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Abstract: Previous research demonstrated that Pseudomonas chlororaphis subsp. aureofaciens strain M71, a plant growth promoting bacteria (PGPB), exerts beneficial effects on plant metabolism and primes defense mechanisms against biotic stresses in tomatoes. We designed an experiment to assess whether root colonization with P. chlororaphis is also able to improve tolerance to water stress in tomatoes. Our results show that inoculation with P. chlororaphis stimulates the antioxidant activity of well-watered tomatoes while maintaining a steady-state level of reactive oxygen species (ROS), increases the expression of genes encoding for the biosynthesis of leaf terpenes, and alters the ABA/IAA phytohormone balance, in turn affecting plant shape (number of leaves) and height (length of internodes), without altering photosynthesis. Upon exposure to mild water stress conditions, the priming effect induced by P. chlororaphis inoculation allowed tomatoes to fine tune the concentration of reactive oxygen species (ROS) and modulate ABA/IAA levels that improved water use efficiency (WUE) and biomass accumulation.

Dear Editors,

herewith I submit our manuscript entitled "Root colonization by *Pseudomonas chlororaphis* primes tomato (*Lycopersicum esculentum*) plants for enhanced tolerance to water stress".

In this work, we widen the investigation on the ability of *Pseudomonas chlororaphis*, a plant growth promoting bacteria (PGPB) already known to be effective against fungal pathogens of tomato, to also improve tolerance to water stress in this plant species. To this purpose, i*n vivo* phenotyping measurements were combined with targeted expression analysis of genes coding for metabolites generally involved in stress responses, quantification of the antioxidant capacity, production of reactive oxygen species (ROS) and hormone profiles. Our results showed that root colonization with *P. chlororaphis* induce a priming phase in tomatoes mainly characterized by: a) a systemic alteration of the ABA/IAA phytohormonal balance that strongly affect plant shape and size; b) an activation of genes involved in both the MEV- and MEP- pathways; c) regulation of ROS homeostasis through stimulation of the antioxidant enzymes activity. Upon exposure to mild water stress conditions, tomatoes primed with *P. chlororaphis* were able to modulate ROS signaling and to improve WUE, enhancing biomass accumulation. The information provided by our study further promotes the exploitation of soil microorganisms as a sustainable strategy to maintain crop yields under future harsh environmental conditions.

Your sincerely,

Dr. Federico Brilli

*Title page

Title:

Root colonization by *Pseudomonas chlororaphis* primes tomato (*Lycopersicum esculentum*) plants for enhanced tolerance to water stress

Running Title: Plant Growth Promoting Bacteria and water stress in tomato

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Abstract

Previous research demonstrated that *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71, a plant growth promoting bacteria (PGPB), exerts beneficial effects on plant metabolism and primes defense mechanisms against biotic stresses in tomatoes. We designed an experiment to assess whether root colonization with *P. chlororaphis* is also able to improve tolerance to water stress in tomatoes. Our results show that inoculation with *P. chlororaphis* stimulates the antioxidant activity of well-watered tomatoes while maintaining a steady-state level of reactive oxygen species (ROS), increases the expression of genes encoding for the biosynthesis of leaf terpenes, and alters the ABA/IAA phytohormone balance, in turn affecting plant shape (number of leaves) and height (length of internodes), without altering photosynthesis. Upon exposure to mild water stress conditions, the priming effect induced by *P. chlororaphis* inoculation allowed tomatoes to fine tune the concentration of reactive oxygen species (ROS) and modulate ABA/IAA levels that improved water use efficiency (WUE) and biomass accumulation.

Keywords:

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Introduction

The extent of the agricultural soil affected by water stress, and progressively more exposed to degradation and loss of fertility is predicted to increase due to climate change (IPCC, 2014). There is an urgent need for alternative, eco-friendly strategies helping conventional agriculture to maintain high productivity, meeting the increasing food demand (Tilman et al. 2002). Among the most promising technologies, the development of soil inoculants containing beneficial microbial strains (Orhan et al. 2006; Coleman-Derr and Tringe, 2014) can contribute to reduce external chemical inputs (i.e. fertilizers and pesticides) while maintaining adequate soil fertility and sustaining crop yields in marginal, arid and semi-arid land (Shaharoona et al., 2008; Adesemoye and Kloepper, 2009).

A wide diversity of naturally occurring plant growth promoting bacteria (PGPB) colonizes the root surfaces, as well as inner tissues of many plant taxa (Bashan et al. 1993; Tilak et al. 2005), exerting beneficial effects on plant metabolism (Kloepper et al. 2004; van Loon et al. 2007) and growth (Glick et al. 2007). Some PGPB can improve the nutritional status of plants, e.g. promoting the uptake of phosphorus (Richardson and Simpson, 2011) and other soil minerals (Mantelin and Touraine, 2004), and stimulating photosynthesis (Zhang et al. 2008; Stefan et al. 2013). PGPB can influence plant production of phytohormones (Tsavkelova et al. 2006) by inducing the synthesis of signaling molecules that modulate the level of auxins (i.e. indole 3-acetic acid, IAA) (Spaepen et al. 2008; Ali et al. 2010), gibberellins (GAs, Bottini et al. 2004), cytokinins (CKs, Timmusk et al. 1999), abscisic acid (ABA, Porcel et al. 2014) and ethylene (Ping and Boland, 2004; Glick, 2005; Hardoim et al. 2008). In particular, PGPB-induced synthesis of IAA and GAs can alter plant shape and size by promoting both cell elongation and division, hence increasing shoot and root growth (Shimizu-Sato and Mori, 2001; Bai et al. 2003; Spaepen et al. 2007). A correct balance among plant hormones might be required to sustain efficient PGPB colonization, and to induce the development of a diverse shape following stimulation of belowground

50 (root) biomass and shoot growth that, in turn, increases the uptake of nutrients and CO_2,
51 thus improving plant performances (Dimkpa et al. 2009; Bettini et al. 2010; Gagné-
52 Bourque et al. 2015).
53 Physiological, transcriptional and metabolic changes stimulated by the colonization with
54 PGPB can also prime plants for enhanced defense ahead of any stress event (Mauch-
55 Mani et al. 2017). Indeed, several PGPB elicit plant mechanisms of induced systemic
56 tolerance (IST) and activate responses against biotic stresses via either salicylic acid or
57 jasmonic acid pathways (Conrath et al. 2015), also involving the production of complex
58 blends of volatile organic compounds (VOC) (Ryu et al. 2004). Priming effects induced by
59 PGPB also improve plant performances under various abiotic stress conditions (Meena et
60 al. 2017; Balestrini et al. 2018) through regulation of phytohormone homeostasis (Yang et
61 al. 2009; Timmusk et al. 2014; Rolli et al. 2015; Vuruklonda et al. 2016) and maintenance
62 of a reactive oxygen species (ROS) steady-state level that enable ROS signaling functions
63 (Gururani et al. 2013). However, more investigations are required to elucidate whether the
64 same changes induced by PGPB on plant metabolism are able to enhance
65 responsiveness to both abiotic and biotic stresses.
66
67 *Pseudomonas chlororaphis* is a much-used PGPB (Shen et al. 2012). Various strains of *P.*
68 *chlororaphis* have already been employed as efficient inoculants against phytopathogenic
69 organisms (Hu et al. 2014; Han et al. 2005; Kozdroj et al. 2004; Thomashow et al. 1990).
70 In fact, *P. chlororaphis* strains synthesize several antibiotics of the phenazine group,
71 siderophores and proteases (Raio et al. 2011, 2017; Puopolo et al. 2013), possessing
72 effective biocontrol activity (Chin-A-Woeng et al. 2003), and also produce VOC capable of
73 enhancing plants defense against pathogens (Han et al. 2006). However, the capacity of
74 *P. chlororaphis* to ameliorate the adverse effects of abiotic stresses has been
75 demonstrated only in Arabidopsis exposed to drought (Cho et al. 2012).

76 In this study, *P. chlororaphis* subsp. *aureofaciens* strain M71 was used to colonize roots of
77 *Lycopersicum esculentum* (tomato), one of the most cultivated crop worldwide, and a plant
78 sensitive to water stress (Dodds et al. 1997). Although this strain of *P. chlororaphis* has
79 already demonstrated to be effective against fungal pathogens of tomato (Chin-A-Woeng
80 et al. 2003; Puopolo et al. 2011), its ability to prime tolerance in the same plant species
81 has been not yet investigated. Therefore, we aimed at evaluating whether inoculation with
82 *P. chlororaphis* primed defenses and enhanced performances of tomato plants exposed to
83 a mild water stress under controlled laboratory conditions, simulating an event frequent in
84 the field. *In vivo* phenotyping measurements were combined with targeted expression
85 analysis of genes coding for metabolites generally involved in stress responses,
86 quantification of the antioxidant capacity, and hormone profiles. Our objectives were to: i)
87 characterize the changes induced in tomato following *P. chlororaphis* inoculation under
88 optimal growth conditions, and particularly those that may prime tolerance; ii) compare the
89 response of *P. chlororaphis*-inoculated and non-inoculated tomatoes under a mild soil
90 water stress and, iii) identify the VOC produced by *P. chlororaphis* that may act as
91 signaling molecules in promoting the interaction with tomato plants.

92 **Materials and Methods**

93

94 *PGPB strain, plant material, growth conditions and application of soil water stress*

95 *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71 (Raio et al. 2011), naturally
96 resistant to rifampicin (Rif +), was employed. *P. chlororaphis* was grown on PPMD agar
97 medium (Wood et al. 1997) amended with 100 ppm rifampicin for 48 h at 25 ± 2°C. Then
98 the bacterial cells were scraped from the agar surface and suspended in sterile distilled
99 water (SDW) to obtain a $1x10^7$ colony forming unit (cfu) ml^{-1} suspension.

