Impact of irrigation water deficit on two tomato genotypes grown under open field conditions: From the root-associated microbiota to the stress responses

Fabiano Sillo,[1] Giovanni Marino,[1] Elisabetta Franchi,[2] Matthew Haworth,[1] Elisa Zampieri,[1] Ilaria Pietrini,[2] Danilo Fusini,[2] Carmelo Mennone,[3] Mauro Centritto,[1,4] Raffaella Balestrini[1,4]

[1]Institute for Sustainable Plant Protection - National Research Council of Italy (CNR), Torino and Sesto Fiorentino (FI), Italy; [2]ENI S.p.A., R&D Environmental & Biological Laboratories, S. Donato Milanese (MI), Italy; [3]ALSIA Polo di Pantanello, Bernalda (MT), Italy; [4]ENI-CNR Water Research Center 'Hypatia of Alexandria', Research Center Metapontum Agrobios, Bernalda (MT), Italy

Highlights

- *Two tomato genotypes were studied under water deficit in a pilot field trial.*
- *The two genotypes responded differently to water stress from eco-physiological and transcriptomic points of view.*
- *The two genotypes recruited diverse root-associated microbiota, particularly under water deficit.*

Correspondence: Raffaella Balestrini, Institute for Sustainable Plant Protection - National Research Council of Italy (CNR), Strada delle Cacce 73, Torino, Italy. E-mail: raffaella.balestrini@ipsp.cnr.it

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See online Appendices for additional Figures and Tables.

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Abstract

In the context of the climate change scenario in the Mediterranean, natural root-microorganism associations have an impact on the resilience and productivity of crops, and the exploitation of these interactions represents innovative, cost-effective and sustainable crop adaptation strategies. An open field experiment with two commercial Italian tomato cultivars was performed. The soil bacterial communities associated with the two commercial Italian tomato genotypes were characterized alongside their physiological and molecular responses under well-watered and moderate water deficit (100% and 75% of crop evapotranspiration) treatments. The two genotypes showed contrasting responses to water deficit, primarily through diverse rhizosphere microbiota recruitment under the two irrigation treatments.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically valuable food crops worldwide (Costa and Heuvelink, 2018). Tomato is an excellent species for both basic and applied crop research, and also a model system for fruit development (Quinet *et al*., 2019) due to its completely sequenced genome (The Tomato Genome Consortium, 2012) and routine transformation technology and genetic tools (Tomato Genetics Resource Center, 2019). Europe is one of the most important global tomato producers, where more than 85% of tomatoes are cultivated outdoors, mainly in Mediterranean countries (Costa and Heuvelink, 2018). Tomatoes grown in open fields are exposed to climatic conditions, where increased drought and heat waves are expected to have a dramatic negative impact (Cramer *et al*., 2018). Tomato is drought-sensitive, experiencing reduced yields during water deficit (Kissoudis *et al*., 2015). Previous research has endeavoured

to identify stress-tolerant varieties (Landi *et al*., 2017). Nonetheless, despite the efforts of researchers and breeders, the development of new genotypes adapted to stress has not been achieved, probably due to the high complexity of the traits involved (Landi *et al*., 2017; Costa *et al*., 2019). Furthermore, the degree of tolerance to water deficit stress of a crop is not simply a function of plant genotype, but also influenced by the presence and the interaction with associated microorganisms in the soil rhizosphere (Calleja-Cabrera *et al*., 2020). Natural root-associated microorganisms affect the resilience and productivity of crops, and the exploitation of these associations represents an innovative, cost-effective, sustainable strategy to enhance climate-resilience (de Vries *et al*., 2020). Despite the importance of these microbial communities to plant growth, information on the mechanisms driving microbiome assembly and composition remains unclear, as well as the interaction of these microbial communities with different plant genotypes (Cheng *et al*., 2020). Improving knowledge of community composition and species diversity of rhizosphere microbiomes associated with different plant genotypes is crucial to maintain a healthy rhizosphere environment and improve plant health and productivity (Lei *et al*., 2019).

The development of next-generation sequencing (NGS) technology has facilitated analysis of the influence on plant health of the composition and assembly of the soil rhizosphere alongside root-associated microbiota dynamics. The composition and assembly of rhizosphere microorganisms is generally influenced by plant species, temporal variation and environmental effects (Dodds and Rathjen, 2010). Several papers have recently focused on the identification of microbiota associated with tomato roots in the rhizosphere (Chialva *et al*., 2018; Chialva *et al*., 2019; Cordovez *et al*., 2021). Cheng *et al*. (2020) performed a comprehensive assessment of community structure and composition, and the variation of the tomato rhizosphere and root-associated microbiota in different genotypes and soil environments, especially soils with amended nutrient levels. These authors confirmed that different genotypes varied in their tendency to shape the microbiome, selectively enriching the microbiome with specific microorganisms (Cheng *et al*., 2020). A metatranscriptomic approach was used on roots collected from tomato plants grown on different native soils to reveal the taxonomic and functional diversity of the associated microbiota (Chialva *et al*., 2019). Cordovez *et al*. (2021) have provided information regarding shaping of the rhizosphere microbiome by tomato genotypes. Analysis of amplicon sequence variants showed that genera belonging to *Acidovorax*, *Massilia* and *Rhizobium* were enriched in the late successional rhizosphere microbiome associated with the wild tomato genotype, while the rhizosphere of a modern tomato cultivar was enriched in the genus *Pseudomonas*. It is worth noting that the loss of *Firmicutes* and *Actinobacteria* in the rhizosphere of the modern tomato cultivar promotes incidence of bacterial wilt disease (Lee *et al*., 2021). Drought also affects root microbiome composition, mainly increasing the relative abundance of *Actinobacteria*. These microorganisms can grow under different environmental stress conditions and can have an important role in alleviating damage caused by abiotic stress and promoting plant growth (Sandrini *et al*., 2022 and references therein). Nevertheless, the rhizosphere microbial response to drought varies between host plant species. Interestingly, host-specific changes in the relative abundance of endosphere *Streptomyces* have been associated with enhanced host drought tolerance (Fitzpatrick *et al*., 2017).

As soil water availability declines, plants generally reduce stomatal conductance (g_s) to decrease transpiration water-loss. Stomatal closure increases the diffusive resistance to CO_2 uptake (Centritto *et al*, 2003), leading to a decline in photosynthesis (P_N) that is evident in gas exchange and chlorophyll fluorescence measurements (Killi *et al*., 2017; Marino *et al*., 2020). Hydraulic and chemical root-to-shoot signals have been proposed to play a role in this drought response (Davies *et al*., 2000; Brunetti *et al*., 2020), and in modifying plant photosynthetic and stomatal sensitivity to fluctuating growth conditions to optimize water use efficiency (Gerardin *et al*., 2018; Durand *et al*., 2019). Inoculation with specific plant growth promoting rhizobacteria can affect the drought response of plants (Yang *et al*., 2009; Brunetti *et al*., 2021). Understanding the effect of these rhizosphere microbes on the drought response of plants and the interaction between different plant genotypes with rhizosphere microbial communities can contribute to the development of climate resilient sustainable crop production.

An integrated approach to examine plant physiological and root-rhizosphere responses to water deficit in two contrasting tomato genotypes was used to: i) assess the physiological response of the tomato varieties to water deficit; ii) quantify differences in the root rhizosphere microbial communities to tomato genotype and differences in soil water availability; iii) identify potential interactions between root rhizosphere composition and the physiological response of the tomato genotypes to water deficit; and iv) highlight potential exploitation of root-rhizosphere microbial communities in developing more climate resilient field-based tomato cultivation.

Materials and methods

Experimental design

The experiment was conducted under field conditions in Metaponto at the '*Azienda Pantanello*' (Basilicata, Southern Italy) in Summer 2020. One-month old tomato plantlets (two varieties: *Solanum lycopersicum, cv*. Impact F1 and *cv*. Contact F1, hereafter named Tondo (round) and Lungo (long), respectively) were bought from a local nursery and planted in the field. Sixteen plots (6×3 m in size, 36 plants × plot, plant density=2 plants/m^2, distance 1 m × 30 cm) were spatially arranged following a 2×2 simple factorial design with 4 replicates, to test two irrigation treatments and two genotypes. The experiment was conducted from July to September 2020 (6 July 2020, sowing - 5 September 2020, last fruit harvesting), and two different irrigation treatments were considered: control (R1=irrigated with 100% of the estimated crop evapotranspiration) and limited water supply (R2=75% of the control treatment). All plants were initially watered adequately to avoid water stress until the plants were well established. From the 1st of August, the two irrigation treatments were applied until the end of the experiment. A drip irrigation system was adopted for water supply (5 drip irrigators *per* m^2). Weather was monitored and recorded using a meteorological station. Rainfall, temperature (min-max) and relative humidity were registered during the three months of the trial and were reported in Figure 1. The irrigation treatments were performed using the following method: the daily calculation of the tomato crop evapotranspiration (ETc), according the formula ETc=ET0 × Kc, where ET0 is the reference evapotranspiration according to Penman-Monteith's equation derived from the daily weather data and Kc is the crop coefficient for the tomato crop, which was adjusted for the environmental conditions and crop growth stage (Allen *et al*., 1998) as follows: 0.6 until 35 days after transplant (DAT), 1.15 between 36 and 70 DAT, and 0.7 from 71

DAT till the last fruit harvest. The sum of daily ETc, excluding the useful rainfall, was equal to 40% of the maximum available water in the 0-40 cm soil depth, where most of roots are expected to grow.

