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RESEARCH PAPER

Mycorrhization and chemical seed priming boost tomato stress tolerance by changing primary and defence metabolic pathways

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Abstract

Priming modulates plant stress responses before the stress appears, increasing the ability of the primed plant to endure adverse conditions and thrive. In this context, we investigated the effect of biological (i.e. arbuscular mycorrhizal fungi, AMF) agents and natural compounds (i.e. salicylic acid applied alone or combined with chitosan) against water deficit and salinity on a commercial tomato genotype (cv. Moneymaker). Effects of seed treatments on AMF colonization were evaluated, demonstrating the possibility of using them in combination. Responses to water and salt stresses were analysed on primed plants alone or in combination with the AMF inoculum in soil. Trials were conducted on potted plants by subjecting them to water deficit or salt stress. The effectiveness of chemical seed treatments, both alone and in combination with post-germination AMF inoculation, was investigated using a multidisciplinary approach that included ecophysiology, biochemistry, transcriptomics, and untargeted metabolomics. Results showed that chemical seed treatment and arbuscular mycorrhizal symbiosis modified the tomato response to water deficit and salinity triggering a remodelling of both transcriptome and metabolome, which ultimately elicited the plant antioxidant and osmoprotective machinery. The plant physiological adaptation to both stress conditions improved, confirming the success of the adopted approaches in enhancing stress tolerance.

Keywords: Arbuscular mycorrhizal symbiosis, chemical priming, metabolomics, salinity, transcriptomics, water deficit.

Abbreviations: ABA, abscisic acid; AM, arbuscular mycorrhizal; AMF, arbuscular mycorrhizal fungi; A_N, assimilation; CCI, chlorophyll content index; CHI, chitosan; CTRL, control; DEG, differentially expressed gene; E, leaf transpiration; g_s, stomatal conductance; IAA, indol-3-acetic acid; JA, jasmonic acid; MYC, inoculated; NMYC, not inoculated; NS, not stressed; Ψ_{stem}, stem water potential; RNA-seq, RNA sequencing; RWC_{soil}, soil relative water content; SA, salicylic acid; SS, salt stress; WS, water stress; T_0, initial time point; T_f, final time point.

Introduction

Intensive agriculture progressively exacerbates plants' exposure to environmental stressors, adversely impacting both crop productivity and quality yield (Francini and Sebastiani, 2019; Fróna *et al.*, 2021). In recent years, ongoing climate change has worsened the frequency and severity of heat waves and drought events, accompanied by a general decrease in or discontinuity of rainfall on agricultural ecosystems across the planet (Rosenzweig *et al.*, 2014; Horton *et al.*, 2016). A further problem occurring in combination with prolonged drought spells is a high risk of salt stress (Qadir *et al.*, 2014), resulting from reduced irrigation and precipitation and heat-driven evaporation.

The challenge of ensuring yield and quality of crops under this complex and worrying scenario may be tackled by developing and implementing sustainable measures that can endow target plant species with superior stress tolerance, thereby fostering the robustness and resilience of cropping systems. To this purpose, various strategies have been applied, such as traditional breeding and recently gene editing techniques, which, however, still encounter applicability restrictions in several European countries (Hu and Xiong, 2014). An alternative and valuable solution is represented by priming agents able to stimulate the innate plant defence system (Mauch-Mani *et al.*, 2017; Alagna *et al.*, 2020). A plant's perception of the priming stimulus triggers the establishment of a complex array of signalling routes, involving epigenetic, transcriptional, post-transcriptional, metabolic, and physiological modifications (Hilker and Schmülling, 2019), ultimately leading to an overall improvement of the plant's resistance or tolerance to stress (Mauch-Mani *et al.*, 2017).

Priming through chemical compounds applied at the seed and seedling stage is very promising but further elucidation is needed to understand the mechanisms behind it (Savvides *et al.* 2016; Gohari *et al.*, 2020, 2024; Tripathi *et al.*, 2024). Among these compounds, chitosan (CHI) and salicylic acid (SA) have emerged as seed priming agents able to promote plant tolerance to abiotic stresses (Hidangmayum *et al.*, 2019; Chakma *et al.*, 2021; Kulak *et al.*, 2021). CHI is a poly-(D-glucosamine) derived from chitin, and as a priming agent, it has been shown to elicit the ability of plants to endure stress conditions, especially against biotic stresses (Iriti and Varoni, 2015). However, its beneficial impact in triggering abiotic stress responses in different plant species has also been reported (Balusamy *et al.*, 2022; Hidangmayum *et al.*, 2023; Hidangmayum and Dwivedi, 2023). CHI's effects on plants subjected to water stress and salinity are associated with the regulation of cellular osmotic pressure by promoting the availability or uptake of water and essential nutrients (Hidangmayum *et al.*, 2019). On the other hand, SA, a well-known plant hormone, plays a key role in the control of plant responses to both biotic and abiotic stresses (Rivas-San Vicente and Plasencia, 2011). SA is known to regulate diverse physiological processes, including stomatal closure, photosynthesis, and the synthesis of some stress-related proteins and antioxidants (Janda *et al.*, 2014), and it has proven to be effective against water deficit conditions (Chakma *et al.*, 2021). Together, CHI and SA may offer complementary strategies for priming plants against abiotic stresses. Their combined use as seed priming agents could therefore represent a promising approach for enhancing crop resilience under challenging environmental conditions.

Additionally, mounting experimental evidence shows that biological agents, such as root-associated microorganisms, can put plants into a primed state that enables them to react quickly and robustly to stress exposure (Balestrini *et al.*, 2018; Alagna *et al.*, 2020). Arbuscular mycorrhizal fungi (AMF), which are obligate biotrophs currently assigned to the subphylum *Glomeromycotina* (Spatafora *et al.*, 2016), establish mutualistic symbioses with the roots of about the 80% of plant species, including the most important crops (Balestrini and Lumini, 2018; Balestrini *et al.*, 2021). AMF play a crucial role in the acquisition and translocation of essential mineral nutrients using their extraradical mycelium, while receiving carbon compounds in return (Smith and Read, 2008). The interaction with AMF can improve plant adaptation to various stress conditions, including water and salt stresses (Balestrini *et al.*, 2018; Bastías *et al.*, 2022). Root symbiosis with AMF positively affects plant physiological parameters, biochemical pathways, and gene expression, resulting in an improved stress tolerance of the host plant (Bastías *et al.*, 2022). Several works investigated transcriptomic changes occurring in different species of agronomic interest exposed to environmental stresses during arbuscular mycorrhizal (AM) symbiosis (Porcel and Ruiz-Lozano, 2004; Recchia *et al.*, 2018; Balestrini *et al.*, 2019; Zhang *et al.*, 2019; Moradi Tarnabi *et al.*, 2020). Moreover, untargeted metabolomic approaches have successfully been applied to explore the whole metabolic changes controlling the interaction between the host plant and AMF. Differences in the metabolic profiles of roots and leaves from mycorrhizal crops have emerged, allowing the identification of compounds potentially involved in stress tolerance (Rivero *et al.*, 2018; Tugizimana *et al.*, 2018; Liang *et al.*, 2021).