100 Seeds of *Lycopersicum esculentum* belonging to the Italian heirloom cv. Costoluto
101 fiorentino were disinfected by immersion in a 0.7% NaClO solution kept on a rotary shaker
102 for 5 min at room temperature (RT) (Appenroth et al. 2006). To be inoculated with the
103 bacterial strain, seeds were washed three times with SDW and dried under a laminar hood
104 before being transferred to Petri dishes containing the bacterial suspension previously
105 prepared, and let at RT for 1h. In order to determine the final concentration of
106 bacteria/seed, five seeds were placed in 4.5 ml of saline solution (0.8% NaCl) and kept on
107 a rotary shaker for 30 minutes. The suspension was serially diluted and aliquots collected
108 from each dilution were spread on PPMD agar medium amended with 100 ppm rifampicin.
109 Plates were then incubated for 48 h at 25 ± 2°C. At the end of the incubation period,
110 bacterial colonies were counted and the number of cfu/seed was calculated. This
111 procedure was replicated five times.

112 PGPB-inoculated seeds were then placed in a 12 x 10 x 10 cm plastic pots filled with
113 commercial soil (previously autoclaved two times at 121°C x 30 min). Non-inoculated
114 seeds were also disinfected with the same procedure used for those inoculated, washed
115 with SDW and placed in plastic pots containing the same commercial soil used for PGPB-
116 inoculated seeds. All pots, each containing one seed, were placed in a growth chamber at
117 23 ± 2 °C under a photosynthetic photon flux density (PPFD) of 500 μmol photons m^{-2} s^{-1}

118 during the day (14 h) cycle and watered daily with distilled water to soil water capacity. To
119 maintain a constant and effective concentration in the soil, *P. chlororaphis* was added
120 once again to the PGPB-inoculated plants one month after seeding. The soil around each
121 plant stem was drenched with 5 ml of a suspension containing $1x10^7$ cfu ml^{-1}, whereas 5
122 ml of SDW were added to the non-inoculated plants.
123 Two months after emergence, 30 plants (15 inoculated and 15 non-inoculated) were
124 water-stressed by withholding water, while other 30 plants (15 inoculated and 15 non-
125 inoculated) were well-watered during the whole experiment. The intensity of water
126 deprivation was evaluated daily by monitoring the soil water content (SWC %) with a soil
127 water sensor (WET-1, Eijkelkamp Agrisearch Equipment BV Giesbeek, the Netherlands).
128 A constant and gradual rate of soil water deprivation (SWC decreasing daily by about 10%
129 compared to the value measured the previous day) was ensured by adding water to the
130 pots drying more rapidly. The water stress lasted 9 days and ended when SWC reached ~
131 50% of its initial value, producing a water stress that can be defined of mild intensity, as
132 also assessed by plant relative water content (RWC) (Ximenéz et al. 2015; Su et al. 2016)
133 (Table 1). The four treatments will be referred as: well-watered non-inoculated (CTRL),
134 well-watered inoculated (B), water-stressed non-inoculated (WS) and water-stressed
135 inoculated (B + WS).
136 At the end of the water stress, the concentration of *P. chlororaphis* on tomato roots was
137 determined through dilution plating method on PPMD agar medium amended with 100
138 ppm of rifampicin and 200 ppm of cycloheximide. In particular, the concentration of *P.*
139 *chlororaphis* was determined as the average number of cfu gr^{-1} of roots of three replicated
140 samples per treatment and accounted for $4.1x10^3$ g^{-1} of roots in well-watered plants, and
141 $3.9x10^4$ g^{-1} of roots of water-stressed plants.
142

143 *Plant biometrics, gas exchange and chlorophyll fluorescence measurements*

144 Before applying water stress, 10 inoculated and 10 non-inoculated tomato plants were
145 harvested to measure the main biometrical parameters: number of leaves and internodes
146 per plant, length of internodes (cm), leaf relative water content (RWC %), plant dry weight
147 (gr) and partitioning between above- and below-ground dry weight (%).
148 Before and after water stress application, gas exchange was measured in fully expanded
149 mature leaves with a portable system equipped with a fluorimeter (LI-COR 6400, LI-COR
150 Biosciences Inc., NE, USA). Leaves were clamped in the 2 cm^2 LI-COR cuvette and
151 exposed to PPFD of 500 µmol photons m^{-2} s^{-1}, temperature of 25°C, [CO_2] of 400 ppm
152 (achieved by fully scrubbing CO_2 from ambient air with soda lime and replacing it with the
153 LI-COR 6400 CO_2 injector system) and relative humidity (RH) ranging between 40 - 50%.
154 Instantaneous photosynthesis (A), stomatal conductance (gs), intercellular CO_2
155 concentration (Ci) and transpiration (Tr) were measured 10–15 min after reaching steady-
156 state conditions, as shown by von Caemmerer and Farquhar (1981). Water Use Efficiency
157 (WUE) was calculated as WUE = A / Tr. Photosynthetic response to Ci, and the rate of
158 CO_2-saturated photosynthesis (A_{sat}), were measured by increasing CO_2 concentration
159 step-wise from 0 to 1200 ppm with the LI-COR 6400 controller (Sharkey et al. 2007).
160 Leaf fluorescence was also measured in dark-adapted leaves to calculate the ratio
161 between variable and maximal fluorescence (Fv/Fm) which represents the maximum
162 quantum yield of photosynthesis.
163

164 *RNA extraction, cDNA synthesis and Real-Time RT-PCR*

165 At the end of the experiment, mature leaf samples were collected, immediately frozen in
166 liquid nitrogen and stored at -80°C for molecular analysis. RNA was extracted according to
167 Chang et al. (1993). Genomic DNA was removed using the Turbo DNA-free^{TM} reagent
168 (Ambion, Austin, TX, USA) following the manufacturer's instructions. Absence of genomic
169 DNA was verified by one-step reverse-transcription PCR (RT-PCR) with primers specific

for the tomato elongation factor (Table S1). cDNA synthesis was performed using the SuperScript II® Reverse Transcriptase and 800 ng of total RNA, following the supplier's protocol (Invitrogen Ltd, Paisley, UK). At the end of the reaction, cDNA was diluted 1:10 for quantitative gene expression analysis (RT-qPCR). Oligonucleotide sequences are listed in Table S1. RT-qPCR was performed with the Rotor-Gene Q (Qiagen) apparatus. The reactions were carried out in a final volume of 15 µl with 7.5 µl of Rotor-Gene™ SYBR® Green Master Mix, 5.5 µl of a mix of forward and reverse primers (diluiting 16 µl of each primer at 10 µM stock concentration in 168 µl of water) and 2 µl of cDNA (diluted 1:10). RT-qPCR cycling program consisted of 10 min/95 °C holding step followed by 40 cycles of two steps (15 s/95 °C and 1 min/60 °C). Expression of target transcripts was quantified after normalization to the geometric mean of the endogenous control genes, tomato elongation factor and ubiquitin (*LeEF* and *LeUBI*). Gene expression was calculated as expression ratios (relative quantity) with respect to controls. All reactions were performed with three biological and two technical replicates.

Determination of proline, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) content and antioxidant (superoxide dismutase and catalase) activity

Extraction and determination of proline were performed on the same leaf samples collected for molecular analysis, according to Bates et al. (1973) with some modifications. Briefly, leaf samples (20 mg) were extracted with ethanol:water (70:30, v/v). Extracts were held for 20 min at 95° C, with 1 ml of ninhydrin reagent: 1 % ninhydrin (w/v) in glacial acetic acid 60 % (v/v), ethanol 20 % (v/v). Proline content was measured with a spectrophotometer (Perkin Elmer, MA, USA) at 520 nm and calculated against a proline standard curve (5-2-1-0.5-0.2 mM of proline in 40:60 ethanol:water, 40:60 v/v). Data were expressed as µmol g^{-1} fresh weight (FW).

195 Endogenous H_2O_2 content was determined according to the method of Velikova et al.
196 (2000), modified for a micro-plate reader. Frozen leaf powder (0.25 g) was homogenized in
197 an ice bath with 1 ml 0.1 % (w:v) TCA. The homogenate was centrifuged at 12 000 x g for
198 15 min at 4 °C. Aliquots of 100 µl from each tube were placed in 96-well plates and 50 µl
199 of 10 mM potassium phosphate buffer (pH 7.0) and 100 µl of 1 M KI were added to each
200 well. The plate was briefly vortexed, incubated at RT for 30 min and the absorbance
201 readings were taken at 390 nm. The relative absorbance (calculated as the sample
202 absorbance subtracted from the absorbance of the same supernatant aliquot without KI)
203 was used to determine the content against a H_2O_2 standard curve, prepared by dilution of
204 reagent grade, 30% H_2O_2 (Sigma-Aldrich, Milano, Italy). Previously, the concentration of
205 H_2O_2 in the standard solutions was calibrated using absorbance at 240 nm and an
206 extinction coefficient of 43.6 M cm^{-1}. Data were expressed as µmol g^{-1} FW.
207 As for the quantification of lipid peroxidation in tomato leaves, the thiobarbituric acid (TBA)
208 test, which determines malondialdehyde (MDA) as an end-product of lipid peroxidation,
209 was used. Frozen leaf powder (0.25 g) was homogenized in 1 ml 0.1 % (w:v) TCA
210 solution. The homogenate was centrifuged at 12000 x g for 15 min and 0.5 ml of
211 supernatant was added to 1 ml 0.5 % (w:v) TBA in 20 % TCA. The mixture was incubated
212 at 95° C for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath.
213 Tubes were briefly centrifuged at 10000 x g for 10 min, then 200 µl aliquots from each tube
214 were placed in 96-well plates, and the absorbance of supernatant was read at 532 nm in a
215 micro-plate reader. The absorbance at 600 and 440 nm of the same aliquot of leaf sample
216 without TBA was subtracted to avoid overestimation of MDA. The amount of MDA–TBA
217 complex (red pigment) was calculated using an extinction coefficient of 155 mM cm^{-1}, and
218 expressed as nmol MDA g^{-1} FW.
219 Enzymes were extracted from 50 mg of leaves after homogenization in 100 µl of extraction
220 buffer [cold 50 mM K-phosphate buffer (pH 7) containing 1 mM ethylenediamine tetra