The four treatments considered: Tondo cv, well-watered (R1-T) or subjected to a limited water supply (R2-T); Lungo cv, well-watered (R1-L) or subjected to a limited water supply (R2-L). Throughout the experiment, plants received standard fertilization for commercial tomato production typical of the region every ten days. In particular, the total amount of total applied NPK fertilization (corresponding to 170-120-150 kg ha^{-1} respectively) was split into three rates during the crop cycle: 30% at transplanting (as ammonium sulphate), 20% at 30 and 50 days after transplanting (as urea phosphate 18-44 for fertigation), 30% at full fruit set and 20% during fruit development (as 20.20.20 fertilizer). Plant protection was performed according to the cultivation protocols of the Basilicata Region (Italy), using different commercial products at the recommended doses on the product labels, in particular: Abamectin, Exitiazox, Azoxystrobin, Emamectin benzoate and *Bacillus amyloliquefaciens*.

Twenty-five days after the imposition of water stress in R2 (last irrigation: 08/20/2020), the response of the plants was assessed by eco-physiological measurements, and soil and roots samples were collected for the identification of the soil microbial communities associated with the different treatments. Leaves were also collected, instantly frozen in liquid N_2 after sampling and then stored at –80°C for molecular analyses of stress marker genes.

Meteorological data from a weather station positioned at the Azienda Agricola Pantanello were also collected to follow the climatic conditions during the crop growth (Figure 1A and B). At the end of the experiment, product quantity was evaluated by summing up the two harvests (Figure 2 and Table S1). Some fruits were also collected to assess production and to test genes involved in fruit quality (Christou *et al*., 2019). Additionally, pooled soils from the four treatments (R1-T, R2-T, R1-L and R2-L) were sent to the Agri-Bio-Eco Laboratori Riuniti (Pomezia, RM, Italy) for standard chemical-physical analyses in addition to a sample of soil collected from the field adjacent to the plots, to evaluate the natural soil characteristics independent of the crop cultivation (Table S2).

Eco-physiological measurements

Leaf gas exchange and chlorophyll fluorescence

Eco-physiological measurements were performed during the fruit

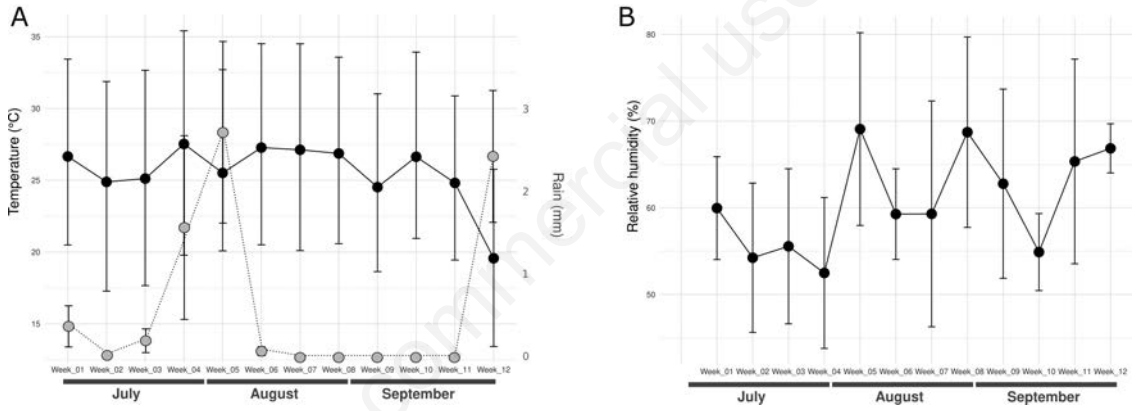

Figure 1. Meteorological data. A) Rain (dashed line, grey dots) and average (max-min) temperature (black line, black dots) reported for each week in the three months (July, August and September) of the experiment. B) Relative humidity reported for the three months of the experiment.

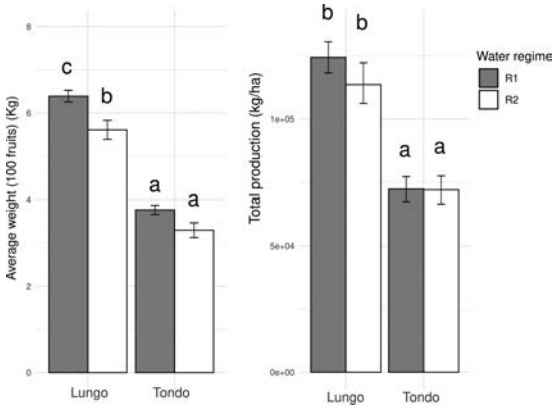

Figure 2. Production data in terms of average total production (kg/ha ± standard error) and average weight of 100 fruits (kg ± standard error), *per* water treatment (R1, R2) and genotype (Lungo, Tondo). Letters are plotted in the barplots according to outcomes of Tukey's test. Tondo, Impact F1; Lungo, Contact F1.

development stages and irrigation was stopped during the measurement campaign. Point measurements of simultaneous leaf gas exchange and chlorophyll fluorescence (ChlF) parameters were performed on one fully expanded tomato leaf per plot in full sunlight between 09:00 and 11:00 a.m. using a LiCor Li6400XT fitted with a 6400-40 2 cm^2 leaf cuvette (Li-Cor, Inc., Nebraska, USA). Conditions in the leaf cuvette were: photosynthetic photon flux density (PPFD) of 2000 µmol m^{-2} s^{-1}, leaf temperature of 35°C, [CO_2] of 400 µmol mol^{-1} and relative humidity of 60%. Leaves were placed in the cuvette and allowed to acclimatize to the conditions for 20 min before gas exchange and ChlF parameters were recorded. The multi-phase fluorescence setting was used with an initial saturating pulse of 8000 µmol m^{-2} s^{-1} to determine the actual quantum efficiency of photosystem II (ΦPSII) (Genty *et al*., 1989; Loriaux *et al*., 2013):

$$\Phi PSII = \frac{F_m' - F_s}{F_m'} \quad (1)$$

where F_m' is the maximal fluorescence and F_s is the steady state fluorescence under light adapted conditions.

Dark-adapted chlorophyll fluorescence

Chlorophyll fluorescence was performed on the uppermost fully expanded leaf of six tomato plants per plot. A Hansatech Handy-PEA (plant efficiency analyser) fluorimeter (Hansatech, King's Lynn, UK) was used for transient analysis of chlorophyll a fluorescence. Leaves were dark adapted for 30-minutes and then exposed to a saturating light pulse (intensity >3500 µmol m^{-2} s^{-1}, excitation light of 650 nm) (Strasser *et al*., 2004). This resulted in a polyphasic chlorophyll fluorescence transient: O (20-50 µs), J (2 ms), I (30 ms) and P (peak). A summary of the theoretical basis and analysis of OJIP curves is given in Strasser *et al*. (2004) and Kalaji *et al*. (2016). The OJIP curves were analysed using Biolyzer 4 HP v.3 (Bioenergetics Laboratory, University of Geneva, Switzerland). The parameters extrapolated from the OJIP curve are: F_v/F_m, the maximum quantum yield of photosystem II; F_o, minimum fluorescence yield in dark-adapted conditions; F_m, maximum fluorescence yield in dark-adapted conditions; φD_o, quantum yield of energy dissipation (F_o/F_m) at time 0; ΨE_o, the probability that harvested excitation energy is utilised for electron transport to the primary plastoquinone A acceptor of PSII; F_v/F_o, an indicator of the activity of the oxygen evolving complex on the donor side of PSII; ΔV_{IP}, a relative measure of the I to P phase of the chlorophyll a fluorescence transient indicating the efficiency of the electron chain flux through photosystem I (PSI); δR_o, efficiency of electron carriers in reducing end electron acceptors at the PSI acceptor; F_v/F_m, maximum quantum yield of PSII photochemistry; φE_o initial quantum yield of electron transport at time 0; ABS/RC, absorption of chlorophyll antennae per reaction centre; ET_o/RC, the electron flux beyond plastoquinone A per reaction centre; TR_o/RC, the flux of trapped energy per reaction centre leading to the reduction of plastoquinone A; DI_o/RC, the flux of energy dissipated for each reaction centre; RC/CS_o, the density of PSII plastoquinone A reducing reaction centres; φR_o, quantum yield of the reduction of final stage acceptors at the PSI stage; PI_{ABS}, a performance index based on the photochemical and non-photochemical energy absorption of chlorophyll antennae; PI_{TOT}, a performance index incorporating the concentration of reaction centres, the quantum yield of PSII photochemistry, capacity for uptake of electrons between PSII and PSI and the efficiency of electron transfer from reduced intersystem electron acceptors to the final stage PSI electron (Strasser *et al*., 2004).