Tomato (*Solanum lycopersicum* L.) is one of the most important crop species worldwide (Cortina and Culiáñez-Macià, 2005) with a global production of 186.1 million metric tonnes in 2022 (FAOSTAT database, http://www.fao.org/faostat/en/#data). The availability of an updated reference genome sequence and the recent release of the genomes of many commercial and wild tomato genotypes make this species an ideal model for physiological and functional genomic studies aimed at identifying stress tolerance mechanisms and stress marker genes for breeding purposes (Wang *et al.*, 2024). Water limitation was reported to have a negative effect on tomato photosynthetic activity (Galmés *et al.*, 2013; Chitarra *et al.*, 2016) and nutrient uptake, resulting in an overall decrease in fruit production (Ma *et al.* 2019; Sardans *et al.*, 2020). Soil salinity has

also been shown to influence tomato growth and fruit yields (Roșca *et al.*, 2023, and references therein). Therefore, tomato represents a model crop for studying the role of AM symbiosis in the regulation of abiotic stress responses. Several authors reported a positive impact of AM symbiosis in tomato tolerance to water deficit through different mechanisms (Chitarra *et al.*, 2016; Rivero *et al.*, 2018; Duc *et al.*, 2018, 2023; Balestrini *et al.*, 2019; Leventis *et al.*, 2021; Zhang *et al.*, 2024). Nonetheless, the application of AMF as stress priming agents is still a challenge in agriculture, mainly due to poorly consistent results (Santos *et al.*, 2019). In this context, the crop genotype, timing, and intensity of different single and/or combined stresses represent variables that can significantly affect the interaction of plants with beneficial microbes (Pascale *et al.*, 2020). Furthermore, native AMF of a particular soil or site are often reported to be more effective than non-native ones, probably as they are adapted to soil features such as nutrient concentrations, or to environmental constraints such as drought (Pellegrino *et al.*, 2011). Within this scenario, other poorly explored aspects concern the establishment of stress tolerance signals from combined AMF and abiotic priming treatments.

This study was focused on verifying the effectiveness of chemical priming [i.e. SA and CHI+SA)] applied to tomato seeds prior to germination, in combination with an AMF inoculum in the soil and considering diverse abiotic stress conditions (water deficit and salinity). A multidisciplinary approach, involving physiological measurements, transcriptomic, biochemical, and untargeted metabolomic analyses, was followed to decipher the mechanisms involved in the tomato response to priming and AM symbiosis.

Materials and methods

Plant material and growth conditions

Solanum lycopersicum cv. Moneymaker (MM) seeds (Semiorto Sementi, Sarno, Italy) were treated with natural compounds by the Cyprus University of Technology (Galviz-Fajardo *et al.* 2020). Seeds were treated with CHI, SA or CHI+SA as potential priming agents, while untreated seeds were used as control (CTRL).

A homogeneous and clear solution of low molecular weight CHI (CAS no.: 9012-76-4) was prepared by dissolving 0.1 g of CHI in 85 ml of deionized water (dH_2O) along with 100 µl of acetic acid (0.5%), followed by stirring for 1 h. To achieve a concentration of 50 µM SA, 10 ml of 500 µM SA was prepared in dH_2O and added to the homogeneous CHI solution while stirring. Tripolyphosphate (TPP) was used to crosslink the CTI nanocarrier, calculated based on the amount of CHI used. In this regard, to form CHI nanoparticles and encapsulate SA, 0.4 g of sodium tripolyphosphate (CAS no.: 7758-29-4) was dissolved in 5 ml of dH_2O. The addition of TPP resulted in crosslinking of CHI nanoparticles in the form of coagulation, which was continued by stirring the nanocarriers overnight (Hassanpour *et al.*, 2021). The CHI-alone treatment consisted of an identical approach, omitting the step in which SA was prepared in dH_2O and added to the homogeneous CHI solution while stirring. Seed priming was performed by soaking tomato seeds in the two different solutions (SA and CHI+SA) for 12 h at 25 °C in the dark. After soaking, the seeds were spread on a filter paper to air-dry at room temperature. Once the primed seeds were dried, they were ready to be sown.

Treated and untreated seeds were germinated in the presence or absence of an AMF inoculum in the soil. A crude inoculum of *Funneliformis mosseae* spores, extraradical mycelium, and colonized roots (MycAgro Lab, Bretenière, France) was used. Seeds were placed in pots (0.7 litre) filled with pumice at the bottom, followed by coconut fibres and a ring with sterilized (at 180 °C for 3 h) quartz sand and coconut fibres at the top (not inoculated, NMYC). For AMF (MYC) pots, the inoculum was mixed with the sterile sand ring, using 100 ml of inoculum with 300 ml of sterile sand (1:4) for four pots. The pots were watered two times a week with tap water and once a week with ½ Hoagland solution (Hoagland and Broyer, 1936), starting 1 month after sowing. Plants were cultivated in a glasshouse, with mean daily temperatures of 24.9±5.35 °C, relative humidity of 42.3–61.8% and maximal photosynthetic photon flux density (PPFD) of 900–1200 µmol photons m^{-2} s^{-1}. When necessary, a photoperiod of 12 h light–12 h dark was obtained with halogen lamps to guarantee a minimum PPFD of 500–600 µmol photons m^{-2} s^{-1}. During the physiological trial, one-third of the plants were subjected to water stress (WS), one-third to salt stress (SS), and the remaining third were maintained fully irrigated and used as control (not stressed, NS). For each treatment (CTRL, SA, CHI, CHI+SA, MYC, NMYC) and condition (NS, WS, SS), eight biological replicates were used, for a total of 144 plants.

Water deficit time course

Water deficit was imposed by withholding irrigation on 2-month-old plants belonging to the different experimental groups. Dynamic changes in stomatal conductance (g_s) rates were monitored over time on WS and NS plants using a portable porometer/fluorometer (LI-600, LI-COR, Lincoln, NE, USA). Leaf vapour pressure deficit values were checked using the same instrument, which also allowed calculation of transpiration rate (E). Daily changes in water loss were also inspected, by measuring the soil relative water content (RWC_{soil}, %) gravimetrically on potted plants exposed to water deprivation according to Pagliarani *et al.* (2022). Additionally, the stem water potential (Ψ_{stem}) was measured on NS and WS plants at the end of the trial (final time point, T_f) as described in Pagliarani *et al.* (2022), using a portable pressure chamber (1505D PMS Instrument Company, Albany, OR, USA). The water stress time course was stopped when RWC values were around 40% and g_s rates were ≤0.05 mol H_2O m^{-2} s^{-1}. At the beginning (initial time point, T_0) and end (final time point, T_f) of the experiment, plant height, leaf number, and basal stem diameter of all plants were measured. The chlorophyll content index (CCI) was also determined on the second and third fully developed leaves from the apex, using a portable chlorophyll meter (SPAD 502; CCM-200, Opti-Sciences). Two leaves per plant were analysed, taking three readings per leaf.

