinity (S)	*	**	**	*	**	**
S ₀	0.23	142.2	228.8	0.19	263.4	181.3
S ₁	0.27	128.9	191.8	0.24	222.8	145.5
Pyraclostrobin (PBF)	ns	*	*	ns	ns	*
Py ₀	0.25	130.3	202.0	0.22	240.5	155.8
Py ₁	0.25	140.8	218.6	0.21	245.7	171.0
S x PBF	ns	*	*	ns	ns	*

ns, *, ** indicate *F* test not significant or significant at *P* < 0.05 and *P* < 0.01, respectively.

Table 5
Effect of salinity and Pyraclostrobin based fungicide (PBF) treatments on total and marketable yield, fruit mean weight, fruits affected by blossom-end rot and shape index in 2010 and 2011.

Treatments	Yield		Fruit mean weight (g)	Blossom-end rot (kg plant ⁻¹)	Shape index (mm mm ⁻¹)
	Total (kg plant ⁻¹)	Marketable (kg plant ⁻¹)			
2010					
Salinity (S)	**	**	**	*	ns
S ₀	2.381	2.195	62.0	0.049	1.26
S ₁	1.959	1.898	50.6	0.081	1.24
Pyraclostrobin (PBF)	*	*	*	ns	ns
Py ₀	2.098	1.978	54.8	0.077	1.24
Py ₁	2.242	2.115	57.8	0.053	1.26
S x PBF	*	*	*	ns	ns
2011					
Salinity (S)	**	**	**	*	ns
S ₀	2.816	2.165	65.0	0.039	1.24
S ₁	1.922	1.530	52.2	0.057	1.30
Pyraclostrobin (PBF)	*	*	*	ns	ns
Py ₀	2.268	1.765	56.8	0.049	1.28
Py ₁	2.470	1.930	60.4	0.049	1.26
S x PBF	*	*	*	ns	ns

ns, *, ** indicate *F* test not significant or significant at *P* < 0.05 and *P* < 0.01, respectively.

Table 7
Effect of salinity and Pyraclostrobin based fungicide (PBF) treatments on Hunter chromatic features of epicarp and pulp of tomato fruits in 2010 and 2011. L*, a* and b* represent brightness, redness and yellowness, respectively.

Treatments	Epicarp				Pulp			
	L*	a*	b*	a*/b*	L*	a*	b*	a*/b*
2010								
Salinity (S)	*	*	**	ns	ns	*	*	ns
S ₀	42.9	33.6	31.9	1.1	35.0	29.9	21.5	1.4
S ₁	45.9	35.2	36.6	1.0	36.7	31.6	23.9	1.3
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns	ns	ns	ns
Py ₀	44.8	34.7	34.0	1.0	35.9	31.5	22.5	1.4
Py ₁	44.0	34.0	34.5	1.0	35.7	30.0	22.9	1.3
S x PBF	ns	ns	ns	ns	ns	ns	ns	ns
2011								
Salinity (S)	ns	*	*	ns	ns	*	*	ns
S ₀	43.2	32.1	31.6	1.0	34.1	28.5	21.2	1.3
S ₁	44.0	34.4	34.7	1.0	34.4	30.2	24.2	1.3
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns	ns	ns	ns
Py ₀	43.9	33.9	33.2	1.0	34.5	30.5	23.3	1.3
Py ₁	43.3	32.6	33.0	1.0	34.0	28.3	22.1	1.3
S x PBF	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, ** indicate *F* test not significant or significant at *P* < 0.05 and *P* < 0.01, respectively.

Table 6
Effect of salinity and Pyraclostrobin based fungicide (PBF) treatments on fruit dry matter (DM), total soluble solids (TSS), titratable acidity (TA), pH and firmness (Fi) in 2010 and 2011.

Treatments	DM (g 100 g f.w.)	TSS (°Brix)	TA (mg 100 g ⁻¹ juice)	pH	Fi (kg cm ⁻²)
2010					
Salinity (S)	ns	ns	ns	ns	ns
S ₀	9.6	7.4	0.36	4.32	4.7
S ₁	9.9	7.5	0.35	4.19	4.6
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns
Py ₀	9.7	7.5	0.38	4.24	4.7
Py ₁	9.8	7.4	0.32	4.27	4.6
S x PBF	ns	ns	ns	ns	ns
2011					
Salinity (S)	**	**	*	ns	ns
S ₀	6.5	5.5	0.38	4.27	4.1
S ₁	7.7	6.5	0.48	4.21	4.5
Pyraclostrobin (PBF)	ns	ns	ns	ns	ns
Py ₀	6.9	5.9	0.44	4.24	4.2
Py ₁	7.3	6.1	0.42	4.24	4.4
S x PBF	ns	ns	ns	ns	ns

ns, *, ** indicate *F* test not significant or significant at *P* < 0.05 and *P* < 0.01, respectively.