Stomatal kinetics and photosynthetic response to varying light conditions

The change in g_s and P_N over time in response to changes in PPFD was measured following the protocol of Haworth *et al*. (2018a) on the fully expanded uppermost leaf of one randomly selected tomato plant *per* plot. The leaf was placed in the Li6400XT leaf cuvette under conditions identical to those used for point measurements of photosynthesis for 20 min until steady state g_s and P_N were achieved. The gas exchange parameters were recorded using the 'autolog' function every 10 s for a further 30 min and throughout the subsequent changes in light intensity. The LED lights within the cuvette were then dimmed from PPFD 2000 µmol m^{-2} s^{-1} to 400 µmol m^{-2} s^{-1} for 30 min before PPFD returned from 400 µmol m^{-2} s^{-1} to 2000 µmol m^{-2} s^{-1} for a further 30 min. This measurement shows potential varietal and irrigation differences in photosynthetic and stomatal responses to varying light conditions that may occur during cultivation in heterogeneous environments.

Analysis of foliar chlorophyll, flavonoids and nitrogen

A Dualex (Force-A, Orsay, France) optical leaf-clip sensor was used to non-destructively measure the chlorophyll and polyphenol content of one fully expanded leaf from six tomato plants per plot. The sensor uses the transmittance ratio of far-red and near-infrared light to estimate chlorophyll content on a unit area basis. The screening effect of polyphenols on chlorophyll fluorescence is used to estimate the content of flavonoids on a unit-less scale of 0 to 3. The nitrogen balanced index (NBI) was used to indicate plant nitrogen status as a ratio of chlorophyll to flavonoids within the leaf (Cartelat *et al*., 2005).

Metabarcoding: sampling, DNA extraction and sequencing

Roots were collected from a single plant for each replicate (1 plant × 4 replicates, *i.e.*, 4 samples for treatment). Soil samples were collected from three points in each plot (on a diagonal axis) and pooled to obtain 16 samples, each formed by three subsamples. Then a pool for each treatment (mixed a soil from the 4 replicates) was done to obtain 4 soil samples (R1-T, R2-T, R1-L, R2-L). In detail, 3 ng of the genomic DNA was obtained by extraction of about 500 mg of soil samples through the Fast DNA® Spin Kit for Soil (MP Biomedicals) and about 200 mg of roots through the Fast DNA® Spin Kit (MP Biomedicals; Table S3). DNA was quantified by Qubit® 2.0 fluorometer (Invitrogen) and amplified using the 16S Metagenomics Kit (Thermo Fischer Scientific). The amplification program was set up as follows: 95°C for 10 min, followed by 25 cycles at 95°C per 30 s, 58°C for 30 s, and 72°C for 20 s, a final hold time for 7 min at 72°C and a cooling step at 4°C. Although DNA had been extracted from soil of the four treatments, one sample (R1-L) was then discarded due to amplification problems. The subsequent purification of the amplicons, the preparation, and the sequencing of the libraries followed the standard protocols for the Ion GeneStudio S5 Systems (*i.e.*, Ion Chef ™ System and Ion GeneStudio S5 Sequencer) provided by the manufacturer. The run is based on the workflow Metagenomics 16S w1.1 handling the Database Curated microSEQ®16 S and the reference Library 2013.1. The primers detected both ends to obtain 250 bp sequences. Alignment in Torrent Suite™ Software (version 5.16) was performed using the torrent mapping alignment program (TMAP). The sequences that occurred only once in the entire dataset were removed, and the representative sequences were defined with a 97% similarity cut-off. When a reading is mapped to multiple locations, the mapping with the best mapping score is used. If there is more

than one such mapping, a random mapping with a mapping quality of zero is used. In the output BAM file is recorded the percentage of reads which pass all filters (*i.e.*, enrichment, no template, clonal and polyclonal discrimination, % of test fragments, % of adapter dimer, and % of low quality). After classifying the operational taxonomic unit (OTU) representative sequences, the output has been elaborated to obtain a relative abundance (%) of each OTU in the total amounts of the entire sample. Diversity within samples (α-diversity) was calculated using both Chao1 and Shannon indexes, while diversity among samples (β-diversity) was calculated by Bray Curtis and reported in a two-dimensional principal coordinates analysis (PCoA). The OTU table was used as input for MicrobiomeAnalyst (Chong *et al*., 2020) for visualization and statistical assessment of data. Data were filtered to remove low quality and not informative features, by setting '4' as minimum count of features (10% of prevalence in samples).

Quantitative gene expression analysis of leaves and fruits

Expression changes of the target transcripts were quantified on leaf samples and fruits (at least three independent biological replicates) by RT-qPCR. Three leaves from two plants (total collected leaves for each plot are six, avoiding the youngest and the oldest leaves) within each treatment were pooled to form a biological replicate that was immediately frozen in liquid nitrogen, and stored at –80°C. Five mature fruits were collected in each plot and then a single fruit for each plot was used as single biological replicate. Total RNA was extracted from each biological replicate using the Spectrum Plant Total RNA extraction kit (Sigma-Aldrich) with slight modifications. RNA quantity was checked using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific), RNA samples were then treated with TURBO™ DNase kit (Thermo Fisher Scientific), and genomic DNA contamination was checked before proceeding with cDNA synthesis by one-step RT-PCR using *SlEFα1*-specific primers of tomato (Table S4). First-strand complementary DNA was then synthesized starting from 1 mg of total RNA using the SuperScriptII - Reverse Transcriptase (Invitrogen) procedure following the manufacturer's instructions. Reactions were carried out in the ConnectTM Real-Time PCR Detection System (Bio-Rad Laboratories), and the SYBR Green method (Power SYBR Green PCR Master Mix; Biorad) was used to quantify the amplification results. Thermal cycling conditions were as follows: an initial denaturation phase at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Specific annealing of primers was checked using dissociation kinetics performed at the end of each RT-qPCR run. The expression of tomato target transcripts was quantified after normalization to two reference genes in leaves (elongation factor, *SlEFα1,* and a protein encoding a clathrin adaptor complexes medium subunit/endocytic pathway, *SlCAC*; Expósito-Rodríguez *et al*., 2008), as well as in fruits (a SAND family gene, *SlSAND*, and *SlCAC*, Expósito-Rodríguez *et al*., 2008). Gene expression data were calculated as expression ratios (relative quantity) to control R1 plants for each genotype. Gene-specific primers are listed in Table S4 (Corrado *et al*., 2007; Digilio *et al*., 2010; López-Ráez *et al*., 2010; Porcel *et al*., 2014; Rounis *et al*., 2015; Chitarra *et al*., 2016). Expression of target transcripts was quantified after normalization to the geometric mean of the endogenous control genes previously described. All reactions were performed using three independent biological and two technical replicates. Particularly, transcriptional level in leaves for water stress marker genes coding for a dehydrin (*SlTAS14*), a gene encoding a Pyrroline-5-carboxylate synthetase (*SlP5CS*) involved in proline biosynthesis, a gene coding for a 1-aminocyclopropane-1-carboxylic acid oxidase (*SlACO4*) involved in the ethylene biosynthetic pathway, a gene (*SlNCED1*) involved in the biosynthesis of the non-volatile isoprenoid ABA, two genes (*SlLOXC* and *SlLOXD*) encoding different lipoxygenase isoforms that participate in the synthesis of jasmonic acid (JA), was evaluated in addition to a gene coding for a protein kinase (*SlSnRK2*) with a role in abiotic stress response. Conversely, genes involved in the metabolism of compounds considered important fruit quality attributes have been considered. Previously published primers (Christou *et al*., 2019) for the sucrose synthase *(SlSuSys*) were used in addition to primers for two carotenoids-related genes (ζ-carotene desaturase, *SlZDS*; lycopene β-cyclase, *Slb-LCY*).

Statistical analyses

The SPSS statistical software package (version 22; SPSS, IBM, Armonk, New York, USA) and R (version 4.2.1) were used to run statistical analyses. Analysis of variance (ANOVA) of the experimental data was performed using the SPSS software and R. A two-way analysis of variance (ANOVA) was performed to assess genotype and irrigation effects. When the two-way ANOVA indicated that either genotype (T and L), irrigation (R1 and R2), or their interaction were significant, comparison of the means was performed using one-way ANOVA with an LSD *post-hoc* test (eco-physiological data) or the Tukey HSD test (production and gene expression), adopting a probability level of $P \leq 0.05$. For gene expression data, significant differences within each genotype based on R1 and R2 comparison were revealed by a Student's *t*-test. The statistical assessment of difference in taxa abundance among samples was performed by PermANOVA (100 permutations) with adjusted P-value cut-off (FDR) set at 0.05 (differences were considered significant for P values of 0.05 or below). Reads were submitted to NCBI Sequence Read Archive (SRA) under BioProject PRJNA868565 (BioSample accessions SAMN30254369-SAMN30254388).