Salt stress experiment

After a month and half from sowing, salt stress was imposed by supplying tomato plants with a solution of NaCl (50 mM), providing 30 ml for each pot. The NaCl concentration was gradually increased every 3 d first to 100 mM, and then to 150 mM, until reaching the maximum concentration (200 mM), according to Pollastri *et al.* (2018). The maximum concentration was maintained for 11 d, administered every 3 d. After 20 d of stress, the experiment was stopped and changes in the main ecophysiological parameters were determined on SS and NS plants. Rates of g_s, E, and assimilation (A_N) were measured in the morning (between 09.00 h and 12.00 h) on each plant, using a portable infrared gas analyser (LCpro-SD system, ADC BioScientific Ltd, Hoddesdon, UK) equipped with a 6.25 cm^2 leaf chamber. Three random, fully expanded, non-senescing leaves exposed to direct sunlight were analysed working with artificial irradiation (1200 µmol photon m^{-2} s^{-1}) and setting the chamber temperature at 25 °C. CO_2 values were those of greenhouse conditions (450 ppm).

Sampling procedure

At the end of the WS and SS experiments, leaves and roots collected from stressed (WS or SS) and NS plants were immediately frozen in liquid nitrogen and stored at −80 °C. Half of the root system was used for the mycorrhizal colonization assessment.

Assessment of mycorrhizal colonization

After 2 months from seedling inoculation, mycorrhizal colonization was checked on three plants for each priming treatment and condition (NS, WS, or SS). Roots were gently washed with tap water, then incubated in a KOH (10%) solution for 10 min at 80 °C, followed by an overnight staining at room temperature with 0.1% cotton blue in lactic acid. Roots were then gently washed with tap water three times and stored in lactic acid. Twenty randomly selected root fragments (1 cm in length) were mounted on microscope slides (three slides per biological replicate). A total of 60 root fragments were observed per biological replicate. The level of mycorrhizal colonization was determined using the method described by Trouvelot *et al.* (1986), based on staining root samples to identify and quantify the fungal structures (e.g. hyphae, arbuscules, vesicles) under an optical microscope.

Phytohormone quantification

Analysis of plant hormones [SA, jasmonic acid (JA), abscisic acid (ABA), indol-3-acetic acid (IAA)] was carried out by LC/MS as described (De Ollas *et al.* 2021). Briefly, 10 mg of ground dry leaf tissue (n=3) was extracted in 1 ml of ultrapure water, after spiking with 25 µl of a solution containing 1 mg l^{-1} of [$^2H_2$]gibberellin A7, [$^2H_6$]ABA, dihydrojasmonic acid, and [$^{13}C_6$]SA, and 0.1 mg l^{-1} of [$^2H_5$]IAA in a ball mill (MillMix20, Domel, Železniki, Slovenija). Extracts were centrifuged at 11 200 *g* and 4 °C for 10 min and the supernatants recovered and their pH adjusted to 3 with 30% acetic acid. Afterwards, all extracts were partitioned twice against 1 ml of diethylene ether, recovering the organic layer on each occasion. The combined organic layers were evaporated under vacuum (SpeedVac, Jouan, Saint Herblain Cedex, France) and the dry residue resuspended in 500 µl of a 1:9 methanol:water solution by sonication. The resulting solutions were filtered through 0.2 µm polytetrafluoroethylene membrane syringe filters (Albet S.A., Barcelona, Spain). Filtered extracts were separated by reversed phase UPLC (Acquity SDS or Alliance 2695, Waters Corp., Milford, MA, USA) using a C18 column (Luna Omega Polar C18, 100 mm×2.0 mm, Phenomenex, CA, USA) and an acetonitrile and water gradient (both supplemented with formic acid) at a constant flow rate of 0.3 ml min^{-1}. The mass spectrometer (TQ-D, Micromass Ltd, UK) was operated in negative ionization electrospray mode and the different plant hormones were detected according to their specific transitions, using a multiresidue mass spectrometric method. Hormone quantification was performed with Masslynx v. 4.0 software (Micromass Ltd, Manchester, UK) after external calibration with standard samples containing known amounts of each plant hormone.

Proline content

Proline content was determined according to Bates *et al.* (1973) and Shabnam *et al.* (2016), with slight modifications. Briefly, leaf tissues (10 mg dry weight) from three biological replicates were extracted by homogenization in 1 ml of 1% aqueous sulfosalicylic acid (w/v). One ml of plant extract was reacted with 400 µl acid ninhydrin reagent (1.25% ninhydrin in 80% glacial acetic acid) in a water bath at 100 °C for 30 min or 60 min. The reaction was stopped by incubating the samples on ice for 10 min. Proline content was determined by reading the sample absorbance at 520 nm using a multi-mode reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, USA).

RNA extraction, library preparation, and sequencing

Total RNA was extracted from 0.1 g of leaf material according to the cetyltrimethylammonium bromide-based method of Chang *et al.* (1993). Quantity and quality of the extracted RNA samples were determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was assessed on an RNA 6000 Nano Labchip using a Bioanalyzer 1000 (Agilent Technologies, Santa Clara, CA, USA) prior to library preparation. Only samples showing a RNA integrity number >8 were processed for sequencing. Library preparation and RNA sequencing were performed at an external service (IGATech s.r.l Laboratories, Udine, Italy). In detail, a Universal Plus mRNA-Seq kit (Tecan Genomics, Redwood City, CA, USA) was used for library preparation following the manufacturer's instructions and sequencing was performed on Illumina NovaSeq 6000 apparatus (Illumina, San Diego, CA, USA). Trimming of lower quality bases and adapters was performed using ERNE software according to Del Fabbro *et al.* (2013).

RNA-seq data analysis

For alignment, reads were mapped onto the reference genome GCF_000188115.5_SL3.1 (Hosmani *et al.*, 2019, Preprint) using STAR v. 2.7.10 (Dobin *et al.*, 2013), a splice junction mapper designed for RNA-seq reads, under default parameters. The software htseq-count v. 2.0.2 (Anders *et al.*, 2015) was utilized to count the overlapping of reads with genes. The data were then used to identify differentially expressed genes (DEGs) using the DESeq2 package v1.34.0 (Love *et al.*, 2014). The variance on read count was calculated based on three biological replicates per condition by applying a negative binomial distribution to model the count data, thereby identifying genes showing significant expression changes among the different tested conditions. The DEG identification was performed after normalization of the count data and correction for multiple testing, both accounted by DESeq2, through the Wald test. During DESeq2 analysis, the shrinkage estimation of effect size (logarithmic fold change estimates) was used, to generate more accurate $\log_2$ fold change ($\log_2$FC) estimates considering the variability among replicates. A cut-off of the *P*-adjusted value <0.05 was used to classify a DEG in comparison with the reference [i.e. untreated (CTRL) in the NS condition and without AMF inoculation (NMYC)]. Both the identified DEGs and all transcripts of the tomato (*Solanum lycopersicum* L.) transcriptome were annotated through Blast2GO v5.2.5 (Conesa *et al.*, 2005) to obtain an updated functional annotation and to assign the corresponding Gene Ontology (GO) terms. A gene class functional enrichment analysis was then conducted using Blast2GO to reveal the biological processes, pathways, or other functional categories that are enriched among the identified DEGs.