This apparent contradiction can be explained by the increase in Chl content that results in photosynthetic efficiency improvement. Contrasting results were obtained on rice (Debona et al., 2016), soybean, barley, and wheat (Nason et al., 2007). Several experimental findings

Table 8
Effect of salinity and Pyraclostrobin based fungicide (PBF) treatments on yield and biomass water use efficiency (WUE_y and WUE_b, kg m⁻³) in the two years.

Treatments	WUE _y		WUE _b	
	2010	2011	2010	2011
Salinity (S)	ns	ns	**	ns
S ₀	25.2	4.3	28.3	5.8
S ₁	25.3	4.3	22.6	5.4
Pyraclostrobin (PBF)	*	ns	*	ns
Py ₀	24.6	4.1	24.7	5.5
Py ₁	25.9	4.4	26.6	5.7
S x PBF	ns	ns	ns	ns

ns, *, ** indicate *F* test not significant or significant at $P < 0.05$ and $P < 0.01$, respectively.

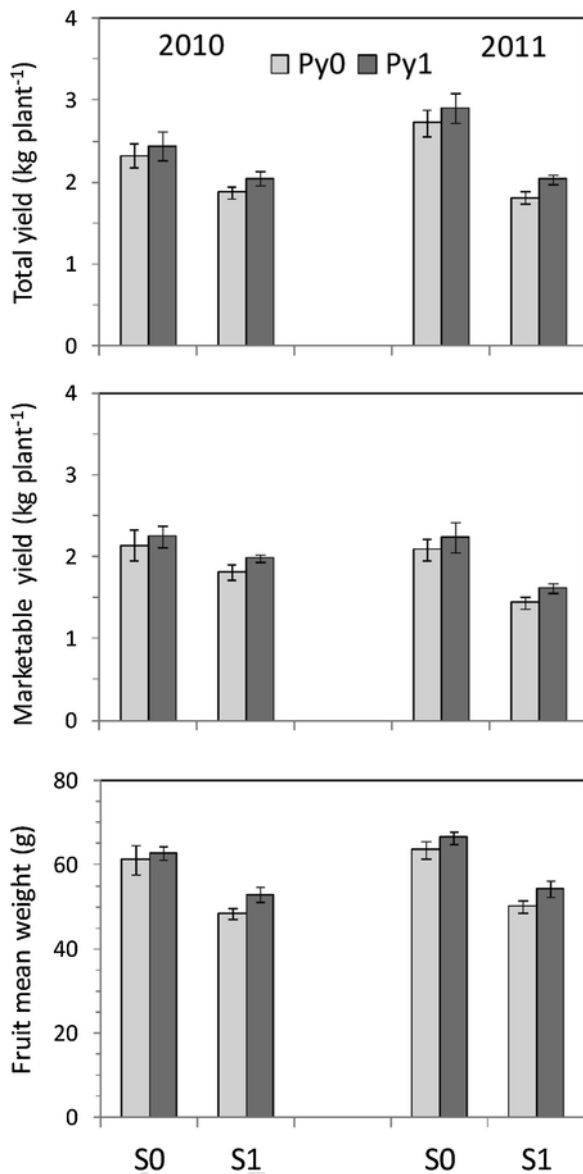


Fig. 3. Interactive effect of salinity level (S₀ and S₁) and Pyraclostrobin based fungicide (PBF) treatment (Py₀ and Py₁) on tomato yield and fruit mean weight in 2010 and 2011. Vertical bars indicate SD (n = 7).