Results

Eco-physiological measurements

Leaf eco-physiological parameters were significantly affected by the irrigation treatments (irr.), with the exception of flavonoid content and NBI. However, there was no significant difference in the genotype (var.) × irrigation treatment interaction (Figure 3). Reduced soil water availability induced 26.3% and 40.5% reductions in P_N in Tondo and Lungo tomato genotypes respectively. A one-way ANOVA with an LSD *post-hoc* test, to identify homogeneous groups (Table S5), showed a statistically significant difference in P_N rates between the two irrigation treatments was only observed in the Lungo genotype (Figure 3A). The Tondo genotype exhibited a proportionally larger 40.3% reduction in g_s in comparison to the 31.5% decline observed in Lungo. However, it should be noted that these differences in g_s between R1 and R2 irrigation treatments were not statistically significant at the 0.05 level (Figure 3B). Both genotypes exhibited lower ΦPSII under a reduced level of irrigation (Figure 3C). Chlorophyll content was significantly lower in R1-T, while no difference in chlorophyll content was observed between irrigation treatments in Lungo (Figure 3D). Leaf flavonoid content (Figure 3E) and the nitrogen balance index (NBI) were unaffected by irrigation treatment in both tomato genotypes (Figure 3F). Consistent with ΦPSII (Figure 3C), the OJIP transients of the tomato varieties under contrasting levels of water availability suggest reduced photochemical PSII electron transport in the Lungo genotype grown at the lower irrigation level

(Figure 4A). In contrast, Tondo exhibited greater similarity in OJIP transient profiles at the two R1 and R2 levels of water availability. Energy absorption at the PSII reaction centres (PI_{TOT}) and the flux of energy dissipated at each reaction centre (DI_o/RC) were 36.5 and 33.6% higher in Lungo grown at the lower R2 irrigation treatment. Under reduced water availability, Tondo exhibited a 20.9% increase in the quantum yield of the reduction of the final stage acceptors at the PSI stage (ΦR_o) and a 21.5% reduction in photochemical and non-photochemical energy absorption of the chlorophyll antennae (PI_{ABS}) (Figure 4B). A 'high-low-high' light transition (2000 to 400 to 2000 µmol m^{-2} s^{-1} PPFD) to simulate changing light conditions during intermittent cloud induced contrasting responses between the tomato genotypes when subject to differing levels of water availability (Figure 5). Both genotypes exhibited higher g_s during the light transitions at the higher R1 rate of irrigation. The stimulation in P_N in the R1 plants at high PPFD was greater in Lungo than Tondo. At the lower light intensity, R1 and R2 Tondo plants exhibited identical P_N rates, while P_N was consistently higher in R1 Lungo when compared to its R2 counterparts. Furthermore, the recovery in P_N was more rapid in R1 than R2 Lungo, while R1 and R2 Tondo followed broadly similar rates of P_N recovery.

Bacterial diversity and community structure: stressed *vs* unstressed roots

IonTorrent sequencing was performed on tomato (Tondo and Lungo) roots and soils harvested from unstressed and stressed fields. Due to a problem in DNA amplification for a soil sample (condition R1-L), five libraries were obtained for samples R1-T, R2-T, R2-L and two additional samples outside the experimental field (uncultivated soils, called ExtA and ExtB). From soil, a total of 146,294 raw reads were generated, and 257 OTUs were obtained (Table S6). These preliminary data showed a similar distribution of

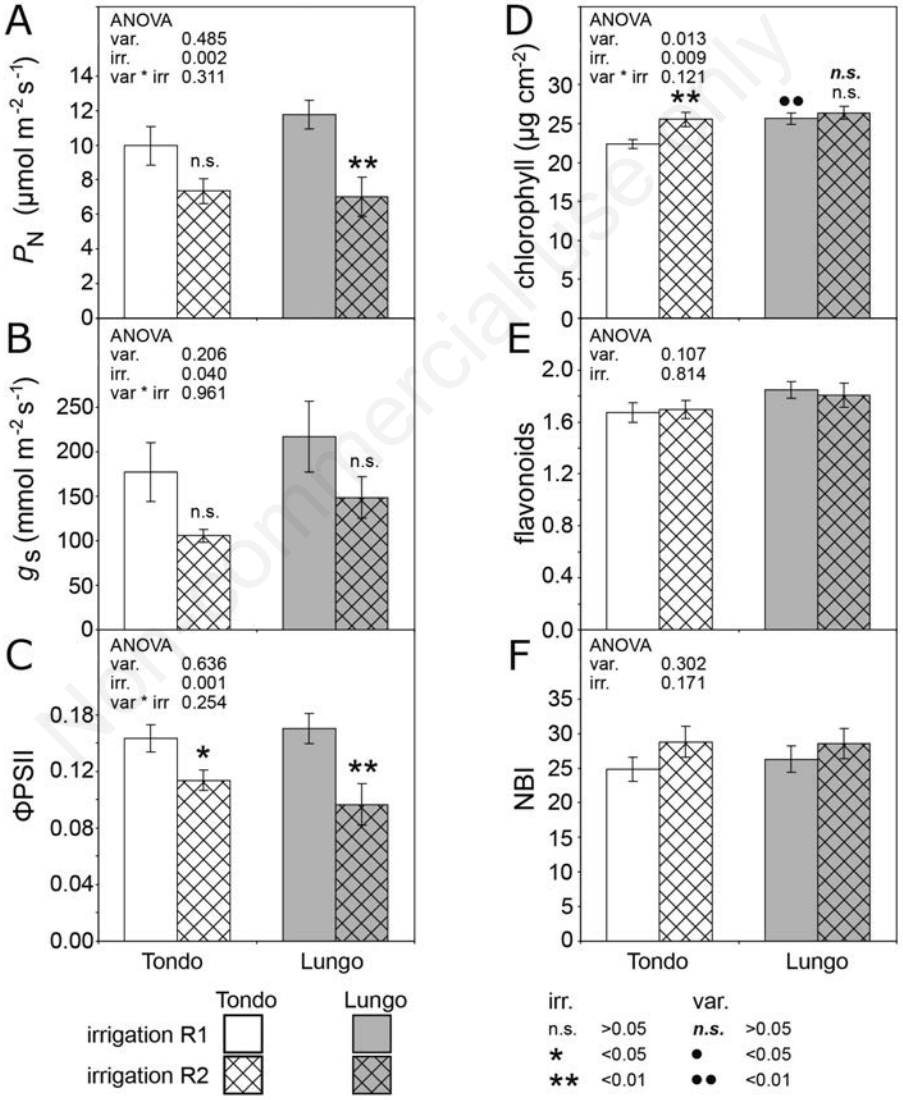

Figure 3. The effect of irrigation water treatment (R1 - 100% ETo, open fill; R2 - 75% ETo, hatched fill) on Tondo (white fill) and Lungo (grey fill) tomato genotypes: A) photosynthesis (P_N); B) stomatal conductance of water vapour (gs); C) actual quantum efficiency of photosystem II (ΦPSII); D) foliar chlorophyll content; E) foliar flavonoid content; and F) leaf nitrogen balanced index (NBI). Error bars indicate one standard error either side of the mean. Two-way ANOVA results denote P-values. Information for multiple comparisons using a one-way ANOVA and LSD *post-hoc* test are given in Table S5. Tondo, Impact F1; Lungo, Contact F1.

microbial communities in all three soil samples R1-T, R2-T, R1-L, with phyla Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes as main detected phyla (Figure S1), with only slight differences (but not significant) compared to samples outside the experimental field (Figure S1). Concerning the sixteen root samples, fifteen 16S rRNA gene amplicon libraries were sequenced for prokaryotes (due to a DNA amplification problem in a R2 Lungo replicate, a library was discarded). A total of 525,364 raw reads were obtained. After extensive quality filtering and chimera removal, reads were clustered using QIIME pipeline into 254 OTUs (Table S7) The analysis of rarefaction curves revealed that sample coverage was optimal for all samples (Figure S2). To account for different sample sizes, OTU abundances were standardized to the median sequencing depth (McMurdie and Holmes 2013). Prokaryote alpha diversity, measured using Chao1 (richness) and Shannon (diversity) indexes, revealed high richness and diversity in root samples from both genotypes under R1 irrigation (Figure 6). Interestingly, according to these two indexes, bacterial and archaeal alpha diversity in the roots was significantly impacted by the reduction in irrigation water for Tondo, while this was not observed for the