221 acetic acid (EDTA), 1% (w/v) polyvinyl-pyrrolidone (PVP) and 0.1% (v/v) Triton X- 100] and
222 centrifuged at 4°C for 15 min at 15000 x g. Protein concentration in the extract was
223 determined according to Bradford (1976). Bovine serum albumin was used to generate a
224 standard curve. After protein assay, samples were stored at -20°C until use.
225 Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the
226 inhibition of photochemical reduction of nitro blue tetrazolium (NBT) as described by
227 Giannopolitis and Ries (1977). The reaction mixture (3 ml) contained 50 mM potassium
228 phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA
229 and 0.1 ml of enzyme extract. The reaction took place in a chamber under illumination of a
230 30 W fluorescent lamp at 25°C for 15 min before switching off the light. The control
231 reaction mixture had no enzyme extract. The blank solution had the same complete
232 reaction mixture, but was kept in the dark. One unit of SOD activity was defined as the
233 amount of enzyme required to cause 50% inhibition of the reduction of NBT in 1 min. Data
234 were expressed as U g^{-1} FW.
235 Catalase (E.C.1.11.1.6) activity was evaluated by monitoring the disappearance of H_2O_2 at
236 240 nm (extinction coefficient of 0.036 $mM\,cm^{-1}$) (Aebi 1984) in a reaction mixture
237 containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 20 µl sample supernatant.
238 One unit of activity decomposed 1 µmol of H_2O_2 at pH 7.0 at 25°C in 1 min. Data were
239 expressed as U g^{-1} FW.
240

241 *Determination of the Indol-3-Acetic Acid (IAA) and Abscisic Acid (ABA) levels*

242 The level of the free forms of both IAA and ABA were analysed on the same leaf samples
243 collected for molecular analysis and antioxidant enzyme activity according to Ludwig-
244 Müller et al. (2008). Approximately 0.02 g DW of lyophilized leaf material were extracted
245 with 1 ml isopropanol:acetic acid (95:5, v/v), to which 100 ng each of $^{13}C_6$-IAA and $^{2}H_6$-
246 ABA (OlChemIm Ltd., Olomouc, Czech Republic) were added as internal standards for

247 quantitative mass-spectral analysis. After overnight isotope equilibration at 4°C, the
248 samples were centrifuged for 10 min at 10000 x g, the supernatants were collected and,
249 after a double re-extraction of the pellet with 500 µl extraction solution, were evaporated to
250 dryness with a rotary evaporator. The residues were taken up with 300 µl methanol and
251 methylated using diazomethane, then dried under a gentle N_2 gas stream (Baraldi et al.
252 1988; Biondi et al. 1997). The samples were finally resuspended in 30 µl ethyl acetate and
253 2µl were injected into a GC-MS system (7890A-5975C, Agilent Technologies, US) in
254 splitless mode onto a HP1 capillary column (length 60 m, inner diameter 0.25 mm; film
255 thickness 0.25 µm, Agilent Technologies, US). Helium was employed as a carrier gas and
256 provided at a flow rate of 1 ml min^{-1}, GC injector was set at 280°C and the oven
257 temperature was increased from 90 to 200°C at a rate of 20°C min^{-1}, then at a rate of 8°C
258 min^{-1} until 280°C, followed by 4 min isotermically at 280°C. The source temperature was
259 set at 230°C and ionizing voltage was 70 eV. Ions monitored were: m/z 130, 136 for the
260 base peak (quinolinium ion) and m/z 189, 195 for the molecular ion of the methyl-IAA and
261 the methyl-$^{13}C_6$-IAA, respectively; m/z 190, 194 for the base peak and m/z 162, 166 for the
262 molecular ion of the methyl-ABA and methyl-$^{2}H_6$-ABA, respectively. For absolute
263 quantification, the endogenous hormone levels were estimated from the corresponding
264 peak area by calculating the ratios between m/z 130/136 and m/z 189/195 for IAA, and
265 m/z 190/194 and 162/166 for ABA, according to the principles of isotope dilution (Cohen et
266 al. 1986). The amounts of free IAA and ABA were calculated from three replicated
267 measurements.

268

269 *Analysis of VOC emitted by Pseudomonas chlororaphis subsp. aureofaciens strain M71*

270 *P. chlororaphis* subsp. *aureofaciens* strain M71 was initially revitalized by streaking it onto
271 Luria Broth (LB) agar medium, and then incubated at 25 ± 2°C for 48 h. The fresh bacterial
272 culture was used to prepare a $1x10^9$ cfu ml^{-1} suspension, part of which (500 µl) was added

273 to different 500 ml flasks each containing 50 ml of LB. Flasks were incubated at 25 ± 2°C
274 and production of VOC from *P. chlororaphis* was analysed *in vivo* by sampling the
275 headspace of the flasks 24 h and 48 h after beginning the incubation period. A Proton
276 Transfer Reaction - Mass Spectrometer (PTR-MS) (Ionicon, Innsbruck, Austria), which
277 enables real-time detection of VOC with very low fragmentation and high sensitivity, was
278 used. In PTR-MS analysis, VOC are detected through chemical ionization between
279 molecules of $H3O^+$ produced at high density within an ion source and the VOC present
280 into the air samples, according to their proton affinity. Constant conditions of pressure (=
281 2.2 mbar), temperature (= 50°C) and electrical field (600 v cm^{-2}) were used, resulting in an
282 ionization energy E/N = 130 Td (Lindinger et al. 1998). PTR-MS was operating in 'Scan'
283 mode to screen for all the protonated ions related to VOC (i.e. $VOC\text{-}H^+$) spanning over a
284 range of 200 molecular weight (from m/z 20 to 220). At least four cycles were completed
285 for each measurement, while replacing the sampled air in the flask containing *P.*
286 *chlororaphis* at a rate of 100 ml min^{-1} with the same amount of VOC-filtered air. The
287 background of VOC in the headspace of flasks containing LB without bacteria were also
288 measured, and then subtracted to the measurements taken from flasks containing the *P.*
289 *chlororaphis* strain. All the four cycles recorded within one measurement were added
290 together and each measurement was replicated three times. Measurement uncertainty
291 was calculated as percentage of standard error on the averaged values of the
292 measurements taken 24h and 48h after *P. chlororaphis* incubation.
293
294 *Statistics*
295 Data were subjected to statistical analysis by applying a one-way ANOVA. The Tukey's
296 post-hoc test was used to compare means when ANOVA results were statistically
297 significant ($P < 0.5$) (Systat software Inc. USA). Consistently with previous investigations

298 (Mayak et al. 2003; Sandhya et al. 2010; Bresson et al. 2013; Timmusk et al. 2014; Khan
299 et al. 2015) all data were collected from one independent experiment of water stress.

300 **Results**

301

302 *Characterization of well-watered tomato plants following colonization with P. chlororaphis*

303 Treatment of tomato seeds with *P. chlororaphis* did not affect the percentage of
304 germination which was similar in both inoculated and non-inoculated seeds (~ 79 and ~ 73
305 %, respectively). Biometric characterization of well-watered tomatoes showed that
306 inoculation with *P. chlororaphis* slightly increased biomass accumulation (dry weight) and
307 led to significantly higher number of leaves and longer internodes than in non-inoculated
308 plants (Table 1). However, root colonization by *P. chlororaphis* did not alter photosynthesis
309 (Fig. 1a), stomata opening (Fig. 1b) and sub-stomatal CO_2 concentration (Ci) (Fig. 1c),
310 resulting in similar values of water use efficiency (WUE) (Fig. 1d) as in well-watered non-
311 inoculated tomato leaves. The rate of saturated photosynthesis (A_{Sat}) and the response of
312 photosynthesis to Ci were also similar in inoculated and non-inoculated well-watered
313 tomato plants (Fig. 2a; Table 2), suggesting an unaltered activity of ribulose-1,5-
314 bisphosphate carboxylase/oxygenase (RUBISCO). Steady efficiency of light use was
315 confirmed by measurements of chlorophyll fluorescence in dark-adapted leaves (Fv/Fm) of
316 inoculated and non-inoculated tomato plants (Table 2).

317 Root colonization by *P. chlororaphis* induced large modifications of plant hormones (Fig.
318 3a, b). The leaf content of both IAA and ABA was enhanced, and the ABA/IAA ratio was
319 higher (although not significantly) in inoculated than in non-inoculated plants (Table 3).
320 Despite leaf transcript levels of the ABA-biosynthetic gene *LeNCED1* did not show
321 significant differences in inoculated and non-inoculated plants (Fig. 3c), *P. chlororaphis*
322 affected the methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate
323 (MEP/DOXP) pathway by up-regulating three different monoterpene synthases genes
324 previously reported to be expressed in fully expanded leaves (Falara et al. 2011): TPS5
325 (*LeMTS1*), TPS12 *(LeTPS12)* and TPS20 (*LePHS1*) (Fig. 4a, b, c). The germacrene C

326 synthase gene (*LeGCS*), which encodes a protein involved in the biosynthesis of
327 sesquiterpenes (de Kraker et al. 1998), was also significantly up-regulated in leaves of
328 inoculated tomatoes compared to non-inoculated plants thus highlighting enhanced
329 activation of the mevalonate-farnesyl diphosphate-germacradiene (MEV) pathway by *P.*
330 *chlororaphis* (Fig. 4d).