have highlighted the positive effect of strobilurins on Chl biosynthesis (Ramos et al., 2015; Giuliani et al., 2018; Marek et al., 2018; Schiattone, 2018) as well as on slowing down its degradation (green

effect') (Amaro et al., 2018; Liang et al., 2018). The increase in pigment biosynthesis involves the improvement of nitrate reductase activity by strobilurins (Marek et al., 2018) which, by improving nitrogen metabolism (Viana and Kiehl, 2010), would rise its availability for the plant and, therefore, would also improve the formation of Chl, being its main constituent (Kluge et al., 2014). The increase in nitrate reductase activity associated with the application of strobilurins, beside tomato (Marek et al., 2018), was also found in Japanese cucumber (Amaro et al., 2018), maize (Barbosa et al., 2011) and soybean (Soares et al., 2011). On the other hand, Chl degradation slowing down would be determined by the effect of strobilurins on phytohormone balance. In particular, on one hand strobilurins would reduce the synthesis of ethylene (Giuliani et al., 2019), a hormone notoriously involved in the processes of senescence and degradation of Chl, on the other hand it would promote the increase of cytokinins, involved in the biosynthesis of the pigment, delaying the senescence process (Grossmann et al., 1999; Amaro et al., 2018). Grossmann et al. (1999) and Venancio et al. (2003) reported that in wheat bud tissue, the Kresoxim-methyl strobilurin inhibits the biosynthesis of ethylene through the reduction of 1-aminocyclopropane-1-carboxylic acid (ACC)-synthase activity. The slowing of senescence was also related to the decline of lipid peroxidation as a consequence of the reduction of oxidative stress (Zhang et al., 2010; Amaro et al., 2018; Liang et al., 2018). The enhanced activity of antioxidant enzymes (SOD, POD, CAT, APX) observed in our trial, as a result of the application of PBF, would confirm this latter hypothesis. Senescence is considered a process associated with ROS (Li et al., 2007). To counter the ROS stress, plants have evolved strategies in which antioxidant enzymes play an important role (Hadrarni et al., 2005; Pompeu et al., 2008). The POD, CAT, and APX are key enzymes in the active-oxygen scavenging system able to inhibit ROS accumulation. Actually, SOD catalyses superoxide anion radicals (O₂⁻) dismutation to hydrogen peroxide (H₂O₂) and oxygen (O₂), while the three other enzymes provide to the H₂O₂ elimination, preventing its toxic accumulation (Zhao et al., 2007).

Under salt stress, PBF determined a higher increase in Chl and A compared to the salt control. Similar results were obtained on tomato under water deficit (Giuliani et al., 2018). This can be explained by the increase in the activity of antioxidant enzymes determined by PBF, which may have played primary role in the control of oxidative processes promoted by saline stress. Similar findings were obtained on barley, where the application of PBF inhibited the manifestation of the 'physiological leaf spot', a physiological disease attributable to oxidative stress, due to the doubling of POD activity (Köehle et al., 2003). Also other authors have found the improvement in the activity of the main antioxidant enzymes following the application of strobilurins on ginseng (Liang et al., 2018) and Japanese cucumber (Amaro et al., 2018).

The increase in A and the decline in gs contributed to enhance tomato WUE_i, as observed also by other authors (Ramos et al., 2015; Giuliani et al., 2018; Marek et al., 2018), indicating greater capacity to assimilate CO₂ with low water loss, generating greater water saving and higher synthesis of photo-assimilates. Similar effects after application of strobilurins were observed in grapevine (Diaz-Espejo et al., 2012) and winter wheat (Baranyiova et al., 2014).

As expected, the better physiological performance due to the PBF allowed greater biosynthesis and translocation of photo-assimilated to fruits, resulting in increased tomato yield. These results are consistent with literature reporting evidences that the application of strobilurins in tomato plants promote higher marketable fruit yield (Giuliani et al., 2010; Cantore et al., 2016; Marek et al., 2018; Giuliani et al., 2019). Previous studies have demonstrated the increase in yield determined by strobilurins also in other crops such as Japanese cucumber (Amaro et al., 2018), maize (Shetley et al., 2015), beans (Jadoski et al., 2015), soybean (Joshi et al., 2014), and grapevine (Diaz-Espejo et al., 2012). The increase in fruit yield of PBF treated plants in our trial was higher in saline stress conditions, confirming the best physiological perfor-

mance promoted by PBF. Moreover, the effect of PBF on WUE_y confirmed that the application of strobilurin could significantly improve WUE related to fruit yield (Giuliani et al., 2018, 2019).

Therefore, it can be said that this fungicide, as well as the phytosanitary defence of tomato crop, can contribute to increase its tolerance to salinity, one of the most widespread and harmful abiotic stresses. Similar results were obtained on tomato subjected to water stress (Giuliani et al., 2011; Cantore et al., 2016), an abiotic stress whose response of the plant presents many similarities with the salt stress.