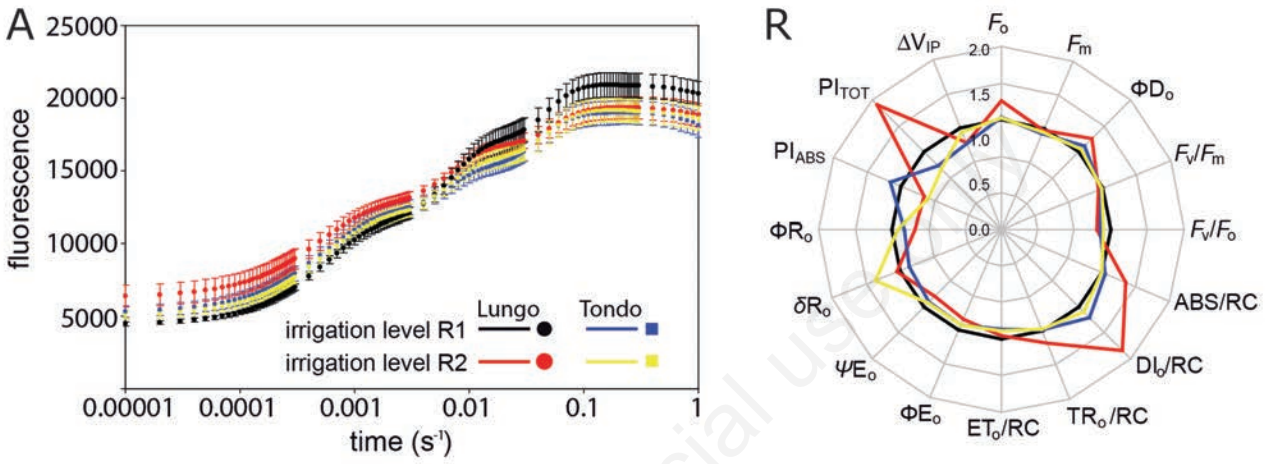

Figure 4. Analysis of the chlorophyll fluorescence transient of the tomato genotypes, Lungo (Contact F1) and Tondo (Impact F1), as influenced by the irrigation treatment (R1 - 100% ETo; R2 - 75% ETo): A) average OJIP induction curves; B) spider plot of parameters (see *Materials and methods* section for definitions and descriptions) extrapolated from the OJIP transient expressed in relation to values of R1 Lungo. Error bars in A) indicate one standard error either side of the mean.

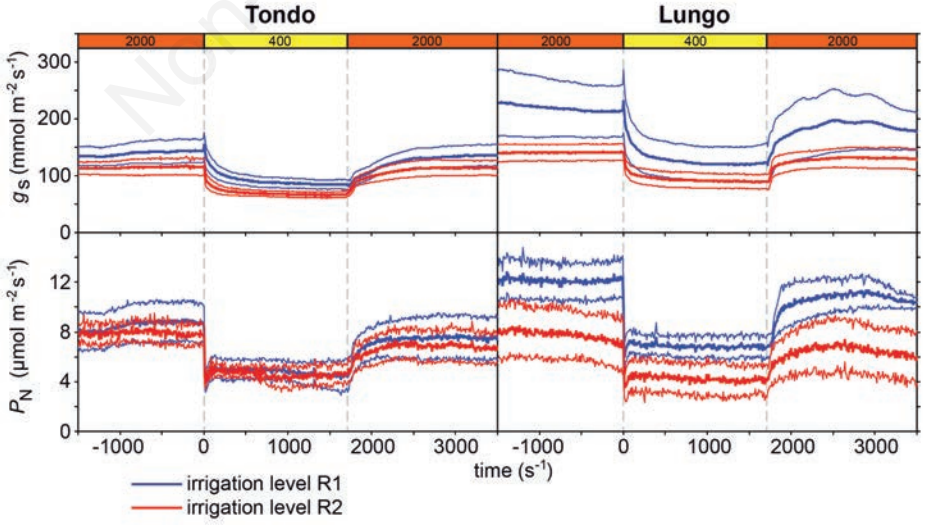

Figure 5. Photosynthesis (PN) and stomatal conductance (gs) sensitivity to variations in PPFD during a simulated intermittent cloudy day of Lungo and Tondo tomato genotypes as influenced by the irrigation treatment (R1 - 100%ETo blue line; R2 - 75% ETo - red line). Levels of PPFD vary from 2000 (orange bar) to 400 (yellow bar) to 2000 (orange bar) μmol m^{-2} s^{-1}. The thicker central line indicates the mean value, the thinner lines either side of the mean indicate +/– one standard error.

Lungo genotype (Figure 6). PCoA resulting from beta-diversity analysis highlighted no significant differences among samples of Lungo genotypes, irrespective of the water treatment, and a slight distinction of samples from R2 irrigation compared to R1 for the Tondo genotype (Figure S3). Bacterial and Archaeal communities largely differed in relative abundances at the phylum level between roots at the two water treatments and genotypes (Figure 6). In both treatments, there was a dominance of Proteobacteria and Actinobacteria. However, highly representative components in each condition and genotype emerged. First, relative abundance was higher in Lungo genotype samples subjected to R2 water treatment than in R1 (Figure 6), while an opposite trend was observed for the Tondo genotype (Figure 6). Both genotypes showed increase in abundance level of Cyanobacteria and Firmicutes (Figure 6), but genotype Tondo showed a significant decrease in the abundance of Bacteroidetes, Alphaproteobacteria, Betaproteobacteria, and Spirochaetes. On the other hand, in roots of the Lungo genotype, an increase of Epsilonproteobacteria, Spirochaetes, and Flavobacteria was detected (Figure 6). At a genus level, Methylovorus showed significant differences in richness between the two genotypes, being significantly abundant in Lungo at both water treatments (R1 and R2), but largely absent in Tondo samples subjected to the R2 irrigation condition (Figure S3).