331 Activation of defense responses in tomato plants following inoculation with *P. chlororaphis*
332 was further investigated at molecular level. Among the genes typically involved in plant
333 defense, two genes encoding lipoxygenase enzymes (*LeLOXC* and *LeLOXD*) expressed
334 in response to cell membrane damage were considered. *LeLOXC* was up-regulated (Fig.
335 5a) and *LeLOXD* was significantly down-regulated in leaves of *P. chlororaphis*-inoculated
336 tomatoes compared to non-inoculated plants (Fig. 5b). On the other hand, the expression
337 of the hydroperoxide lyase gene (*LeHPL*), also induced by the mechanical damage of cell
338 membranes, and of a gene encoding for a phenylalanine ammonia lyase (*LePAL5*), which
339 is putatively involved in SA synthesis, was not affected by the colonization with *P.*
340 *chlororaphis* (Fig. 5c, d). Moreover, the expression of a gene coding for the 1-
341 amynocyclopropane-1-carboxylic acid oxidase (*LeACO4*) involved in ethylene biosynthesis
342 and of a gene (*LeTAS14*) encoding for a dehydrin (DHN) were both unaffected by
343 inoculation with *P. chlororaphis* (Fig. 5e, f).

344 Root colonization by *P. chlororaphis* led to a simultaneous increase in the activity of SOD
345 (Fig. 6a) and CAT (Fig. 6b), and to proline accumulation in tomato leaves (Fig. 6c).
346 Nevertheless, both the content of H_2O_2, (Fig. 6d) and that of MDA (Fig. 6d, e) did not vary
347 in *P. chlororaphis*-inoculated with respect to non-inoculated well-watered tomato plants.

348 In a separate experiment, the blend of VOC emitted from *P. chlororaphis* subsp.
349 aureofaciens M71 strain that might have a role during the interaction with the roots of
350 tomato plants was investigated. After a 24 h incubation, *P. chlororaphis* emitted a mixture

351 of VOC mainly composed of methanethiol (~ 83%) and hydrogen cyanide (~ 7%) and, after
352 48 h, *P. chlororaphis* started to release also dimethyl sulfide (~ 5.4%) (Table S2).

353

354 *Performances of P. chlororaphis-inoculated and non-inoculated tomato plants under water*
355 *stress*

356 The water stress applied in our experiment was mild as SWC was reduced to ~ 50% of its
357 initial value (Ximenéz et al. 2015; Su et al. 2016). However, our cultivar of tomato was
358 highly sensitive to water stress. The leaf relative water content (RWC) decreased by ~
359 25% (Table 1) and the stomatal conductance was largely reduced (Fig. 1b) both in non-
360 inoculated and inoculated plants, compared to well-watered ones. Low stomatal
361 conductance decreased the intercellular CO_2 concentration (Fig. 1c), consequently limiting
362 photosynthesis (Fig. 1a) and improving WUE (Fig. 1d) in both water stressed inoculated
363 and non-inoculated tomato plants, compared to well-watered ones. Nevertheless, neither
364 biochemical nor photochemical impairments were found in *P. chlororaphis* inoculated and
365 non-inoculated water stressed tomato leaves. In fact, similar maximum quantum yield of
366 chlorophyll fluorescence in dark-adapted leaves, and CO_2-saturated photosynthetic rate
367 (Table 2), and similar response to increasing CO_2 concentration (Fig. 2) were measured in
368 water-stressed and well-watered leaves. Interestingly, WUE increased more in *P.*
369 *chlororaphis*-inoculated than in non-inoculated water-stressed plants (Fig. 1d). A higher
370 biomass accumulation (more leaves and longer internodes) was found in *P. chlororaphis*-
371 inoculated than in non-inoculated plants during the water stress period (Table 1).
372 Reduced stomatal conductance of water-stressed tomato leaves was associated to a
373 slightly, not significantly higher concentration of ABA compared to well-watered leaves in
374 both inoculated and non-inoculated plants (Fig. 3b). The ABA/IAA ratio was also higher in
375 water-stressed than in well-watered tomatoes, and this ratio significantly increased in
376 plants that were also inoculated (Table 3).

377 The transcriptional level of *LeNCED1*, involved in the biosynthesis of (the non-volatile
378 isoprenoid-) ABA, increased in water-stressed leaves, although this trend resulted
379 statistically significant only in leaves not inoculated with *P. chlororaphis* (Fig. 3c). The
380 expression levels of genes involved in volatile terpenes biosynthesis (*LeMTS1*, *LeTPS12*,
381 *LePHS1* and *LeGCS*) were generally lower in water-stressed than in well-watered tomato
382 leaves, especially in non-inoculated ones (Fig. 4). In addition, the most evident response
383 to water stress was the increase of the transcriptional levels of *LeTAS14* (a tomato
384 dehydrin encoding gene). The increase of *LeTAS14* was significantly higher in non-
385 inoculated than in *P. chlororaphis*-inoculated water-stressed plants (Fig. 5f). The
386 transcription of the other investigated genes involved in the activation of defense
387 mechanisms was either unchanged or down-regulated as a consequence of water stress
388 (Fig. 5).

389 Upon water stress, the concentration of the osmolyte proline increased significantly in non-
390 inoculated leaves, and even more in *P. chlororaphis*-inoculated ones (Fig. 6c). Moreover,
391 both the concentration of H_2O_2 (Fig. 6d) and MDA (Fig. 6e) increased following water
392 stress, resulting higher in non-inoculated than in *P. chlororaphis*-inoculated plants. As
393 already observed in well-watered plants, inoculation with *P. chlororaphis* further enhanced
394 the activity of both SOD and CAT as compared to non-inoculated, water-stressed tomato
395 leaves (Fig. 6a, b).

396 ## Discussion

397

398 *Pseudomonas chlororaphis* strain M71 is a particularly effective biocontrol agent against
399 biotic stresses (Weller, 2007), especially pathogenic fungi in plants (Raio et al. 2011, 2017;
400 Puopolo et al. 2013), including tomatoes (Puopolo et al. 2011; Chin-A-Woeng et al. 2000).
401 In the present study, we tested whether the physiological, molecular and metabolic
402 changes induced by root colonization with *P. chlororaphis* are able to prime tomato plants
403 for enhanced tolerance to water stress of a mild intensity, a condition that recurrently
404 occurs during the growing season.
405 Our results highlights that *P. chlororaphis* produces a large impact on the secondary
406 metabolism of well-watered tomato plants. In particular, inoculation with *P. chlororaphis*
407 induced changes in the hormonal balance between an inhibitor (ABA) and a promoter
408 (IAA) of growth. Past research has already shown that both IAA (Marulanda et al. 2009;
409 Contesto et al. 2010; Spaepen et al. 2008) and ABA (Cohen et al. 2009; Porcel et al.
410 2014) contents increase in plants inoculated with PGPB. Alteration of either IAA content or
411 ABA/IAA ratio might also affect meristematic activity and cell division rate (Noda et al.
412 2000; Mwange et al. 2005), thus contributing to the observed development of more leaves
413 and longer internodes in *P. chlororaphis*-inoculated than non-inoculated tomato plants.
414 Despite ABA content was enhanced in tomato leaves by *P. chlororaphis* inoculation, the
415 expression of *LeNCED1*, a gene involved in ABA biosynthesis (Burbidge et al. 1999),
416 remained as low as in non-inoculated plants. However, regulation of ABA in plants
417 depends on biosynthesis, catabolism, transport, and compartmentalization in multiple
418 pools both in shoot and roots (Hirayama et al. 2007; McAdam et al. 2016). Moreover, the
419 foliar ABA pool is likely formed from the MEP/DOXP pathway that also originates volatile
420 terpenes (Barta and Loreto 2006). A higher expression of the genes involved in
421 monoterpene biosynthesis suggests that the entire MEP/DOXP pathway (also producing

422 ABA) is activated as a consequence of *P. chlororaphis* inoculation. In fact, *P. chlororaphis*
423 induced in tomato the expression of genes coding for both MEP/DOXP and MEV
424 pathways, and most likely enhanced the biosynthesis of constitutive volatile leaf mono-
425 and sesqui-terpenes (Degenhart et al. 2009; Vranovà et al. 2013) that are involved in
426 defense mechanisms against abiotic and biotic stresses (Loreto and Schnitzler 2010).
427 Consistently with results obtained using other plant-PGPB combinations (Sandhya et al.
428 2010; Kaushal and Wani, 2016), root colonization of tomato plants with *P. chlororaphis*
429 also boosted the synthesis of osmolytes, such as proline in leaves (Theocharis et al.
430 2012), and enhanced the activity of the main leaf antioxidant enzymes (SOD and CAT)
431 without inducing a concurrent increase of proxies for oxidative stress occurrence (H_2O_2)
432 and membrane denaturation (MDA). This indicates that, in well-watered plants, *P.*
433 *chlororaphis* primes the ROS-scavenging capacity of tomatoes, as raising antioxidant
434 capacity helps both to maintain low ROS levels for signaling purposes, and to rapidly
435 respond the need to detoxify the excess of ROS produced under stress conditions (Pastor
436 et al. 2013).
437 We did not find a specific improvement in photosynthesis efficiency in mature leaves
438 attributable to inoculation with *P. chlororaphis* (Xu et al. 2010; Dai, 2013). Indeed, none of
439 the indicators of photosynthetic performances changed in *P. chlororaphis*-inoculated
440 tomatoes, as photosynthesis in RUBISCO-limited (low CO_2) or ETR-limited (high CO_2)
441 conditions was similar to that of non-inoculated plants. Other studies reported a stimulation
442 of photosynthesis in PGPB-inoculated plants (Peng et al. 2002; Zhang et al. 2008; Rolli et
443 al. 2015). However, these studies used different plant species and PGPB strains than
444 those employed in our experiment (Jones et al. 2009). Moreover, our measurements were
445 carried out only on leaves at a mature stage, and we cannot exclude the possibility that the
446 photosynthetic process was temporarily stimulated by *P. chlororaphis*, for example only in
447 young leaves during early growth stage (Stefan et al. 2013). Past research highlighted an