5. Conclusions

The results of the research confirmed the harmful effects of salinity at physiological and productive level and the positive one in terms of tomato quality. 'Coronel' has proved to be a tomato cultivar more tolerant to salinity respect to the tolerance levels reported in classical literature. WUE was negatively influenced by salinity in the second year while in the first one it did not change, demonstrating the influence that also environmental conditions may have on this parameter.

This research confirmed that PBF, besides being a valid fungicide for the phytosanitary defence of many crops, could have biostimulant effects on physiological and biochemical parameters that lead to an increase in yield and water use efficiency of tomato. Furthermore, the hypothesis underlying the research was confirmed. In fact, as well as strobilurin-based fungicides have proven effective in reducing water stress, PBF reduced the negative effects of salt stress on tomato. The results of this research obtained in a controlled environment would require further experimental confirmation at field scale. However, in view of the different experimental findings that have demonstrated the positive effect of strobilurins on the production and attenuation of water stress in open field, it can be reasonably asserted that PBF applied in open field would increase tomato's tolerance to salinity. However, since it is a pesticide, we do not believe its use should be exclusively aimed at reducing the salt stress, but it should be destined mainly for phytosanitary defence and prefer it to other pesticides if the water and/or soil salinity are a problem.

Acknowledgements

The research was financed by BASF Italia s.p.a., Cesano Maderno (MB). We are grateful to Stefano Tarlazzi, Giuseppe Rosa and Gianpaolo Ronga for technical support in crop disease management. In addition, we are thankful to ISPA-CNR colleagues Egidio De Palma for technical assistance in the field, and Pasquale Del Vecchio for the assistance on journals publication policy and copyright.

References

Abdelgawad, H., Zinta, G., Hegab, M., Pandey, R., Asard, H., Abuelsoud, W., 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedling organs. *Front. Plant Sci.* 24, 134–141.

Albacete, A., Ghanem, M.E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I.C., Pérez-Alfocea, F., 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *J. Exp. Bot.* 59, 4119–4131.

Al-Karaki, G.N., 2000. Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress. *J. Plant Nutr.* 23, 1–8.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration. *FAO Irrig. and Drain. Paper* 56. FAO, Rome, 300 p.

Álvarez, S., Gómez-Bellot, M.J., Castillo, M., Bañón, S., Sánchez-Blanco, M.J., 2012. Osmotic and saline effect on growth, water relations, and ion uptake and translocation in *Phlomis purpurea* plants. *Environ. Exp. Bot.* 78, 138–145.

Amaro, A.C.E., Pereira Ramos, A.R., Macedo, A.C., Ono, E.O., Rodrigues, J.D., 2018. Effects of the fungicides azoxystrobin, Pyraclostrobin and boscalid on the physiology of Japanese cucumber. *Sci. Hortic.* 228, 66–75.

Arya, B., Komala, B.R., Sumalatha, N.T., Surendra, G.M., Gurumurthy, P.R., 2018. PGPR induced systemic tolerance in plant. *Int. J. Curr. Microbiol. App. Sci.* (Issue 7), 453–462.

Asadi Karam, E., Keramat, B., 2017. Foliar spray of triacontanol improves growth by alleviating oxidative damage in coriander under salinity. *Ind. J. Plant Physiol.* 22, 120–124.

Balba, H., 2007. Review of strobilurin fungicide chemicals. *J. Environ. Sci. Health B* 42, 441–451.

Baranyiova, I., Klem, K., Kren, J., 2014. Effect of exogenous application of growth regulators on the physiological parameters and the yield of winter wheat under drought stress. *Mendelnet* 442–446.

Barbosa, K.A., Fagan, E.B., Casaroli, D., de Carvalho Canedo, S., Teixeira, W.F., 2011. Aplicação de estrobilurina na cultura do milho: alterações fisiológicas e romatológicas. *Rev. Centro Univ. Patos de Minas* 2, 20–29.

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-Dobrzanski, B., 2002. Review: the strobilurin fungicides. *Pest Manage. Sci.* 58, 649–662.

Battilani, A., Prieto, M.H., Argerich, C., Campillo, C., Cantore, V., 2012. Tomato. In: Steduto, P., Hsiao, T.C., Fereres, E., Raes, D. (Eds.), "Crop Yield Response to Water", Irrigation and Drainage Paper No. 66. FAO, Rome, pp. 192–198, ISBN 978-92-5-107274-5.