Non-targeted polar metabolite profiling

Polar metabolites were separated using hydrophilic interaction liquid chromatography (HILIC) coupled to hybrid quadrupole-time of flight mass spectrometry (QTOF-MS) according to Andrade *et al.* (2021). HILIC separation was performed on a 2.1 mm×100 mm InfinityLab Poroshell 120 HILIC-Z, 1.9 µm (Agilent Technologies) using as solvents acetonitrile:water, 95:5 (v/v) (solvent A) and acetonitrile:water, 2:98 (v/v) (solvent B), both supplemented with ammonium formate, at 0.063% and 0.126%, respectively, at a flow rate of 0.3 ml min^{-1}. During chromatographic runs, column temperature was at 40 °C. Tomato leaf samples (10 mg dry weight) were extracted in triplicate by ultrasonication in 300 µl of 80% aqueous methanol supplemented with kinetin (1 mg l^{-1}) as internal standard for relative quantification. After extraction, samples were centrifuged at 10 000 rpm and 4 °C for 10 min and the supernatants recovered. Subsequently, supernatants were diluted 1:4

with acetonitrile (LC/MS grade) and filtered through 0.2 µm polytetrafluoroethylene syringe filters directly into chromatography vials. Mass chromatographic data were acquired in positive and negative ionization modes within the 50–1000 amu mass range. Nitrogen was used both as nebulization and desolvation gas (60 and 800 l h^{-1} and 350 °C temperature, respectively). During measurements, capillary and cone voltages were set at 3.5 kV and 30 V for positive electrospray and 2.3 kV and 30 V for negative electrospray, respectively. An additional acquisition function to obtain collision-induced dissociation information was set by performing a voltage ramp between 6 and 40 eV. To ensure accurate mass data acquisition, a lockmass reference (Leu-enkephalin, $[M+H]^+$ 556.2771 and $[M-H]^-$ 554.2614) was regularly infused during runs. Data files from each ionization mode were converted to mzML with msconvert (Chambers *et al.* 2012) and processed independently with XCMS (Smith *et al.*, 2006). Mass chromatographic features were annotated and grouped with CAMERA (Kuhl *et al.*, 2012). Peak areas were normalized to internal standard area and actual sample weight before statistical analyses. Significantly altered mass chromatographic features were subsequently identified as individual compounds by matching *m/z* and retention time values with those of authentic standards or tentatively annotated by matching experimental mass spectra in public databases (Metlin, Massbank or HMDB).

Statistical analysis

Statistical analyses were performed using R software (version 4.1.1). For colonization a two-way ANOVA was conducted to determine the effect of priming treatment (T) and stress condition (S). Regarding physiological, biometric (including CCI), and hormonal data (including proline), a three-way ANOVA was conducted to determine the effect of priming treatment (T), AMF inoculation (I), and stress condition (S), and of their interaction (T×I×S). A one-way ANOVA was applied to evaluate the combined effect of priming treatment and inoculation on RWC_{soil} data during the first 4 d of experiment. Standard error of the mean was calculated. Significant differences between means were determined using the Tukey's HSD test ($P \leq 0.05$). Graphs were generated using Prism v.9.5.0 software (GraphPad Software, Boston, MA, USA), while two-dimensional partial least squares discriminant analysis was executed using the MetaboAnalyst 6.0 software. An UpSet plot, heatmaps, and GO-enriched plot were elaborated, respectively, using the upsetplot, pheatmap, and ggplot2/dplyr R packages (R version 4.1.1).

Integration between transcriptomic and metabolomic data

To integrate data from RNA-seq and untargeted metabolomics, the DIABLO [N-integration, multi-block sparse partial least squares discriminant analysis (sPLS-DA)] framework was used (Singh *et al.*, 2019). The input datasets included RNA-seq data (normalized transcript counts of 14 315 genes resulted as DEGs in at least one condition) and metabolomic data (peak areas of 230 significantly altered mass chromatographic features), both normalized using variance stabilizing transformation and $\log_2$ transformed. For designing the model, the optimal number of features was obtained by performing sPLS-DA for each dataset. The final DIABLO model was refined through cross-fold validation repeated 10 times, considering the overall and the balanced classification error rates to identify the optimal number of components. The function *plotArrow()* was used to visualise the level of agreement between the datasets. Correlations between the different types of variables (RNA-seq and metabolomic data) were visualized through the relevant network plots via *network()*, which were inspected and customized by using Cytoscape v3.10.2. In the networks, edges showing weight higher than 0.95 were considered as key edges.

Results

Arbuscular mycorrhizal fungal root colonization

To assess differences in root mycorrhizal colonization, the mycorrhization frequency and arbuscule abundance in mycorrhizal root parts were evaluated (Fig. 1; Supplementary Table S1). No colonization structures were detected in uninoculated roots. Regarding inoculated plants, mycorrhization frequency tended to be higher following SA treatment with respect to other treatments (Fig. 1B), but no differences were observed among the samples in terms of arbuscule abundance (Fig. 1C). Under WS, CHI+SA roots displayed significantly higher mycorrhization frequency values compared with CTRL (Fig. 1B). Differently, the SS condition associated with SA and CHI+SA treatments led to a lower mycorrhization frequency than that of CTRL plants and higher arbuscule abundance values, although this was not statistically significant (Fig. 1C).

Effect of seed priming and mycorrhizal inoculation on tomato eco-physiological responses to water deficit and salinity

Over the whole duration of the water deficit time course (15 d), inoculated (MYC) SA and CHI+SA plants depleted water by transpiration more slowly with respect to both inoculated CTRL and all uninoculated (NMYC) plants, indicating that water extraction dynamics under WS were improved in mycorrhized SA- and CHI+SA-primed plants (Fig. 2A; Supplementary Table S2). This trend was particularly evident during the early times after water withdrawal, especially considering the significant changes occurring in the RWC_{soil} of pots containing CHI+SA_MYC plants. In the absence of seed priming, the inoculation with AMF alone did not alter the plant water loss dynamics, as RWC_{soil} values in CTRL_MYC plants were close, even slightly lower, in the last days of WS imposition than those measured in all NMYC plants (Fig. 2A). Moreover, the analysis of stomatal conductance (g_s) patterns in AMF-inoculated plants (Fig. 2B; Supplementary Table S3) showed that, compared with CTRL_MYC, CHI+SA_MYC and SA_MYC plants were able to maintain higher g_s rates during the first days of water shortage. This finding might therefore reflect a direct effect of seed priming in the regulation of water relations. Notably, this response was observed despite a more pronounced increase in leaf vapour pressure deficit that occurred in these plants (Fig. 2C). However, patterns of leaf transpiration (E) over stress progression were similar to those of g_s (Fig. 2D; Supplementary Table S3), likely indicating that the glasshouse ambient conditions were overall stable during the experimental trial and did not alter the plant physiological response to water deprivation. The combined effect of seed priming agents and AMF inoculation on the monitored parameters was also confirmed at the end of the WS time course (T_f) by comparing the different plant groups in terms