448 improvement of plant productivity following PGPB inoculation. These reports were based
449 on destructive measurements that spanned an extended growth period and integrated
450 photosynthesis at different growth stages (Mayak et al. 2004; Sandhya et al. 2010; Rubin
451 et al. 2017). Thus, our instantaneous measurements of photosynthesis may have not
452 captured the integrated benefit of plant-PGPB interaction.
453 Overall, all the metabolic changes induced by colonization with *P. chlororaphis* did not
454 involve any fitness cost for the well-watered plants (van Hulten et al. 2006), and they can
455 be considered as part of the 'priming phase' that prepare tomato plants for enhanced
456 tolerance upon further occurrence of a stress (Mauch-Mani et al. 2017).
457
458 Indeed, our results showed that primed *P. chlororaphis*-inoculated tomatoes displayed a
459 higher growth and resulted more tolerant to a mild water stress than non-inoculated plants.
460 Consistently with a recent meta-analysis (Rubin et al. 2017), our biometric parameters
461 showed a statistically significant increase in biomass accumulation of *P. chlororaphis*-
462 inoculated tomato plants compared to non-inoculated plants under water stress conditions
463 (Table 1). Despite the high sensitivity of tomato plants to water stress, it should be noted
464 that the mild conditions applied in our experiment did not affect photosynthesis *per se* (Fig.
465 2). In fact, the observed large reduction of photosynthetic CO_2 assimilation rates (Fig. 1)
466 were only caused by increasing diffusive limitations (and thus probably fully reversible) at
467 stomatal and leaf mesophyll scale (Flexas et al. 2004), as no changes both in the light use
468 efficiency and in the CO_2-saturated photosynthetic rate occurred (Table 2).
469 The increase of ABA/IAA ratio induced by *P. chlororaphis* inoculation was more effective in
470 water stressed than in well-watered tomatoes (Table 3). Unfortunately, we were not able to
471 disentangle whether such ABA/IAA increase in inoculated tomatoes was influenced by a
472 direct contribution of ABA and IAA made by *P. chlororaphis*. The ability of IAA to
473 antagonize ABA-induced stomatal closure has already been recognized (Snaith and

474 Mansfield, 1982; Dodd, 2003). The highest ABA/IAA ratio induced by *P. chlororaphis* can
475 explain why stomata closed to a higher extent in water-stressed inoculated than in non-
476 inoculated leaves. Indeed, the increased level of ABA following inoculation with *P.*
477 *chlororaphis* allowed stomata to be more responsive to water stress and this improved the
478 WUE, confirming previous results (Mayak et al. 2004; Timmusk et al. 2014; Cohen et al.
479 2015). Nevertheless, the large drop in stomatal conductance measured in both *P.*
480 *chlororaphis*-inoculated and non-inoculated tomatoes following a ~ 50% decrease in soil
481 water content underlined the ‘water spending’ strategy of tomato (Levitt 1980) which, like
482 other herbaceous plant species, is unable to gradually adjust stomata aperture as water
483 decreases in soil (Brilli et al. 2011). Therefore, an enhanced WUE induced by *P.*
484 *chlororaphis* inoculation represents, especially in tomatoes, an important ecological
485 advantage (Chaves et al. 2002; Flexas et al. 2004) allowing tolerance to water stress
486 (Dimka et al. 2009). At molecular level, the expression of *LeNCED1*, involved in ABA
487 biosynthesis (Burbidge et al. 1999), was up-regulated in both *P. chlororaphis*-inoculated
488 and non-inoculated leaves under water stress. This was in agreement with the fact that an
489 increase of ABA, although non-significant, was observed in both inoculated and non-
490 inoculated leaves of water-stressed tomatoes (Fig. 3).
491 As water stress led to a significantly lower accumulation of H_2O_2 and MDA in inoculated
492 than in non-inoculated tomatoes, it is suggested that priming of *P. chlororaphis* enabled a
493 fine tuning of intercellular ROS concentration (Mittler, 2002; Baxter et al. 2017). Indeed,
494 during water stress, *P. chlororaphis* further stimulated a higher synthesis of proline than in
495 non-inoculated tomatoes, and further enhanced the antioxidant enzymes (i.e. SOD and
496 CAT) that prevented ROS accumulation (Balmer et al. 2015). Maintenance of low ROS
497 levels avoids the initiation of programmed cell death (PCD) and, on the other hand, allows
498 ROS to exert signaling functions and activate other stress responses (Knight et al. 2001),
499 which can effectively improve tolerance of *P. chlororaphis*-inoculated tomatoes to more

500 severe water stress. At molecular level, inoculation of with *P. chlororaphis* determined in
501 water stressed tomatoes a significant down-regulation of *LeTAS14*, a gene encoding for
502 DHN proteins that are usually up-regulated by drought conditions (Supranova et al. 2004).
503 This further confirms that *P. chlororaphis*-inoculated tomatoes experienced a less intense
504 water stress than non-inoculated ones.
505
506 Finally, we confirmed that *P. chlororaphis* actively produce a complex blend of VOC (Kai et
507 al. 2009; Farag et al. 2013) that can act as chemical stimuli to prime plants defenses (Ryu
508 et al. 2004; Han et al. 2006). In particular, a previous study has demonstrated that *P.*
509 *chlororaphis* can induce systemic tolerance to drought in *Arabidopsis thaliana* through the
510 production of 2R,3R-butanediol (Cho et al. 2008). Our analysis showed that *P.*
511 *chlororaphis* can emit a changing blend of VOC mainly consisting of methanethiol, while
512 only a very small percentage of 2R,3R-butanediol was found (Table S2). However, since
513 VOC emission from bacteria is strongly influenced by the growing media, inoculum
514 quantity and species (Blom et al. 2011), the blend of VOC produced by *P. chlororaphis*
515 grown in pure culture may be different than that released during plant-bacterium
516 interaction (Kanchiswamy et al. 2015; Kai et al. 2016). Whether a specific bacterial VOC
517 has a role in priming or eliciting defensive pathways in plants should be tested with more
518 dedicated experiments. Nevertheless, our results highlighted the capacity of *P.*
519 *chlororaphis* to emit a wide range of VOC that might facilitate colonization of different host
520 plant species under various environmental conditions.
521
522 **Conclusions**
523 We demonstrated that *P. chlororaphis* provides tomato plants a broad-spectrum defense
524 response also effective against water stress. Our results showed that root colonization with
525 *P. chlororaphis* induce a priming phase in tomatoes mainly characterized by: a) a systemic

526 alteration of the ABA/IAA phytohormonal balance that strongly affect plant shape and size;
527 b) an activation of genes involved in both the MEV- and MEP-pathways; c) regulation of
528 ROS homeostasis through stimulation of the antioxidant enzymes activity. Moreover, we
529 highlighted that tomatoes primed with *P. chlororaphis* were able to modulate ROS
530 signaling and to improve WUE, enhancing biomass accumulation during a mild water
531 stress. Future research should be designed to test the effectiveness of both single strain
532 and mixtures of PGPB, also in combination with other beneficial microorganisms naturally
533 present in soil (e.g. mychorrizal fungi), to induce protection in plants exposed to multiple
534 (abiotic and biotic) stressors in field conditions.
535

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541

542 **Author contribution statement**

543 BEM and AR conceived and designed the research. FB, SP, AR, RB, LN, PB, AP, BEM
544 conducted experiments and analysed the data. FB, FL and RB wrote the manuscript. All
545 the authors read and approved the manuscript.

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Table

	Soil water content (SWC) $m^3\ m^{-3}$	Leaf Relative Water Content (RWC) %	Dry weight (gr/plant)	Partitioning above/below ground dry weight (%)	n° internodes	Length internodes (cm)	n° leaves / plant
CTRL	0.488 ±0.011^b	82.4 ± 4.3^b	1.71 ± 0.08^c	74.3 / 25.7	10.2±0.4^c	43.4±1.6^b	87.0±1.3^c
B	0.511 ±0.011^b	87.4 ± 3.2^b	1.81 ± 0.06^c	75.1 / 24.9	10.6±0.4^c	52.2±2.3^c	97.1±1.1^d
WS	0.231 ±0.058^a	63.2 ± 5.7^a	0.87 ± 0.09^b	75.1 / 24.9	8.5±0.3^a	32.3±1.8^a	61.3±2.3^a
WS + B	0.188 ±0.005^a	65.9 ± 2.5^a	1.04 ± 0.11^a	73.2 / 26.8	9.6±0.2^b	45.1±1.2^b	73.9±2.4^b

Table 1 – Soil water content and plant biometric data in non-inoculated and PGPB-inoculated tomato plants before (CTRL and B) and after (WS, WS+B) the application of water stress. Values are means ± standard errors; lower case letters indicate significant differences between treatments (n=10; $P < 0.05$).