Boari, F., Cucci, G., Donadio, A., Schiattone, M.I., Cantore, V., 2014. Kaolin influences tomato response to salinity: physiological aspects. *Acta Agric. Scand. - Section B - Plant Soil Sci.* 64 (7), 559–571.

Boari, F., Donadio, A., Pace, B., Schiattone, M.I., Cantore, V., 2016. Kaolin improves salinity tolerance, water use efficiency and quality of tomato. *Agric. Water Manage.* 167, 29–37.

Boari, F., Pace, B., Todorovic, M., De Palma, E., Cantore, V., 2012. Effect of water regime and salinity on artichoke. *It. J. Agron.* 7 (1), 58–63.

Boari, F., Todorovic, M., Albrizio, R., Sellami, M.H., Schiattone, M.I., Cantore, V., 2017. Le strobilurine su pomodoro migliorano le rese e l'uso dell'acqua. *L'Infotere Agrario* 73 (5), 42–45.

Candido, 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.

Cantore, V., Boari, F., Pace, B., 2008. Salinity effects on tomato. *Proc. XVth EUCARPIA tomato. Acta Hortic.* 789, 229–234.

Cantore, V., Boari, F., Vanadia, S., Pace, B., Zacheo, G., Leo, L., De Palma, E., 2008. Evaluation of yield and qualitative parameters of high lycopene tomato cultivars. *Proc. XVth EUCARPIA tomato. Acta Hortic.* 789, 173–179.

Cantore, V., Iovino, F., Pontecorvo, G., 1987. Aspetti climatici e zone fitoclimatiche della Basilicata. *CNRIEF* 2, 49 p.

Cantore, V., Lechkar, O., Karabulut, E., Sellami, M.H., Albrizio, R., Boari, F., Stellacci, A.M., Todorovic, M., 2016. Combined effect of deficit irrigation and strobilurin application on yield, fruit quality and water use efficiency of "cherry" tomato (*Solanum lycopersicum* L.). *Agric. Water Manage.* 167, 53–61.

Cantore, V., Pace, B., Todorovic, M., De Palma, E., Boari, F., 2012. Influence of salinity and water regime on tomato for processing. *Ital. J. Agron.* 7 (1), 64–70.

Cassaniti, C., Romano, D., Flowers, T.J., 2012. The response of ornamental plants to saline irrigation water. In: Garcia-Garibabal, I. (Ed.), *Irrigation Water Management, Pollution and Alternative Strategies*. InTech Europe, Rijeka, Croatia, pp. 132–158.

Cassi, F., Viviano, L., et al., 2006. I Suoli della Basilicata - Carta pedologica della Regione Basilicata in scala 1:250.000. Regione Basilicata - Dip. Agricoltura e Sviluppo Rurale. Direzione Generale.

Caverzan, A., Casassola, A., Brammer, S.P., 2016. Antioxidant responses of wheat plants under stress. *Gen. Mol. Biol.* 39, 1–6.

CIE, 1986. Colorimetry, 2nd ed. Central Bureau of the Commission Internationale de l'Eclairage, Vienne, 1–83, n. 1, 15, 2.

Coella, T., Candido, V., Campanelli, G., Camele, I., Battaglia, D., 2014. Effect of irrigation regimes and artificial mycorrhization on insect pest infestations and yield in tomato crop. *Phytoparasitica* 42, 235–246.

Cuartero, J., Baena, J., Soria, T., Fernández-Munóz, R., 1996. Evolución de la dureza del fruto del tomate, como un componente de la calidad, en cultivares de larga duración y normales cultivados en 5 concentraciones salinas. *Actas de Horticultura* 13, 59–65.

Cuartero, J., Fernández-Munóz, R., 1999. Tomato and salinity. *Sci. Hortic.* 78, 83–125.

Cucci, G., Cantore, V., Boari, F., De Caro, A., 2000. Water salinity and influence of SAR on yield and quality parameters in tomato. *Acta Hortic.* 537, 663–670.

Debona, D., Nascimento, K.J.T., Gomes, J.G.O., Aucique-Perez, C.E., Rodrigues, F.A., 2016. Physiological changes promoted by a strobilurin fungicide in the rice-bipolaris oryzae interaction. *Pestic. Biochem. Physiol.* 130, 8–16.