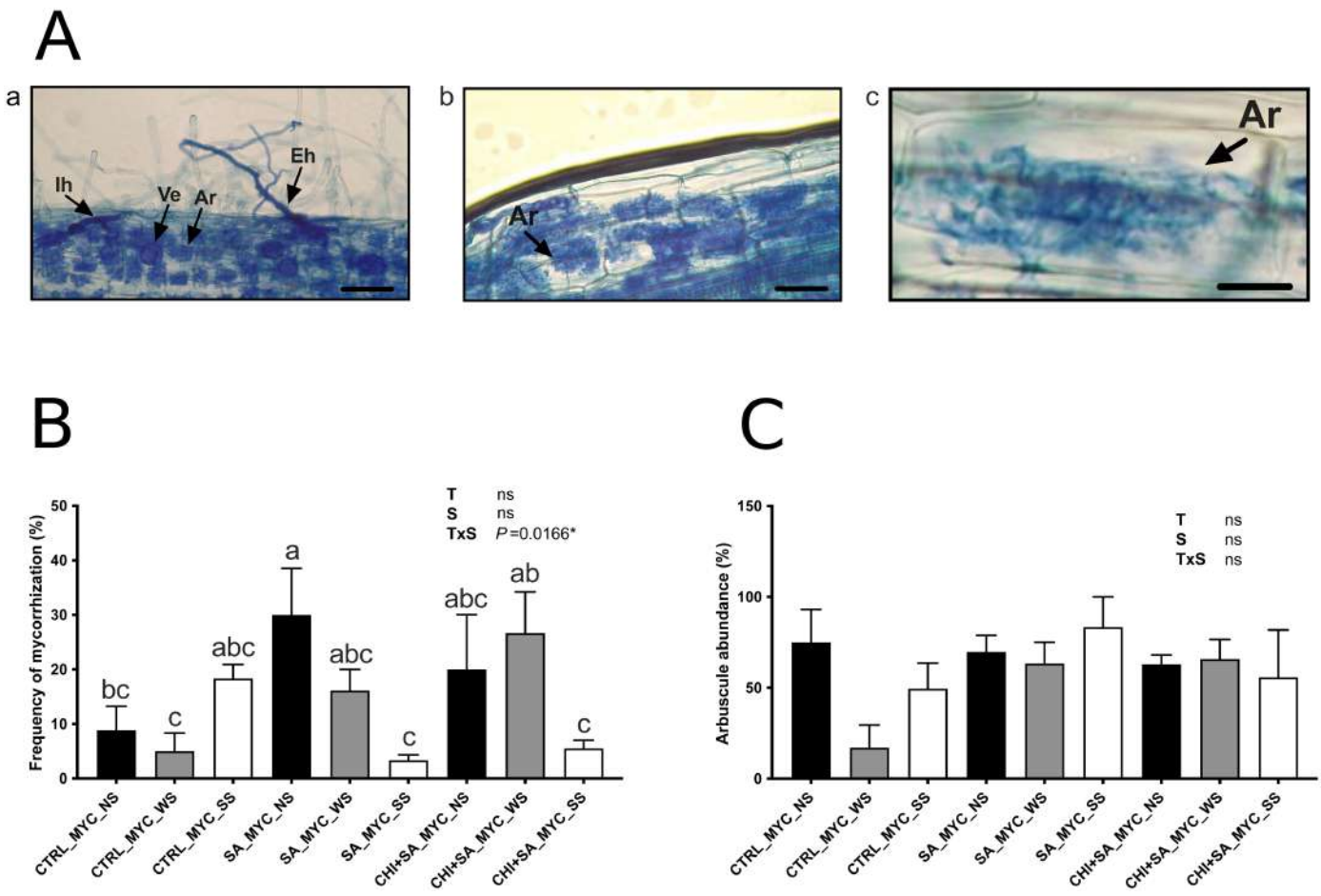

Fig. 1. Arbuscular mycorrhizal fungal (AMF) colonization assessment in tomato potted plants (*Solanum lycopersicum* cv. Moneymaker) inoculated with a commercial inoculum (MycAgro, *Funneliformis mosseae*). (A) Representative images of AMF-inoculated roots. Scale bars 120 µm (Aa), 60 µm (Ab), and 20 µm (Ac). (B) Frequency of mycorrhization in root system. (C) Arbuscule abundance in the colonized root system. Data are means ±SE. Lower case letters above bars denote significant differences as assessed by Tukey's HSD test ($P<0.05$) and are reported only when the priming treatment (T)×stress condition (S) interaction is significant. ns, not significant; *, significant at $P\leq0.05$. Analysis of variance on the single variables is reported in Supplementary Table S1. CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress; WS, water stress.

of Ψ_{stem} (Supplementary Fig. S1), g_s, and E measurements (Fig. 2E; Supplementary Fig. S2). Xylem pressure values were significantly higher in CHI+SA_MYC and SA_MYC plants with respect to all other plant groups subjected to WS. This condition was more accentuated in the comparison between SA_NMYC_WS and SA_MYC_WS plants: the former reached Ψ_{stem} around −0.8 MPa, attesting a water deficit condition for potted tomato plants of this age and size (1.5 months old and height 27 cm), whereas the latter showed a Ψ_{stem} close to −0.2 MPa, not significantly different from that of all NS plant groups (Supplementary Fig. S1; Supplementary Table S3). Although at T_f all WS plants showed g_s rates up to one-fourth lower than those of the corresponding NS controls, stomatal limitation in the inoculated SA_MYC plants was milder, resulting in significantly higher g_s values (Fig. 2E; Supplementary Table S3). The same occurred at for leaf transpiration patterns (Supplementary Fig. S2).

The effect of AMF inoculation and seed priming treatments, alone or in combination, was also noticed by analysing the leaf gas exchanges of tomato plants in response to SS at the end of the treatment with NaCl (200 mM). In the absence of AMF inoculation and stress imposition (NMYC_NS), g_s rates were significantly lower in SA- and CHI+SA-primed plants than in CTRL, likely suggesting an effect of the treatment in the regulation of stomatal opening. Such a condition was also observed upon SS. In fact, while g_s significantly dropped in all NMYC plants but to different extents based on the priming treatment, CHI+SA NMYC SS plants only showed a 3-fold decrease in g_s in comparison with CHI+SA NMYC NS plants. Furthermore, SA NMYC SS experienced a 2-fold, non-significant reduction in g_s with respect to SA NMYC NS plants (Fig. 3A; Supplementary Table S4). These data suggested that the negative effect of SS on stomatal closure was counteracted by chemical priming. In the presence of