	Fv/Fm	A/Ci fitting curve $f(x) = y_0 + ax/(b + x)$	A_{sat}
CTRL	0.763±0.020[a]	y_0 = -11.01±1.28[a] a = 27.65±1.01[a] b = 123.73±17.67[a]	14.22±0.21[a]
B	0.764±0.020[a]	y_0 = -12.02±1.76[a] a = 29.38±1.40[a] b = 115.77±20.31[a]	14.08±0.99[a]
WS	0.791±0.020[a]	y_0 = -10.61±1.43[a] a = 26.94±1.10[a] b = 135.12±22.70[a]	13.07±1.03[a]
WS + B	0.783±0.040[a]	y_0 = -11.81±1.19[a] a = 29.52±0.92[a] b = 127.02±15.51[a]	14.08±0.85[a]

Table 2 – Maximum quantum yield of chlorophyll fluorescence in dark adapted leaves (Fv/Fm), A/Ci fitting parameters and CO_2-saturated photosynthetic rate (A_{sat}) in non-inoculated and PGPB-inoculated tomato plants before (CTRL and B) and after (WS, WS+B) the application of water stress. Values are means ± standard errors; lower case letters indicate significant differences between treatments (n=4; $P < 0.05$;).

	ABA / IAA
CTRL	1.60 ± 0.18[a]
B	2.30 ± 0.30[a]
WS	2.10 ± 0.16[a]
WS + B	3.30 ± 0.15[b]

Table 3 - Ratio between means values of ABA and IAA contents (pmol gFW^{-1}) measured in well-watered non-inoculated (CTRL) and PGPB-inoculated (B), in water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. Values are means ± standard errors; lower case letters indicate significant differences between treatments (n=3; $P < 0.05$).

Supporting information

Table S1 - List of the oligonucleotides used for RT-qPCR analyses.

Name	Primer sequence (5'-3')	Target Gene	Reference
LeEF_F	CTCCATTGGGTCGTTTTGCT	Elongation Factor (*EF*)	Digilio et al. 2010
LeEF_R	GGTCACCTTGGCACCAGTTG		
LeUbi_F	CTTGTTGGGGTAATCCTCAG	Ubiquitin (*UBI*)	Weiss and Egea-Cortines 2009
LeUBi_R	ACGAGAGAACACAAAGCACA		
LeGCS_F	TTGGTGAAGCCTTAACTCAGCC	Germacrene C synthase (*GCS*)	Corrado et al. 2007
LeGCS_R	GCAAATGGTGGTGTGCATCAT		
LeLoxC_F	TTGCCTATGGTGCTGAATGGA	Lipoxygenase C (*LOXC*)	Corrado et al. 2007
LeLoxC_R	TTGCCTATGGTGCTGAATGGA		
LeLoxD_F	TTCATGGCCGTGGTTGACA	Lipoxygenase D (*LOXD*)	Corrado et al. 2007
LeLoxD_R	AACAATCTCTGCATCTCCGG		
LeHPL_F	CCAATCGCGGATCGATTAGAC	Fatty acid hydroperoxide lyase (*HPL*)	Corrado et al. 2007
LeHPL_R	GGCACGTTCGTTCTGAAAACC		
LeACO4_F	TTCGCGCTCACACGGATGCT	1-Amynocyclopropane-1-carboxylic acid oxidase (ACO4)	Porcel et al. 2004
LeACO4_R	CACCTCTAGCTGATCGCCGAGG		
LePAL5_F	AATGGGTGCTAATGGCGAACT	Phenylalanine ammonia-lyase (*PAL5*)	This work
LePAL5_R	CTGCAGGGGTCATCAGCATAG		
LePHS1_F	AAGGAAATCTTGGAAATGAATAGAA	Terpene synthase (*TPS20/PHS1*)	Schilmiller et al. 2009
LePHS1_R	ATAGAAGGAAAGAACAAAAGTCATAA		
LeTPS12_F	CCCAATGGTTAAACAATGATAATC	Terpene synthase (*TPS12*)	Falara et al. 2011
LeTPS12_R	ATCATATAGCCAGCACTTACCATC		
LeMTS1_F	TTTGGGGACATCTTCGGATGAA	Terpene synthase (*TPS5/MTS1*)	van Schie et al. 2007
LeMTS1_R	CTACTCGAGTTACTTGAGAGCGAATGCAAC		
LeTas14_F	CAATACGGCAATCAAGACCAAA	Dehydrin TAS14 (Solyc02g084850.2.1)	Chitarra et al. 2016
LeTas14_R	TGTTTCTTGGACATGGTTTCCA		
LeNCED1_F	ACCCACGAGTCCAGATTTC	9-cis-epoxycarotenoid (NCED1)	Lopez-Raez et al. 2010
LeNCED1_R	GGTTCAAAAAGAGGGTTAGC		

Table S2 – Percentages of single protonated ions related to VOC (or VOC fragments) out of the total sum of protonated ions measured 24 h and 48 h after flask incubation of P. chlororaphis subsp. aureofaciens M71 strain (± percentage uncertainty).

Time / ***m/z (likely compound)***	**After 24 hours**	**After 48 hours**
28 (HCN)	6.7 (± 18.6)	2.4 (± 1.9)
33 (methanol)	0.2 (± 2.4)	0.5 (± 15.2)
43 (protonated fragments i.e. acetic acid)	0.2 (± 27.3)	1.0 (± 31.5)
47 (formic acid/ethanol)	0.3 (± 30.5)	0.3 (± 33.6)
49 (methanethiol)	82.7 (± 22.7)	77.8 (± 29.3)
51 (???)	3.8 (± 23.1)	3.7 (± 31.3)
57 (1-butanol fragment, 2-Methylpropan-2-ol fragment)	---	1.1 (± 29.7)
59 (Acetone)	---	1.1 (± 36.4)
71 (butanoic acid, isoamyl alchol fragment)	---	0.2 (± 41.0)
74 (???)	2.3 (± 48.4)	0.6 (± 0.4)
79 (???)	---	0.4 (± 50.5)
83 (hexenals)	---	0.5 (± 31.2)
91 (diethylsulfide, thioacetic acid methyl ester, 2,3 butanediol)	1.0 (± 40.5)	1.5 (± 36.9)
93 (toluene)	---	0.1 (± 53.5)
95 (dimethyl disulfide)	0.4 (± 39.5)	5.4 (± 49.4)
97 (2,4 – hexadienal, furfural)	---	0.5 (± 52.3)
Other minor VOCs-H+	2.3 (± 0.7)	2.7 (± 0.8)

Figure
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Captions to figures

Figure 1 – Photosynthesis (A, panel **a**), stomatal conductance (gs, **b**), intercellular CO_2 concentration (Ci, **c**) and water use efficiency (WUE, **d**) of well-watered non-inoculated (CTRL) and PGPB-inoculated (B) and of water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. Means $\pm$ standard errors are shown, and lower case letters indicate significant differences between means (n=19 in well-watered and n=9 under water stress conditions; $P < 0.05$).

Figure 2 - Photosynthesis response to increasing intercellular CO_2 concentrations in non-inoculated (black circles) and PGPB-inoculated (white circles) tomato plants. Best -fit curves are shown by solid lines for non-inoculated and by dashed lines for PGPB-inoculated well-watered (**a**), and water-stressed leaves (**b**). Means $\pm$ standard errors (n=4) are shown.

Figure 3 - IAA (**a**) and ABA (**b**) contents in well-watered non-inoculated (CTRL) and PGPB-inoculated (B), and in water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. In panel (**c**) the change of expression of the ABA biosynthetic gene *LeNCED1* is reported in the four treatments as shown above. Means $\pm$ standard errors are shown, and lower case letters indicate significant differences between means (n=3; $P < 0.05$).

Figure 4 - Expression of genes involved in monoterpene *LeMTS1* (**a**), *LeTPS12* (**b**), *LePHS1* (**c**), and sesquiterpenes synthase *LeGCS* (**d**), in well-watered non-inoculated (CTRL) and PGPB-inoculated (B), and in water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. Means $\pm$ standard errors are shown and lower case letters indicate significant differences between means (n=3; $P < 0.05$).

Figure 5 - Expression of stress-dependent genes *LeLOXC* (**a**), *LeLOXD* (**b**), *LePAL5* (**c**), *LeHPL* (**d**), *LeACO4* (**e**) and *LeTAS14* (**f**) in leaves of well-watered non-inoculated (CTRL) and PGPB-inoculated (B), and in water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. Means $\pm$ standard errors are shown and lower case letters indicate significant differences between means (n=3; $P < 0.05$).

Figure 6 – Activity of SOD (superoxide dismutase) (**a**), and CAT (catalase) (**b**), and concentration of proline (**c**), H_2O_2 (**d**) and MDA (**e**) in well-watered non-inoculated (CTRL) and PGPB-inoculated (B), and in water-stressed non-inoculated (WS) and PGPB-inoculated (WS+B) tomato plants. Means $\pm$ standard errors are shown and lower case letters indicate significant differences between means (n=3; $P < 0.05$).