Diaz-Espejo, A., Cuevas, M.V., Ribas-Carbo, M., Flexas, J., Martorell, S., Fernández, J.E., 2012. The effect of strobilurins on leaf gas exchange, water use efficiency and BA content in grapevine under field conditions. *J. Plant Physiol.* 169, 379–386.

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P., 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric.* 83, 369–382.

Ehret, D.L., Ho, L.C., 1986. Effects of osmotic potential in nutrient solution on diurnal growth of tomato fruit. *J. Exp. Bot.* 37, 1294–1302.

Ehret, D.L., Usher, K., Helmer, T., Block, G., Steinke, D., Frey, B., Kuang, T., Diarra, M., 2013. Tomato fruit antioxidants in relation to salinity and greenhouse climate. *J. Agric. Food Chem.* 61, 1138–1145.

Flagella, Z., Cantore, V., Giuliani, M.M., Tarantino, E., De Caro, A., 2002. Crop salt tolerance: physiological, yield and quality aspects. In: In: S.G., Pandalai (Ed.), *Recent Res. Devel. Plant Biol.* Vol. 2, pp. 155–186, ISBN:81-7736-149-X.

Giuliani, M.M., Carucci, F., Nardella, E., Francavilla, M., Ricciardi, L., Lotti, C., Gatta, G., 2018. Combined effects of deficit irrigation and strobilurin application on gas exchange, yield and water use efficiency in tomato (*Solanum lycopersicum* L.). *Sci. Hortic.* 233, 149–158.

Giuliani, M.M., Gagliardi, A., Nardella, E., Carucci, F., Amodio, M.L., Gatta, G., 2019. The effect of strobilurin on ethylene production in flowers, yield and quality parameters of processing tomato grown under a moderate water stress condition in Mediterranean area. *Sci. Hortic.* 249, 155–161.