Gene expression

Leaf transcript levels of six genes potentially involved in the

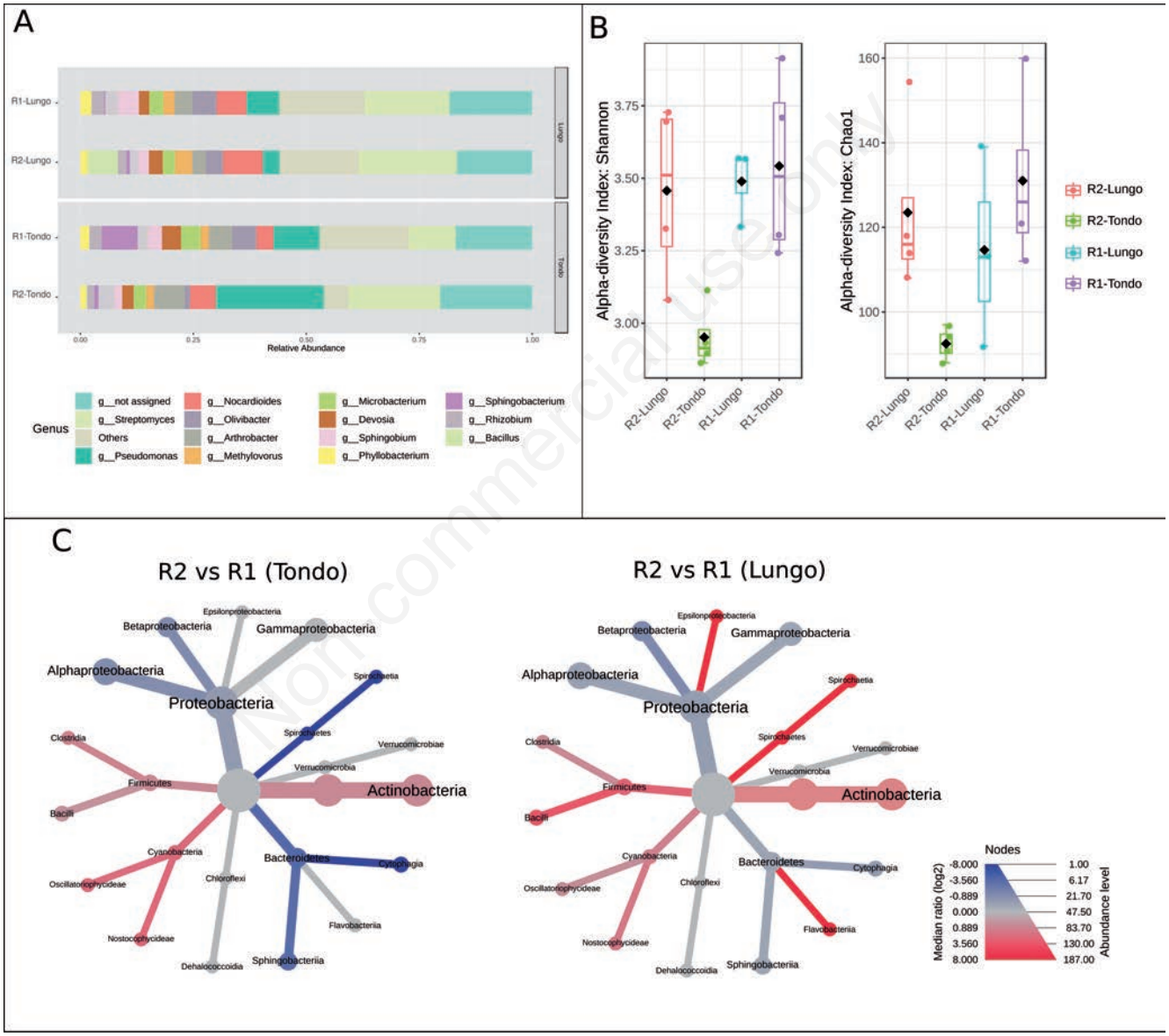

Figure 6. Results of metabarcoding analysis of rhizosphere samples. A) Stacked taxa barplots of bacterial relative abundance at genus level in rhizosphere samples. B) Alpha diversity of rhizosphere samples calculated with Chao1 and Shannon diversity indexes. C) Heat trees of community structure depicting changes in microbiota composition between R2 *vs* R1 (control) conditions, for both tomato genotypes. Size of the nodes is correlated with the abundance of detected taxa and colour of nodes and edges are correlated with the median ratio (log2 transformed) between abundance in each condition. Nodes indicate the hierarchical structure of taxa. Red branches represent a significant increase in R2 compared to R1, while blue branches represent a significant decrease. Ratios between R2 *vs* R1 of microbial communities of Tondo (left side) and Lungo (right side) are reported. Tondo, Impact F1; Lungo, Contact F1.

stress response were evaluated in the four treatments (R1-T, R2-T, R1-L, R2-L; Figures 7 and 8 and Table S8). Particularly, the ABA-biosynthetic gene *SlNCED1* was significantly upregulated in both genotypes under the R2 water treatment (Figure 7). Similarly, the regulation of a gene encoding for a dehydrin responsive to water stress and ABA (*SlTAS14*) and a gene encoding a protein kinase (*SlSnRK2;4*) were both significantly affected by the stress deficit (Figure 7) in the Lungo genotype. In contrast, in Tondo only *SlSnRK2;4* was significantly upregulated upon water deficit, although an upregulation trend was evident also for *SlTAS14*. A gene coding a 1-amynocyclopropane-1-carboxylic acid oxidase (*SlACO4*) involved in producing ethylene was also investigated. It is worth noting that under water deficit, *SlACO4* was significantly upregulated in Lungo while its expression was not significantly affected in Tondo, confirming differences in stress perception in the two genotypes. Among the genes typically involved in plant defence, two genes encoding lipoxygenase enzymes (*SlLOXC* and *SlLOXD*) expressed in response to cell membrane damage were highly upregulated in water deficit conditions, although *SlLOXC* expression values were significantly higher only in Lungo. Interestingly, the gene coding for a protein involved in proline biosynthesis (*SlP5CS*) was upregulated in the Tondo genotype in respect to Lungo in unstressed conditions, indicative of the diverse state of the two genotypes. Expression of genes involved in sugar

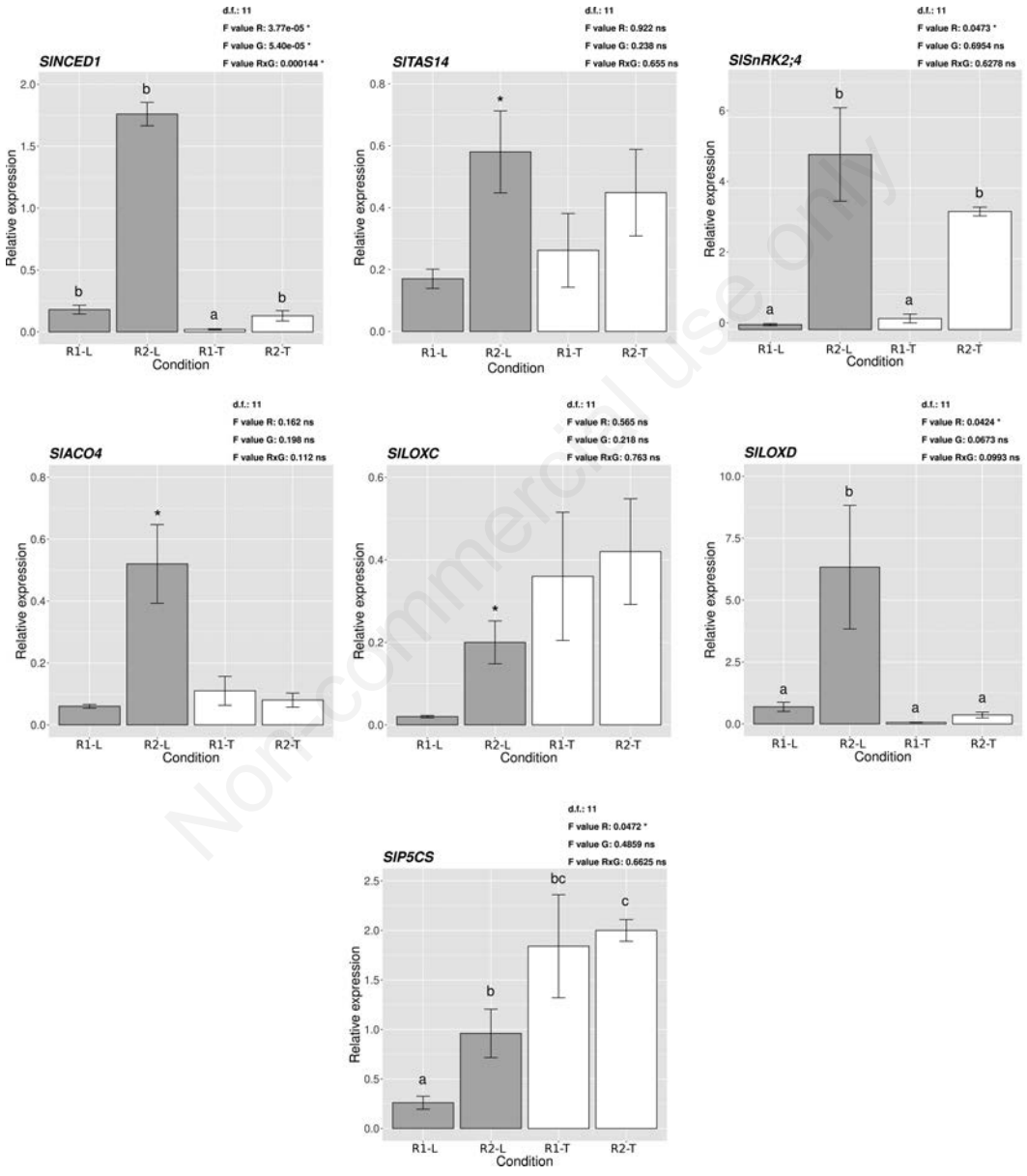

Figure 7. Relative expression levels of genes putatively involved in the response to water deficit of the Lungo (Contact F1) and Tondo (Impact F1) tomato genotypes. Data were normalized with the geometric mean of two endogenous control genes (*SlEFα1* and *SlCAC*). Gene expression data were calculated as expression ratios (relative quantity, RQ) within each condition (means ± SE of three different biological replicates). Significant differences among treatments were statistically assessed by two-way ANOVA test (two factors: genotype - G and water treatment - R) and letters are plotted according to Tukey's HSD post hoc test. F- values of two-way ANOVA are reported. Asterisks represent significant differences within genotype based on R2 *vs* R1 comparison by a Student's *t*-test (P-value ≤0.05).

and carotenoid metabolism in fruits were also evaluated (Figure 8). The results showed that a ζ-carotene desaturase (*SlZDS*) was not significantly regulated among treatments and genotypes, while a lycopene β-cyclase (*Slb-LCY*) was significantly up-regulated only in the Tondo genotype under water deficit (R2). Conversely, sucrose synthase *SlSuSys* was significantly up-regulated in both genotypes under R2 irrigation, with a higher expression value in Lungo in comparison to Tondo (Figure 8).