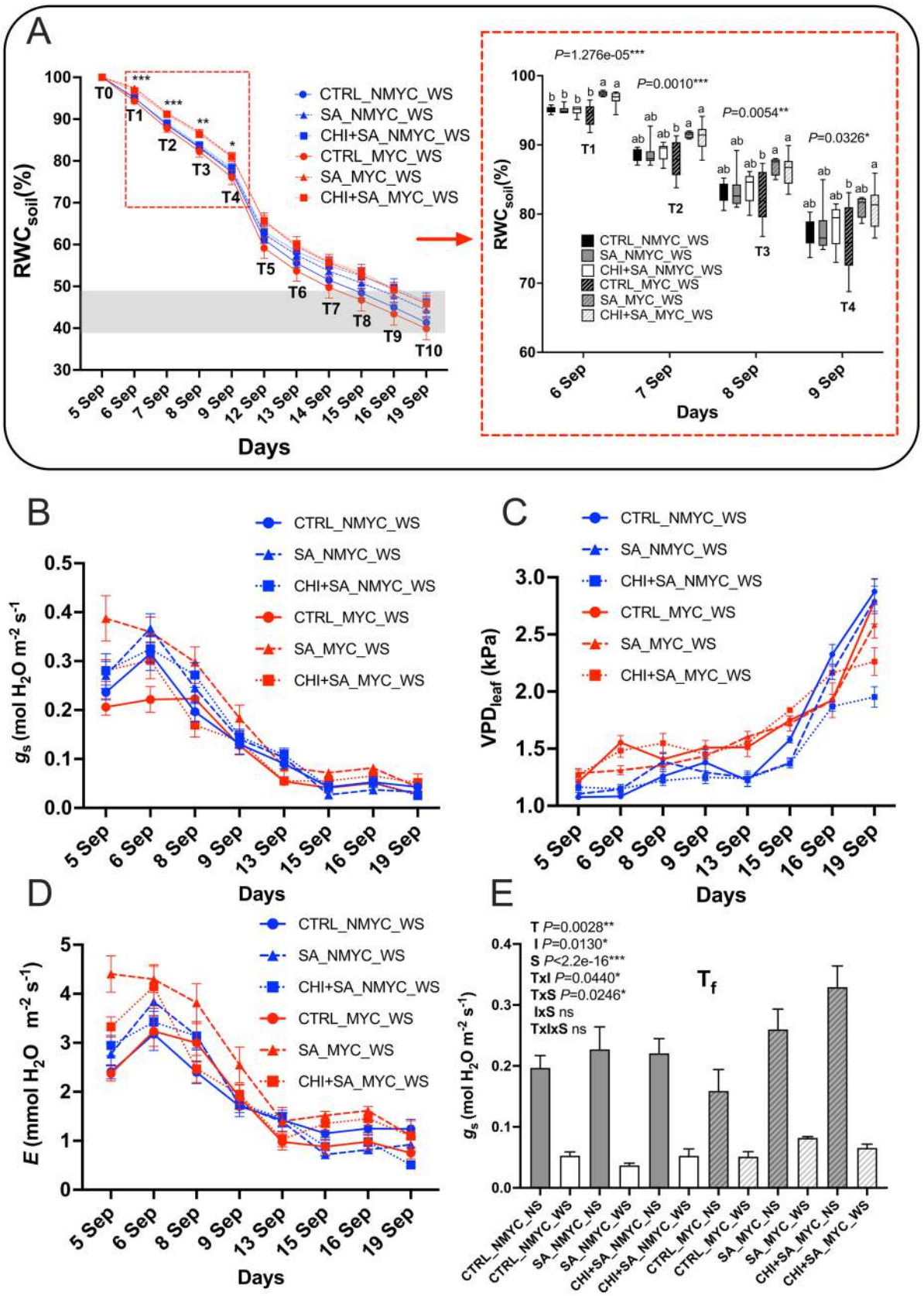

Fig. 2. Effects of priming treatment and arbuscular mycorrhizal fungal inoculation on the physiological performances of tomato (*Solanum lycopersicum* cv. Moneymaker) potted plants during a water stress time course. (A) Changes in soil relative water content (RWC$_{soil}$, %) monitored over the whole

duration of the water stress time course. The inset shows magnified differences in RWC_{soil} values among the diverse groups of plants during the first 4 d after water withdrawal (T1–T5). Asterisks denote significant differences determined by one-way ANOVA on RWC_{soil} data (*P≤0.05, **P≤0.01, ***P≤0.001) with corresponding values reported in the panel inset, while different lower case letters in the inset highlight significant differences among plant groups within each day of trial, as assessed by Tukey's HSD test (P<0.05). (B–D) Dynamic changes in the rates of stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) (B), leaf vapour pressure deficit (VPD_{leaf}, kPa) (C), and leaf transpiration (E, mmol H_2O m^{-2} s^{-1}) (D) monitored over the whole duration of the water stress time course. (E) Rates of g_s measured at the end (T_f) of the experiment. All results are means ±SE. Statistically significant differences were evaluated considering the priming treatment (T)×inoculation (I)×stress condition (S) interaction, and lower case letters are indicated only when the T×I×S interaction is significant, as assessed by Tukey's HSD test (P<0.05). ns, not significant; *P≤0.05, **P≤0.01, ***P≤0.001. Analysis of variance on the single variables is reported in Supplementary Tables S2, S3. CHI, chitosan; CTRL, control, untreated; SA, salicylic acid; MYC, inoculated; NMYC, not inoculated; NS, not stressed; WS, water stress.

AM symbiosis (MYC) and in the absence of stress, the stomata of CTRL plants were significantly more closed than those of CTRL_NMYC plants. Notably, such limitation was not observed in CHI+SA_MYC and SA_MYC plants compared with the respective NMYC plants. Nonetheless, independently of seed priming, MYC plants were all able to maintain g_s rates upon SS conditions, with values close to or even slightly higher than those measured in NS leaves (Fig. 3A). It thus emerged there was a significant effect of the tripartite interaction among priming treatment (T), inoculation (I), and stress condition (S) on the regulation of stomatal closure in response to salinity. This observation was also confirmed by analysing changes in E, which mirrored the trend described for g_s (Fig. 3B). The effect of the T×I×S interaction also significantly affected the plant photosynthetic capacity in terms of A_N. In fact, in the absence of both AMF inoculation and seed priming, net photosynthesis steeply decreased (by more than one-half) in CTRL_SS plants with respect to the CTRL_NS condition (Fig. 3C). Moreover, although under SS there was a trend towards increased A_N in AMF-inoculated plants without priming agents compared with uninoculated plants under the same conditions, the changes were not statistically significant. The A_N levels in SA_MYC_SS and CHI+SA_MYC_SS plants were similar to those in CTRL_NMYC_SS, suggesting that the benefits of priming and presence of AMF under SS were not additive, at least for this parameter (Fig. 3C). In parallel, eco-physiological responses, as well as AMF colonization, were also checked in plants treated with CHI alone (Supplementary Figs S3, S4). Although no statistically significant differences were detected, AMF colonization in those plants was lower, either in terms of frequency of mycorrhization in the unstressed condition (Supplementary Fig. S3A) or in terms of arbuscule abundance upon WS (Supplementary Fig. S3B), in comparison with the CHI+SA treatment. Moreover, no significant changes were observed by analysing stomatal conductance rates at the end of the WS and SS imposition in CHI- and CHI+SA-treated plants (Supplementary Fig. S4A, B). Finally, considering the effect of the combination of seed priming and AMF inoculation, it emerged that, unlike CHI+SA-treated plants, tomato plants treated with CHI alone were not able to maintain g_s levels upon SS (Supplementary Fig. S4B). For these reasons and based on the need to avoid adding further complexity to the analysed processes, we did not consider CHI for further analyses.