- Giuliani, M.M., Nardella, E., Gagliardi, A., Gatta, G., 2017. Deficit irrigation and partial root-zone drying techniques in processing tomato cultivated under Mediterranean climate conditions. *Sustainability* 9 (12), 2197.
- Giuliani, M.M., Nardella, E., Gatta, G., De Caro, A., Quitadamo, M., 2010. Processing tomato cultivated under water deficit conditions: the effect of Azoxystrobin. *Acta Hort.* 914, 287–4.
- Giuliani, M.M., Nardella, E., Gatta, G., Quintadamo, M., De Caro, A., 2011. Processing tomato cultivated under water deficit conditions: the effect of Azoxystrobin. *Acta Hort.* 914, 287–294.
- Grattan, S.R., Grieve, C.M., 1999. Salinity-mineral nutrient relations horticultural crops. *Sci. Hortic.* 78, 127–157.
- Grossmann, K., Kwiatkowski, J., Casper, G., 1999. Regulation of phytohormone levels, leaf senescence and transpiration by the strobilurin kresoxim-methyl in wheat (*Triticum aestivum*). *J. Plant Physiol.* 154, 805–808.
- Hadrami, A.E., Kone, D., Lepoivre, P., 2005. Effect of juglone on active oxygen species and antioxidant enzymes in susceptible and partially resistant banana cultivars to black leaf streak disease. *J. Plant Physiol.* 113, 241e54.
- Hernández, J.A., Almansa, M.S., 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea plants. *Physiol. Plant.* 115, 251–257.
- Hsiao, T.C., Xu, L.K., 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J. Exp. Bot.* 51, 1595–1616.
- Jadoski, C.J., Rodrigues, J.D., Soratto, R.P., dos Santos, C.M., Ribeiro, E., 2015. Ação fisiológica da piraclostrobina na assimilação de CO₂ e enzimas antioxidantes em plantas de feijão condicionado em diferentes tensões de água no solo. *Irriga* 20, 319.
- Jamil, A., Riaz, S., Ashraf, M., Foolad, M.R., 2011. Gene expression profiling of plants under salt stress. *Crit. Rev. Plant Sci.* 30 (5), 435–458.
- Johnson, R.W., Dixon, M.A., Lee, D.R., 1992. Water relations of the tomato during fruit growth. *Plant Cell Environ.* 15, 947–953.
- Joshi, J., Sharma, S., Guruprasad, K.N., 2014. Foliar application of Pyraclostrobin fungicide enhances the growth, rhizobial-nodule formation and nitrogenase activity in soybean (var. JS-335). *Pestic. Biochem. Physiol.* 114, 61–66.
- Kaur, L., Zhawar, V.K., 2016. Antioxidant parameters under salt stress in drought tolerant and susceptible wheat cultivars. *Ind. J. Plant Physiol.* 21, 101–106.
- Khan, N.A., 2006. NaCl-inhibited Chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Biol. Plant.* 47, 437–440.
- Kluge, R.A., Tezotto-Uliana, J.V., Silva, P.P., 2014. Aspectos fisiológicos e ambientais da fotossíntese. *Revista Virtual de Química* 7, 56–73.
- Köchle, H., Grossmann, K., Jabs, T., Gerhard, M., Kaiser, W., Glaab, J., Conrath, U., Seehaus, K., Herms, S., 2003. Physiological effects of the strobilurin fungicide F 500 on plants. In: Lyr, H., Russell, P.E., Dehne, H.-W., Sisler, H.D. (Eds.), *Modern Fungicides and Antifungal Compounds III*. Intercept, Andover, UK, pp. 61–74.
- Li, Z.Z., Niu, W., Qiao, X.W., Ma, L.P., 2007. Anti-oxidant response of *Cucumis sativus* L. to fungicide Carbendazim. *Pestic. Biochem. Physiol.* 89, 49e59.
- Liang, S., Xu, X., Lu, Z., 2018. Effect of azoxystrobin fungicide on the physiological and biochemical indices and ginsenoside contents of ginseng leaves. *J. Ginseng Res.* 42, 175–182.
- Lovelli, S., Potenza, G., Castronuovo, D., Perniola, M., Candido, V., 2017. Yield, quality and water use efficiency of processing tomatoes produced under different irrigation regimes in Mediterranean environment. *Ital. J. Agron.* 12, 17–24.
- Lovelli, S., Scopa, A., Perniola, M., Di Tommaso, T., Sofo, A., 2012. Abscisic acid root and leaf concentration in relation to biomass partitioning in salinized tomato plants. *J. Plant Physiol.* 169, 226–233.
- Maas, E.V., Hoffman, G.J., 1977. Crop salt tolerance – current assessment. *J. Irr. Drain. Div. ASCE* 103, 115–134.
- Maathuis, F.J.M., Ahmad, I., Patishtan, J., 2014. Regulation of Na(+) fluxes in plants. *Front. Plant Sci.* 5, 467.
- Maggio, A., Raimondi, G., Martino, A., De Pascale, S., 2007. Salt stress response in tomato beyond the salinity tolerance threshold. *Environ. Exp. Bot.* 9, 276–282.
- Marek, J., de Azevedo, D., Ono, E.O., Rodrigues, J.D., Faria, C.M.D.R., 2018. Photosynthetic and productive increase in tomato plants treated with strobilurins and carboxamides for the control of *Alternaria solani*. *Sci. Hortic.* 242, 76–89.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25, 239–250.
- Munns, R., James, R.A., Lauchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57, 1025–1043.
- Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.* 59, 651–681.
- Nason, M.A., Farrar, J., Bartlett, D., 2007. Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress. *Pest Manage. Sci.* 63, 1191–1200.