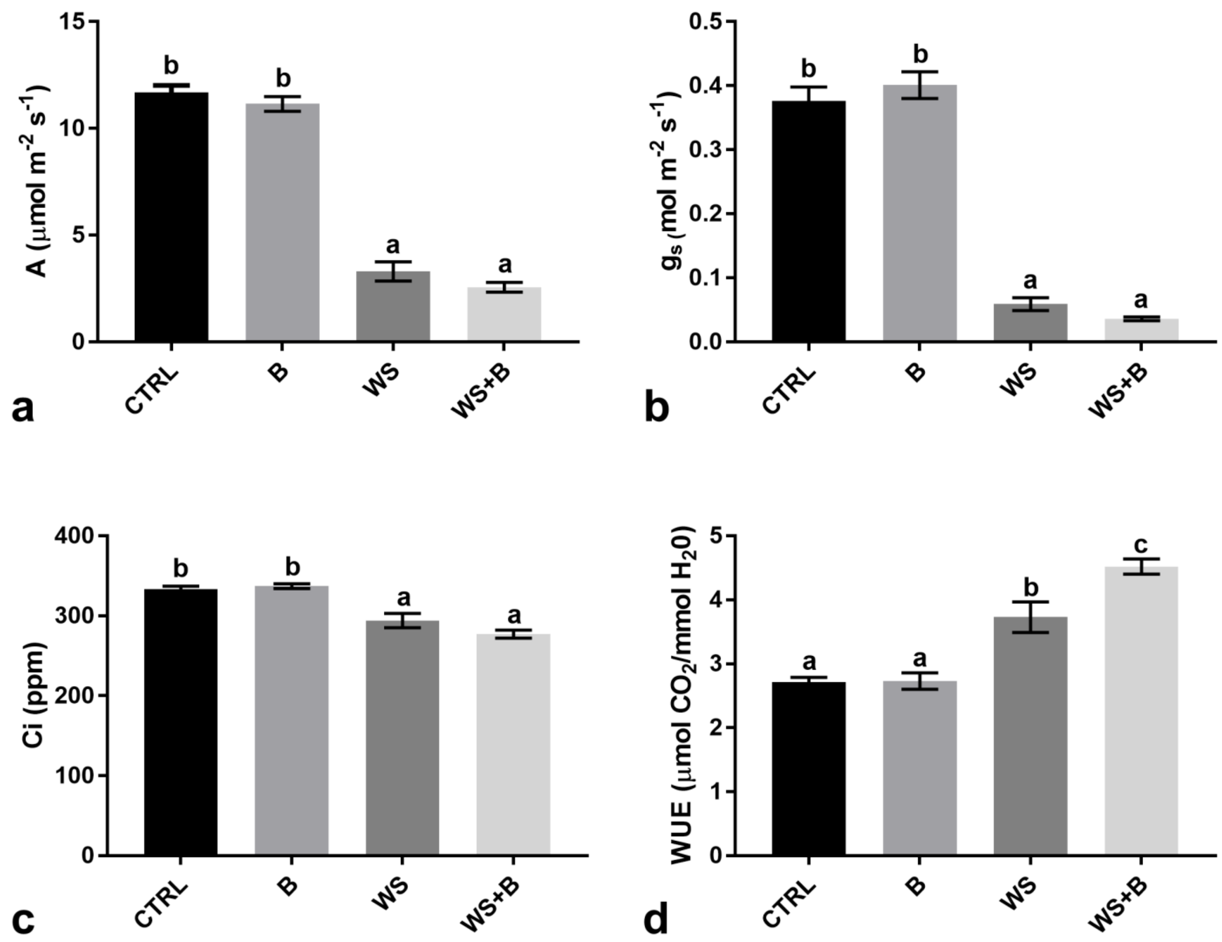
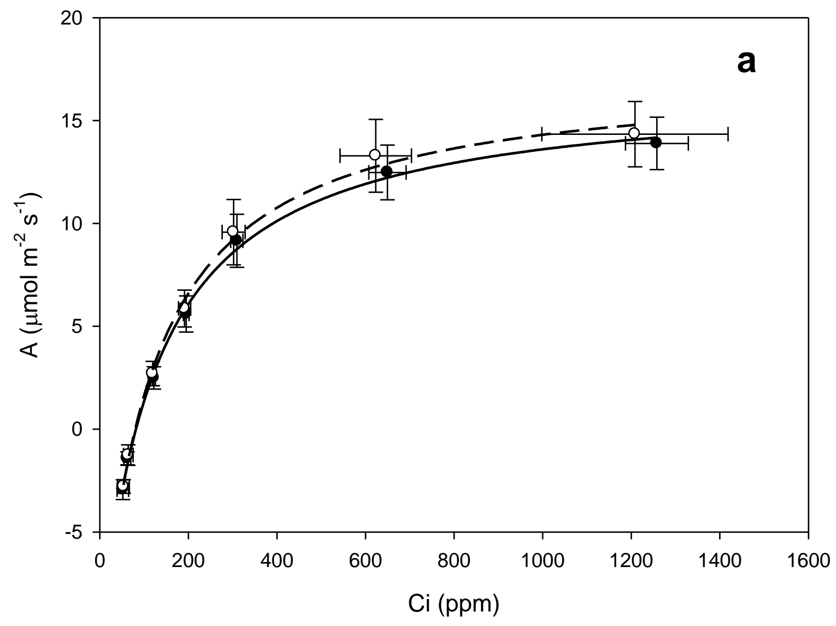