Discussion

In this study, we evaluated the effect of water limitation on the cultivation of two tomato genotypes in open field conditions, following typical crop management for the region. A combined approach has been used, including eco-physiological measurements as well as gene expression and metabarcoding analyses. The impact of water stress on growth, eco-physiological parameters, yield, metabolite production and transcriptomics in tomato have been considered in several investigations (Nuruddin *et al*., 2003; Tahi *et al*., 2007; Sánchez-Rodríguez *et al*., 2011; Giannakoula and Ilias, 2013; Sacco *et al*., 2013; Landi *et al*., 2017; Iovieno *et al*., 2018; Nemeskéri *et al*., 2019; Conti *et al*., 2022), although most of them were performed in controlled conditions. Our results suggest that the two genotypes differently perceived and responded to the imposed stress, as supported by the eco-physiological measurements and target gene expression analysis. Lower soil water availability induced reductions in P_N, g_s and ΦPSII of both varieties of tomato consistent with other water deficit studies (Killi *et al*., 2017, Marino *et al*., 2020). The reduction in P_N was only significant in Lungo, possibly associated with higher expression of a gene (*SlNCED*) involved in the biosynthesis of the stress hormone abscisic acid (ABA) that induces stomatal closure (Zhang *et al*., 1987). The difference in the effect of irrigation level on P_N between the two tomato genotypes might suggest that the higher alpha diversity in bacterial communities associated to R2 Lungo roots did not coincide with the retention of P_N rates under the lower level of irrigation. Under R1 conditions, Lungo exhibited higher rates of P_N than Tondo, suggesting a more conservative water use behaviour in Tondo that is less likely to elicit a significant decline than Lungo. It is noteworthy that Lungo was the only genotype under R2 irrigation conditions to exhibit impaired PSII performance indicative of longer-term biochemical limitations to P_N rather than transient diffusive limitations associated with reduced stomatal and mesophyll conductance (Tahi *et al*., 2007; Flexas *et al*., 2013; Killi and Haworth, 2017). Despite this evidence of reduced photochemical performance in the Lungo genotype, under conditions of changing PPFD, Lungo exhibited a more rapid increase in P_N rates after light conditions transitioned from low to high PPFD. This may be influenced by higher content of foliar chlorophyll on a leaf area basis and more responsive stomata. The Tondo genotype showed little significant difference in P_N and g_s under fluctuating light conditions, consistent with an interpretation of less physiologically active stomata than Lungo and/or more conservative water use. Individuals of the fast-growing grass, *Arundo donax*, with higher leaf-levels of ABA (but no difference in relative water content), showed faster rates of g_s reduction than those with lower leaf [ABA] during stomatal closure (Haworth *et al*., 2018b), but faster physiological stomatal responsiveness was not apparent in terms of the rate of g_s increase during stomatal opening (Figure 5). The lack of difference in rates of g_s reduction observed in this study between the two irrigation treatments, despite apparent differences in the expression of an ABA biosynthesis related gene (*SlNCED*) may be indicative of differences in stomatal physiology between grasses and eudicots (Franks and Farquhar, 2007). Given the different responses of the tomato genotypes in terms of *SlNCED* expression, this may indicate that the differences in the root-zone rhizosphere bacteria community were not influencing stomatal physiological behaviour through foliar [ABA]. Nevertheless, optimisation of photosynthetic carbon gain during transient light conditions is increasingly important to the maximization of crop production (Głowacka *et al*., 2018). The Lungo genotype showed consistently higher rates of P_N and g_s at the different PPFD levels,

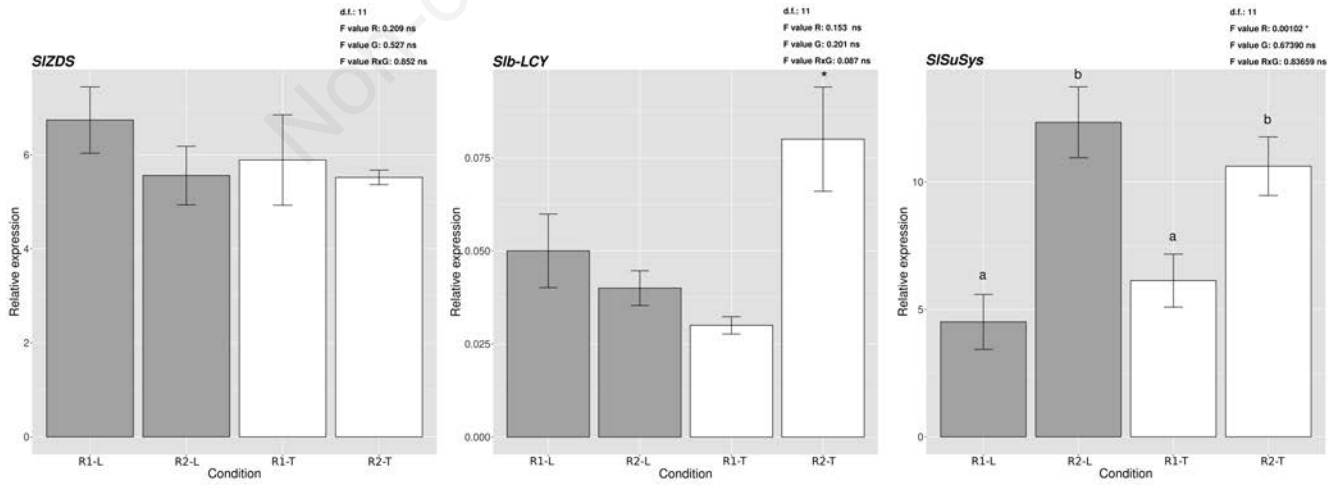

Figure 8. Relative expression levels of genes putatively involved in fruit quality of the Lungo (Contact F1) and Tondo (Impact F1) tomato genotypes. Data were normalized with the geometric mean of two endogenous control genes (*SlSAND* and *SlCAC*). Gene expression data were calculated as expression ratios (relative quantity, RQ) within each condition (means ± SE of three different biological replicates). Significant differences among treatments were statistically assessed by two-way ANOVA test (two factors: genotype - G and water treatment - R) and letters are plotted according to Tukey's HSD post hoc test. F- values of two-way ANOVA are reported. Asterisks represented significant differences within genotype based on R2 *vs* R1 comparison by a Student's *t*-test (P-value ≤0.05).

whereas the Tondo genotype showed comparatively little difference between R1 and R2 irrigation conditions. However, the physiological responses of the tomato genotypes did not exhibit a clear physiological response that could be linked to differences in interactions between tomato genotype, root-associated microbial communities and irrigation status. Under heterogeneous growth conditions in the field, in particular the high summer temperatures during the experimental period, it may not be possible to detect the physiological impact of differences in rhizosphere microbial composition in leaf gas exchange and chlorophyll fluorescence characteristics.

Genes involved in the response to stress factors were significantly up-regulated as a consequence of water deficit, particularly in R2 Lungo in comparison with control R1 Lungo. Conversely, only three genes (*SlSnRK2*, *SlNCED* and *SlLOXD*) were up-regulated in R2 Tondo with respect to R1 Tondo, at a lower level with respect to the Lungo samples. This confirmed a difference in the response of the two genotypes to irrigation deficit and was consistent with the leaf gas exchange data. The two genotypes also showed diverse regulation of some genes in the R1 irrigation treatment, such as a gene involved in the osmolyte proline biosynthesis (*SlP5CS*) that was significantly upregulated in Tondo with respect to Lungo. This result suggested that this genotype was in a 'primed' state due to its genetic features, considering that several of the other considered stress marker genes were not differentially upregulated in the Tondo R1 plants with respect to Lungo R1. However, it is not possible to exclude the possibility that Tondo genotype was suffering the impact of water deficit to a greater extent than Lungo at the R1 irrigation level, considering that irrigation was blocked for one week during sampling. In agreement with previous studies (Iovieno *et al*., 2018), *SlP5CS* was upregulated in water deficit conditions but only in R2 Lungo, suggesting that the difference between the R1 and R2 irrigation levels were more evident in the Lungo genotype, at least for proline biosynthesis. Looking at the other water stress markers genes (Chitarra *et al*., 2016), the most evident response to water limitation was an increase in the transcriptional levels of *SlTAS14* (a tomato dehydrin encoding gene). The transcriptional level of *SlNCED1*, involved in the biosynthesis of the non-volatile isoprenoid ABA, which is considered one of the main regulators of drought stress response in plants, also increased in water-stressed leaves, in both genotypes. These genes are reported to have a diverse gene expression pattern in the presence of rhizosphere-associated microorganism (Chitarra *et al*., 2016; Brilli *et al*., 2019). In parallel, a significant up-regulation of *SlSnRK2;4*, encoding a protein kinase of the SnRK2 family, whose members are a component in ABA signaling (Yang *et al*., 2015), was observed under the R2 irrigation treatment in both genotypes. However, the function for these proteins should be further investigated due to the different roles reported for the diverse members of this family. Notably, overexpression of tomato *SnRK2.1* and *SnRK2.2* led to a decrease in osmotic stress tolerance, suggesting that they may play negative roles in the stress response (Yang *et al*., 2015). A previous experiment in pot conditions, using a sterilized substrate, showed down-regulation of *SnRK2.1* and *SnRK2.2* in water stress. This discrepancy could be due to the tomato genotype, the natural environmental variability and the stress level. It is worth noting that a role for *SlSnRK2;4* in the regulation of root growth of plants affected by salt stress has been reported (McLoughlin *et al*., 2012). The expression of *SlACO4,* coding for an enzyme involved in the conversion of ACC in ethylene, should be also highlighted. This gene appeared to be significantly up-regulated in R2 Lungo, but not in R2 Tondo, with respect to the corresponding controls. Ethylene is considered a stress-related hormone and *ACO* genes, including *ACO4*, are involved in drought response in wild tomato (Egea *et al*., 2018). However, it should be noted that the responding candidate genes might be different depending on the water deficit level.