Biometric features (Supplementary Table S5) indicated that while significant reductions in shoot height were observed following exposure to both stresses and in the presence of AMF inoculation, the overall effects of MYC and seed priming on the other morphometric parameters were generally limited. Particularly, SA_MYC plants upon both stress conditions showed the lowest number of leaves (Supplementary Table S5). Stem diameter was primary affected by the imposed stress, as well as the leaf CCI. For these two parameters, independently of seed priming or AMF inoculation, both WS and SS tomato plants showed significantly lower values compared with NS ones (Supplementary Table S5).

Phytohormones and proline are differently accumulated in tomato leaves based on stress, priming treatment and arbuscular mycorrhizal symbiosis

Changes in the content of the main stress-associated phytohormones, namely SA, JA, ABA, and IAA, were investigated to elucidate the impact of either seed priming treatments or AMF inoculation or their combination on plant responses to the imposed stresses. In NMYC plants, SA concentrations decreased in CTRL and SA leaves upon both stress conditions. SA levels were still high in CHI+SA plants following WS, while they decreased during SS with respect to the corresponding NS samples, suggesting a regulation of the hormone metabolism based on the abiotic stress type (Fig. 4A; Supplementary Table S6). A completely opposite trend emerged from the analysis of MYC_NS leaf samples, which showed a much lower SA accumulation than NMYC_NS plants. Regardless of the priming treatment, SA content in MYC plants was always higher upon both WS and SS in comparison to MYC_NS plants (Fig. 4A). JA levels in NMYC plants were significantly lower in WS and SS leaves, independent of the priming treatment. This phenomenon did not occur in MYC plants, which during WS maintained JA content similar to (CTRL_MYC and CHI+SA_MYC) or even significantly higher than (SA_MYC) the corresponding NS plants. The T×I×S effects were particularly evident in SS plants. A reduction in the hormone levels was in fact noticed upon SS in CTRL_MYC and SA_MYC samples, whereas in CHI+SA_MYC_SS plants JA concentrations were close to those measured in NS and WS conditions (Fig. 4B; Supplementary Table S6).

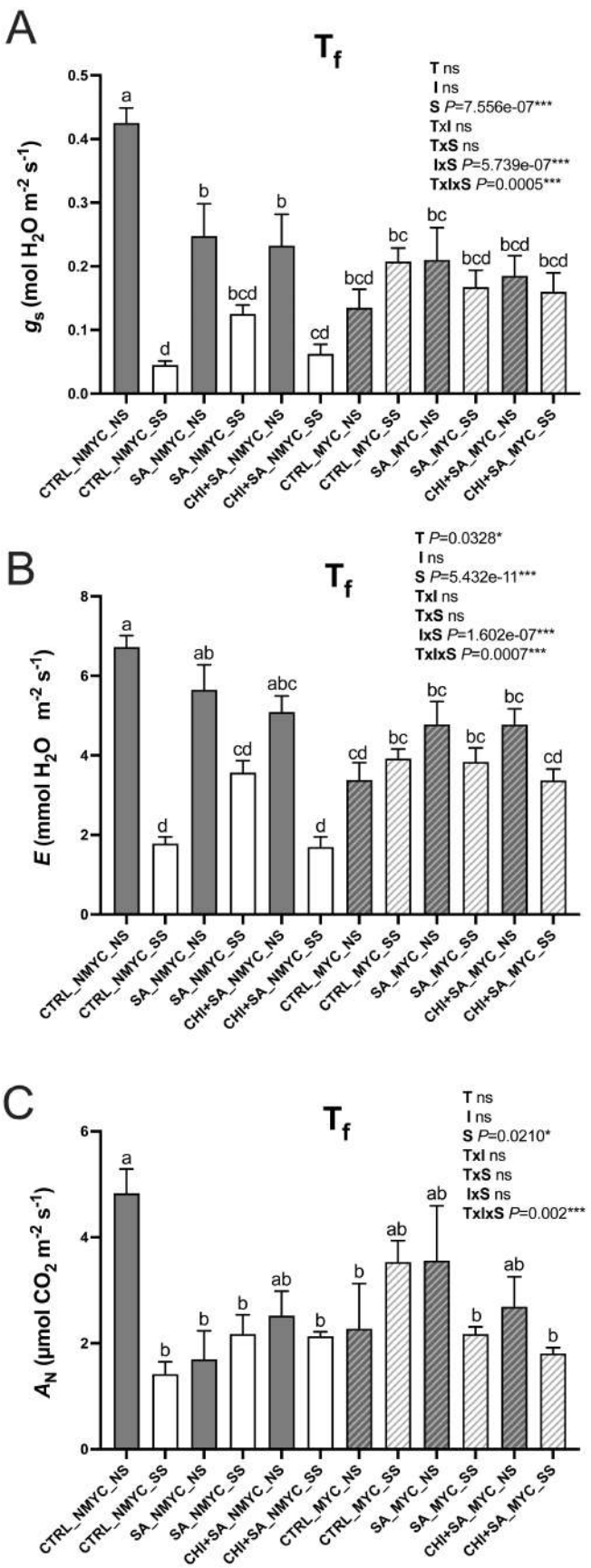

Fig. 3. Effects of priming treatment and arbuscular mycorrhizal fungal inoculation on the physiological performances of tomato (*Solanum lycopersicum* cv. Moneymaker) under salt stress at the end of the experiment. (A) Stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), (B) leaf transpiration (E, mmol H_2O m^{-2} s^{-1}), and (C) assimilation rates (A_N, µmol CO_2 m^{-2} s^{-1}). All results are reported as means ±SE. Lower case letters above bars denote significant differences as assessed by Tukey's HSD test (P<0.05) and are reported only when the priming treatment (T)×inoculation (I)×stress condition (S) interaction is significant. ns, not significant; *P≤0.05, **P≤0.01, ***P≤0.001. Analysis of variance on the single variables is reported in Supplementary Table S4. CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress.

Upon stress conditions, ABA content increased in NMYC plants, with no significant variations between WS and SS. In AMF-inoculated plants, except for a slight, but not significant, increase in CTRL_MYC_WS leaves, ABA levels were similar among all other samples (Fig. 4C; Supplementary Table S6). The only variable that significantly influenced IAA accumulation patterns was the stress condition, particularly salinity both in MYC and NMYC plants (Fig. 4D, Supplementary Table S6). With regard to the osmoprotectant solute, proline, levels of the metabolite increased in NMYC plants based on the stress application, with a steeper accumulation in SS samples (Fig. 4E; Supplementary Table S6). A similar pattern occurred in CTRL plants in the presence of AMF inoculation. The impact of T×I×S interaction was instead evident in the case of inoculated-seed primed plants, but with a different trend based on the seed priming agent. Indeed, in SA_MYC samples proline increased both upon WS and upon SS with respect to NS conditions, whereas in CHI+SA significantly higher concentrations of the solute were measured exclusively upon SS (Fig. 4E; Supplementary Table S6).