- Nxele, X., Klein, A., Ndimba, B.K., 2017. Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *S. Afr. J. Bot.* 108, 261–266.
- Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. *Eco-toxicol. Environ. Saf.* 60, 324–349.
- Polemio, M., 2016. Monitoring and management of karstic coastal groundwater in a changing environment (Southern Italy): a review of a regional experience. *Water* 8, 148.
- Pompeu, G.B., Grato, P.L., Vitorello, V.A., Azevedo, R.A., 2008. Antioxidant isoenzyme responses to nickel-induced stress in tobacco cell suspension culture. *Agric. Sci. China* 65, 548e52.
- Ramos, R.P., Amaro, A.C.E., Macedo, A.C., Souza, E.R., Rodrigues, J.D., Ono, E.O., 2015. Acúmulo de carboidratos no desenvolvimento de tomateiro tratado com produtos químicos. *Semina. Ciências Agrárias* 36, 705–718.
- Reina-Sánchez, A., Romero-Aranda, R., Cuartero, J., 2005. Plant water uptake and water use efficiency of greenhouse tomato cultivars irrigated with saline water. *Agric. Water Manage.* 78, 54–66.
- Romero-Aranda, M.R., Soria, T., Cuartero, J., 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Sci.* 160, 265–272.
- Santamaria, P., Cantore, V., Conversa, G., Serio, F., 2004. Effect of night salinity level on water use, physiological responses, yield and quality of tomato. *J. Hort. Sci. Biotechnol.* 79 (1), 59–66.
- Scheidleger, A., Grath, J., Lindinger, H., 2004. Saltwater intrusion due to groundwater over-exploitation EEA inventory throughout Europe. Cartagena, Spain. 18th Saltwater Intrusion Meeting 125 p.
- Schiattone, M.I., 2018. Interventi agronomici per migliorare la produzione, la qualità e l'efficienza d'uso dell'acqua e dell'azoto della ruchetta [*Diplotaxis tenuifolia* (L.) DC.]. PhD Thesis. Università degli Studi della Basilicata, Potenza (Italy), 191 p.
- Schiattone, M.I., Viggiani, R., Di Venere, D., Sergio, L., Cantore, V., Todorovic, M., Perniola, M., Candido, V., 2018. Impact of irrigation regime and nitrogen rate on yield, quality and water use efficiency of wild rocket under greenhouse conditions. *Sci. Hortic.* 229, 182–192.
- Schiattone, M.I., Candido, V., Cantore, V., Montesano, F.F., Boari, F., 2017. Water use and crop performance of two wild rocket genotypes under salinity conditions. *Agric. Water Manage.* 194, 214–221.
- Sergio, L., De Paola, A., Cantore, V., Pieralice, M., Cascarano, N.A., Bianco, V.V., Di Venere, D., 2012. Effect of salt stress on growth parameters, enzymatic antioxidant system, and lipid peroxidation in wild chicory (*Cichorium intybus* L.). *Acta Physiol. Plant.* 34, 2349–2358.
- Shetley, J., Nelson, K.A., Stevens, W.G., Dunn, D., Burdick, B., Motavalli, P.P., English, J.T., Dudenhoefter, C.J., 2015. Corn yield response to Pyraclostrobin with foliar fertilizers. *J. Agric. Sci.* 7, 18–34.
- Sirault, X.R.R., James, R.A., Furbank, R.T., 2009. A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography. *Funct. Plant Biol.* 36, 970–977.
- Soares, L.H., Fagan, E.B., Casaroli, D., Andrade, D.M.D., Soares, A.L., Martins, K.V., Rocha, F.J.D., 2011. Aplicação de diferentes estrobilurinas na cultura da soja. *Revista da FZVA* 18, 78–97.
- Ünlükara, A., Kurung, A., Kesmez, G.D., Yurtseven, E., Suarez, D.L., 2010. Effects of salinity on eggplant (*Solanum melongena* L.) growth and evapotranspiration. *Irrig. Drain.* 59, 203–214.
- Venancio, W.S., Tavares Rodrigues, M.A., Begliomini, E., de Souza, N.L., 2003. Physiological effects of strobilurin fungicides on plants. *Publ. UEPG Ciência Exatas e da Terra; Ciências Agrárias e Engenharias.* Ponta Grossa 9 (3), 59–68.
- Viana, E.M., Kiehl, J.C., 2010. Doses de nitrogênio e potássio no crescimento do trigo. *Bragantia* 69, 975–982.
- Wu, Y.-X., von Tiedemann, A., 2001. Physiological effects of azoxystrobin and epoxiconazole on senescence and the oxidative status of wheat. *Pest. Biochem. Physiol.* 71, 1–10.
- Zhang, H.Z., Guo, L., Ye, J., Zhang, L., Wang, Q., Li, F., Zhang, X., Cao, X., Xu, M., Hao, L., Zheng, Y., 2018. Responses of leaf stomatal traits and gas exchange process of cherry tomato to NaCl salinity stress. *Trans. Chin. Soc. Agric. Engin.* 34 (5), 107–113.
- Zhang, P., Senge, M., Dai, Y., 2017. Effects of salinity stress at different growth stages on tomato growth, yield, and water-use efficiency. *Commun. Soil Sci. Plant Anal.* 48 (6), 624–634.
- Zhang, Y.J., Zhang, X., Chen, C.J., Zhou, M.G., Wang, H.C., 2010. Effects of fungicides JS399-19, azoxystrobin, tebuconazole, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. *Pest Biochem. Physiol.* 98, 151–157.
- Zhao, H., Dai, T.B., Jing, Q., Jiang, D., 2007. Leaf senescence and grain filling affected by post-anthesis high temperatures in two different wheat cultivar. *Plant Growth Regul.* 51, 149e58.