The quality of fruit under diverse deficit irrigation treatments have been already studied to assess the most efficient management strategies for fruit crops. A decrease in fruit weight associated with water deficit has been reported in several studies (Miller *et al*., 1998; Pérez-Pastor *et al*., 2007; Terry *et al*., 2007; Rodrigues *et al*., 2010; Arji *et al*., 2016; Lobos *et al*., 2016). Lobos *et al*. (2016) demonstrated that although severe water limitation decreased fruit weight and crop production, the imposition of a 25% reduction in water did not negatively influence fruit quality and levels of antioxidants in blueberries. In the present study, data on total production (kg of fruits for four plots) are limited, although yield did not show significant differences between R1 and R2, a greater decrease was evident in Lungo (199 kg in L-R1 *vs* 182 in R2) and the weight of 100 fruits was significantly lower in R2 Lungo in comparison to R1 Lungo, in agreement with the fact that when fruit water accumulation is reduced, such as under water deficit, tomato fruit weight is decreased (Machado *et al*., 2022). It is also worth noting that the stress led to a significant upregulation of a gene coding for a sucrose synthase in fruits collected from R2 plants, in agreement with previous works looking at the gene expression of homologous genes in soybean and rice upon drought conditions (Du *et al*., 2020). These enzymes have been reported to play a prominent role in modulating sink strength in plants under water deficit conditions and increasing the concentration of hexose sugars that are osmoprotectant molecules, putatively involved in plant response to oxidative stress (Coleman *et al*., 2009).

Interestingly, the differences in eco-physiological and molecular responses were mirrored by diverse bacterial communities associated with the two genotypes, mainly under water limitation. In recent years, several efforts have been also made to elucidate tomato rhizosphere composition (Larousse *et al*., 2017; Chialva *et al*., 2018, 2019; Lee *et al*., 2021). It has been reported that the tomato rhizosphere microbiome is mainly composed of Proteobacteria, Actinobacteria, and Bacteroidetes (Cheng *et al*., 2020; Lee *et al*., 2019; Poudel *et al*., 2019). In our work, Proteobacteria, Actinobacteria and Bacteroidetes were also the main phyla of root-associated samples in R1 conditions in both genotypes. Interestingly, one of the more abundant genera in the R1 (the genus *Sphingobium*) has been documented as one of the dominant genera in tomato roots (Pii *et al*., 2016). However, in R2 conditions, Firmicutes replace a large proportion of Bacteroidetes in both genotypes, but especially in R2 Lungo soil samples. The observed Firmicutes enrichment in R2 treatments seems to be consistent with those reported in the roots of sorghum and rice affected by drought (Santos-Medellín *et al*., 2021). In addition, the relative abundance of Actinobacteria increased in both genotypes when affected by water deficit. Interesting, it has been documented that Actinobacteria might enhance plant vigour and confer tolerance to drought (Franco-Correa and Chavarro-Anzola, 2016; Naylor *et al*., 2017), and they have been reported to be favoured under drought conditions (Naylor and Coleman-Derr, 2018; Breitkreuz *et al*., 2021). At the genus level, the main genera detected in both genotypes at R1 and R2 were *Nocardioides*, *Pseudomonas*, *Streptomyces*, *Sphingobacterium*, *Sphingobium*, *Microbacterium* and *Olivibacter*. The abundance level of the first three groups increased in R2 conditions, while the abundance of the remaining four genera decreased during water deficit. Notably, in experiments on maize subjected to drought, an increased abundance of genera belonging to Actinobacteria such as *Streptomyces* and *Nocardioides*

has also been reported (Zhang *et al*., 2021). Fitzpatrick *et al*. (2017) demonstrated that drought led to an increase in the mean relative abundance of endosphere Streptomycetaceae, which belong to Actinobacteria, although this effect changed among plant species. These authors suggested that modifications in the relative abundance of specific bacterial taxa, mainly Streptomycetaceae, might be associated with an increased tolerance to drought (Fitzpatrick *et al*., 2017). In our experiments, it is worth noting that the relative abundance of the *Streptomyces* genus increased in R2, mainly in the Tondo genotype. Remarkably, *Pseudomonas* genus, which also increased in water deficit conditions (R2) only in Tondo, has been shown to have a high efficiency as plant growth promoting bacteria (PGPB) for different crops (Zhang *et al*., 2020), in addition to a role in alleviating the effects of water scarcity in plants (Yasmin *et al*., 2022). It is worth noting that the *Bacillus* genus also increased but only in R2 Lungo. This genus harbours strains capable of increasing plant stress tolerance (Ashraf *et al*., 2004; Marulanda *et al*., 2010; Tiwari *et al*., 2011; Vardharajula *et al*., 2011; Sorty *et al*., 2016). Conversely, other bacteria with potential as PGPB, such as *Microbacterium* and *Olivibacter* (Cordovez *et al*., 2018) decreased under water deficit, suggesting that these strains could be more sensitive to water limitation in soil or to any change in the composition of root exudates. In addition, the class Flavobacteria was significantly more abundant in R2 Lungo compared to R1 Lungo. A role in plant function has been hypothesized for members of this class, *e.g*., *Flavobacterium* (Kolton *et al*., 2016). The significantly higher abundance of *Methylovorus* genus in R1 Lungo and R2 Lungo compared to R1 Tondo and R2 Tondo suggests that the Lungo genotype seems to be able to recruit a bacterial taxa belonging to a methylotrophic bacterial group known to play a putative role in plant growth promotion, crop yield and soil fertility under reduced soil water availability (Kumar *et al*., 2019).

Taken together, our results suggest that the microbiome in tomato roots is shaped by water deficit, and the selection of taxa potentially involved in promoting tolerance to drought in open field conditions may occur. This is more evident for the Lungo genotype that showed a significant higher alpha diversity in the R2 condition compared to Tondo. These results are in keeping with previous papers reporting that plants can shape their belowground microbiome, as shown by the fact that diverse plant genotypes host specific microbial communities when grown on the same soil (Berendsen *et al*., 2012). Drought-induced plant responses, including physiological and molecular changes, could be responsible for the effects of plants on the root-associated (endosphere) microbiome (Fitzpatrick *et al*., 2017), and changes in root exudation may be considered to be relevant within this context (Williams and deVries, 2019). The plant host shapes the rhizosphere microbiome community, including root-associated microorganisms, by regulating root exudates and root phenotypes, as observed in an increasing number of investigations across diverse plant species (Bulgarelli *et al*., 2015; Edwards *et al*., 2015; Schlemper *et al*., 2017; Walters *et al*., 2018; Singer *et al*., 2019; Qu *et al*., 2020). Root exudates, which can vary considerably according to plant age, developmental stage and genotype, constitute a food source for the microorganisms inhabiting the rhizosphere, *i.e.*, the thin layer of soil strictly adhering to roots, and the root endosphere (Escudero-Martinez and Bulgarelli, 2019). It has been demonstrated that plants with diverse growth patterns significantly differed in their root exudation characteristics, and this influences the selection of rhizosphere-associated microorganisms (Williams and deVries, 2019). In tomato, changes in the composition of root exudates may also have an impact on the growth of plant pathogens and PGPB, such as *Bacillus* and *Pseudomonas* species (Ngalimat *et al*., 2021). Although drought can affect the composition of root exudates and volume (Williams and deVries, 2019), the impact of water deficit on the chemical and microbiological responses in the rhizosphere remains a comparatively unexplored topic (Williams and deVries, 2019). It has been suggested that plants can influence rhizosphere-associated microorganisms to enhance water relations and that the modifications induced by drought might be environment- and genotype-dependent (Williams and deVries, 2019).

Conclusions

The use of eco-physiological and molecular approaches indicated that, in real agronomic conditions, where plant growth is influenced by multiple environmental factors, the two tomato genotypes responded differently to water shortage through shifts in the balance between growth and resilience mechanisms. The two genotypes showed diverse responses to water deficit through different allocation of resources. The Lungo genotype was more responsive in terms of photosynthetic and stomatal behaviour alongside the expression of stress related genes. In terms of microbial diversity, water management (control regime *vs* moderate deficit irrigation) was crucial in driving the assembly of microbial communities in tomato with a strong effect in the root compartment, although with contrasting outcomes between the two genotypes. The diversity level in the root-associated bacterial community remained high in Lungo roots independent of the water irrigation regime. Conversely, the diversity of the bacterial community was reduced in Tondo under water limitation, whereas these conditions favoured the presence of taxa reported to be involved in drought tolerance. These results, obtained in a natural environment, potentially serving as a reservoir of beneficial microbial communities, could be useful for the exploitation of these microorganisms towards the development of a root microbiome adapted to specific plant genotypes and soil conditions.

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