Figure 1



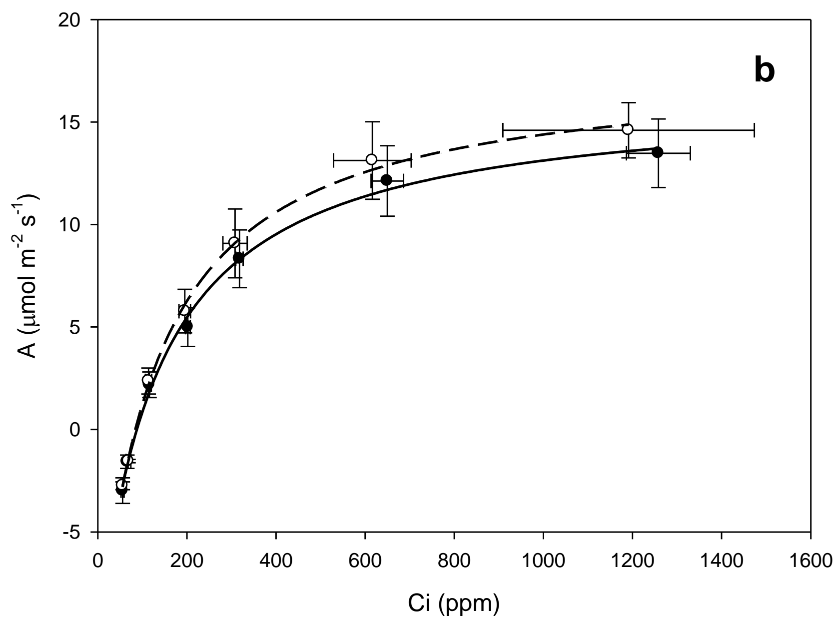


Figure 2

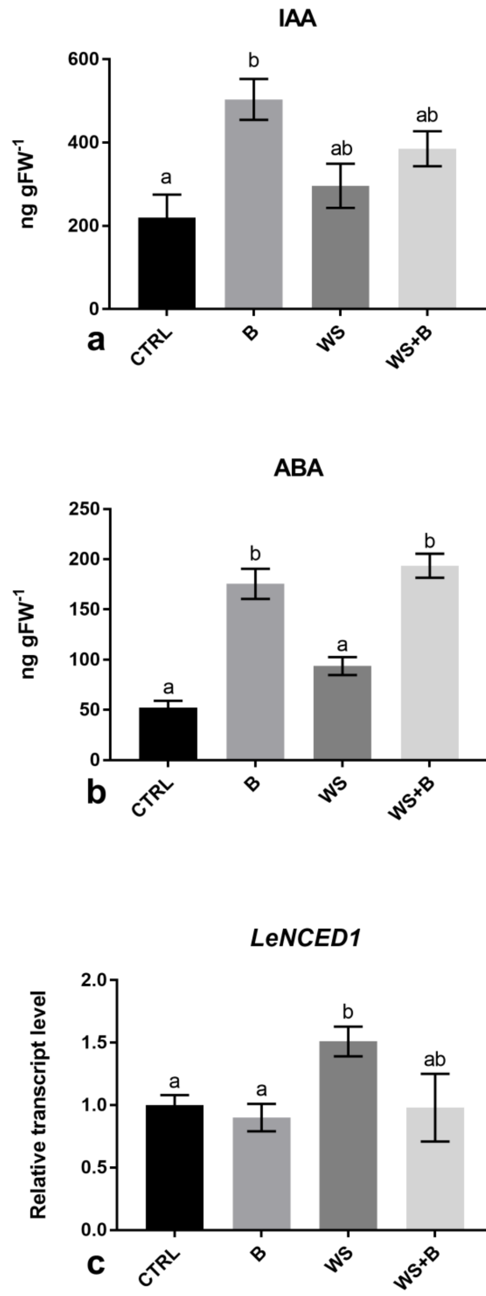


Figure 3

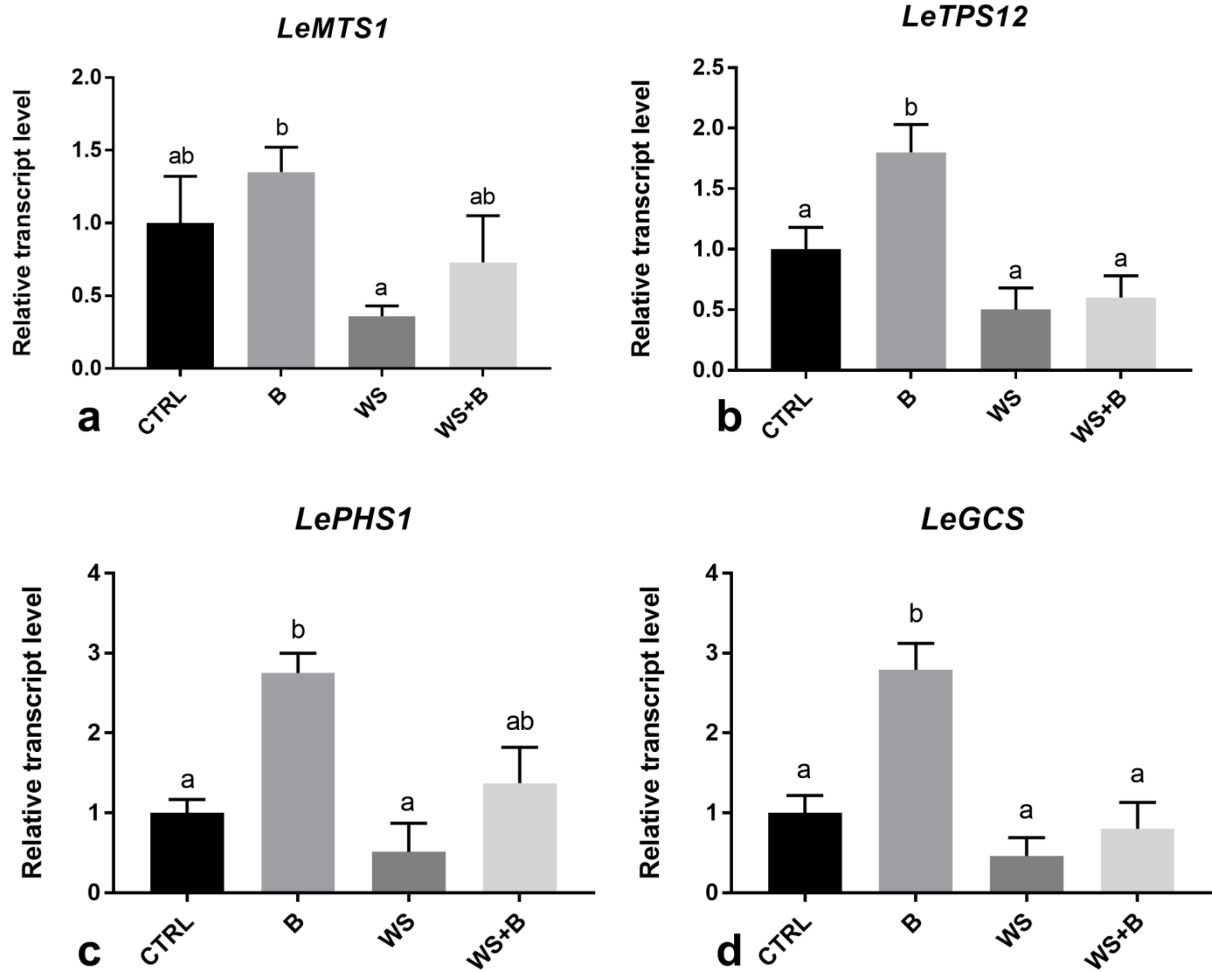


Figure 4

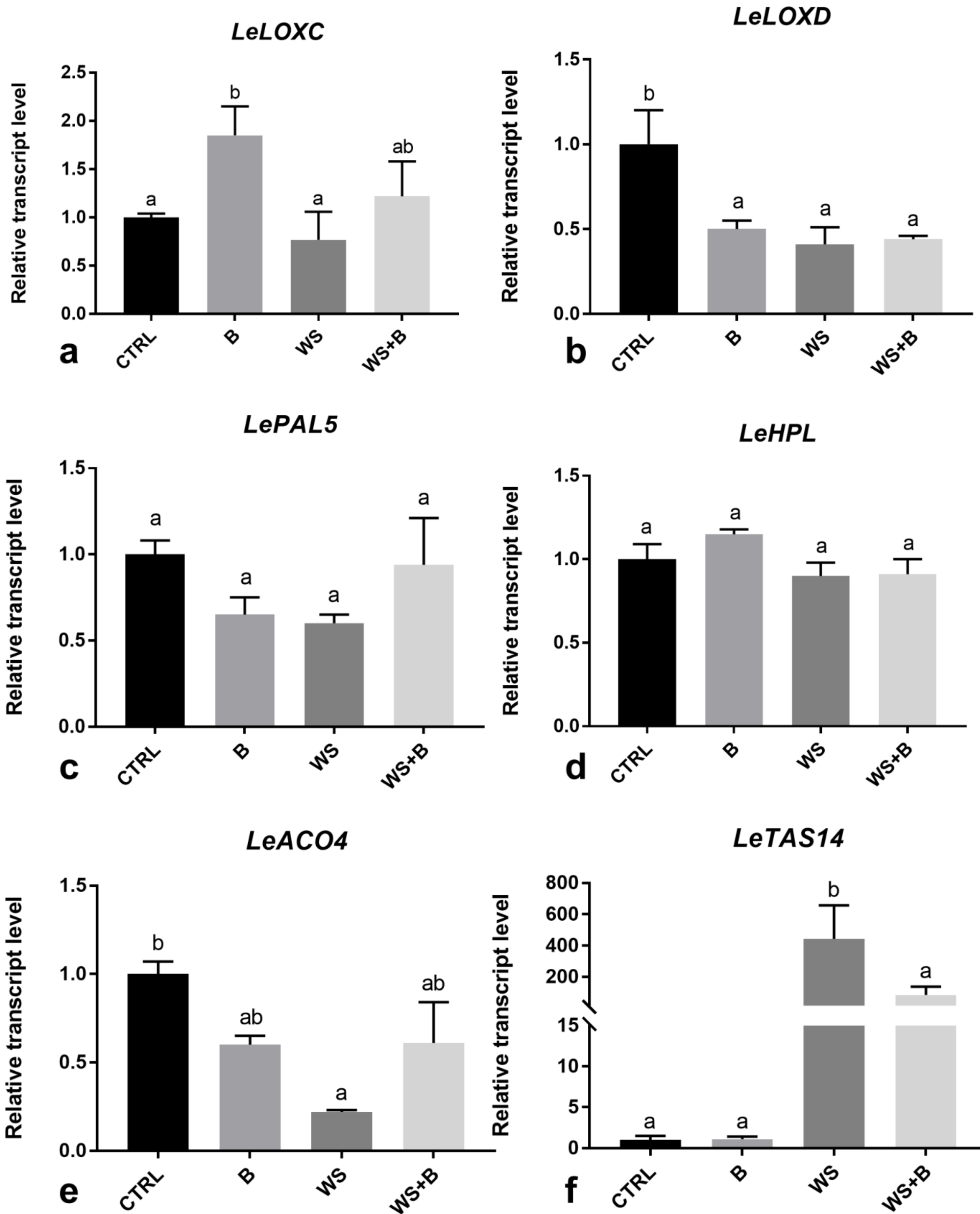


Figure 5

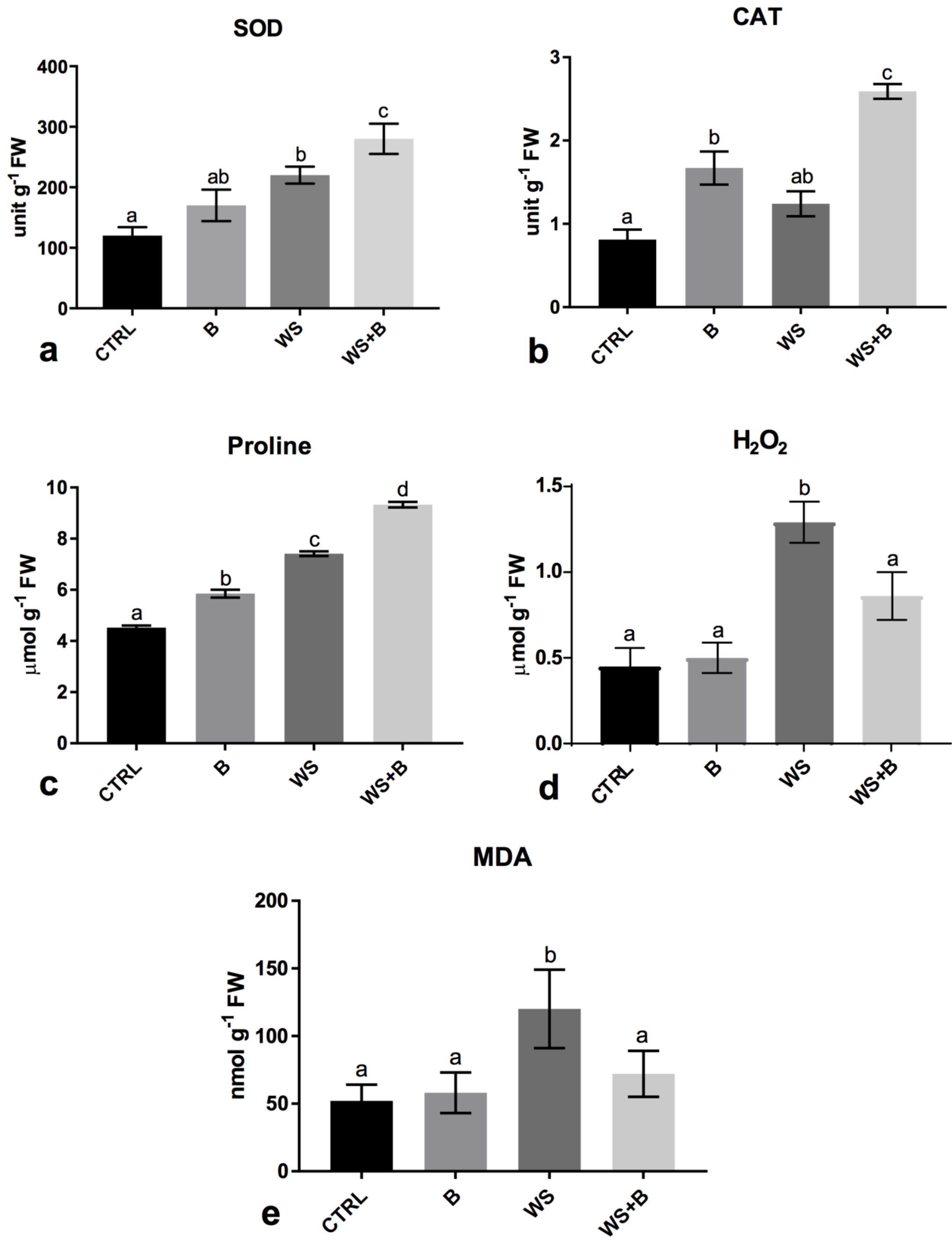


Figure 6