General overview of tomato leaf transcriptomic profiles

To investigate systemic transcriptional changes, a transcriptomic approach via RNA-seq was performed. After Illumina NovaSeq 6000 sequencing of 54 libraries, a total of 1 857 600 000 raw reads were generated. The mean percentage of mapped reads obtained from all analysed samples to the reference tomato genome was 88.5% (Dryad Dataset S1; https://doi.org/10.5061/dryad.dbrv15fb9). Total read counts for each sample are reported in Dryad Dataset S2. A total of 14 318 genes (out of the 37 604 annotated *Solanum lycopersicum* genes) were differentially regulated across all samples. Complete lists of identified DEGs in the 17 conditions in comparison with the control (CTRL_NMYC_NS) are reported in Dryad Datasets S3–S19. A significant transcriptomic remodelling occurred following stress exposure, and particularly in plants subjected to SS, with SA_MYC_SS and CHI+SA_MYC_SS showing the highest number of DEGs (7150 and 6890, respectively; Fig. 5A; Dryad Dataset S20). CHI+SA_MYC_SS was the condition showing the highest number of up-regulated DEGs (3512 genes; Fig. 5A), while the highest number of down-regulated genes was detected in SA_MYC_SS leaves (3696 genes; Fig. 5A). NMYC_NS plants *plus* CHI+SA and SA were had limited leaf transcriptomic reprogramming compared with

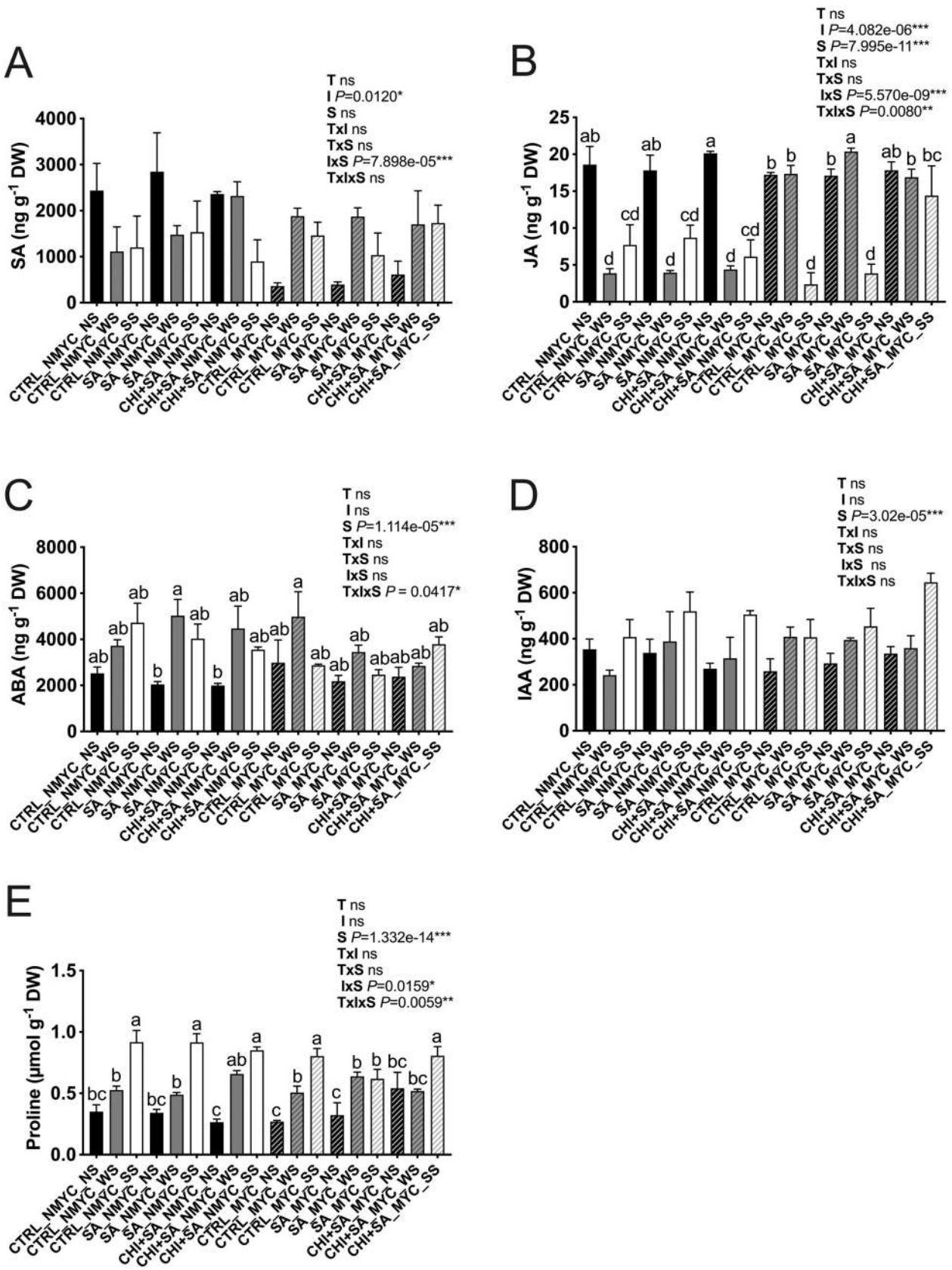

Fig. 4. Content of phytohormones and proline in leaves of tomato (*Solanum lycopersicum* cv. Moneymaker) potted plants. Concentrations of salicylic acid (SA) (A), jasmonic acid (JA) (B), abscisic acid (ABA) (C), indole-3-acetic acid (IAA) (D), and proline (E). For phytohormones, data are expressed as

ng g^{-1} dry weight (DW); for proline, data are expressed as μmol g^{-1} DW. All results are the mean ±SE. Lower case letters above bars denote significant differences as assessed by Tukey's HSD test ($P<0.05$) and are reported only when the priming treatment (T)×inoculation (I)×stress condition (S) interaction is significant. ns, not significant; *$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$. Analysis of variance on the single variables is reported in Supplementary Table S6. CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress; WS, water stress.

CTRL_NMYC_NS (166 and 107 up- and down-regulated genes for CHI+SA_NMYC_NS, and 171 up- and 69 down-regulated genes for SA_NMYC_NS; Fig. 5A).

From the identification of DEGs in all samples compared with the control condition (CTRL_NMYC_NS), two distinct DEG clusters were detected (Fig. 5B). Cluster I harboured DEGs mainly up-regulated by both water deficit and salinity in MYC plants and mainly down-regulated in the NS condition (Fig. 5B). Cluster II included DEGs primarily up-regulated in NS plants, and particularly in the absence of AMF inoculation (Fig. 5B).

A GO enrichment analysis allowed grouping of the identified up- and down-regulated DEGs into functional classes based on biological process (Supplementary Figs S5, S6). In the NS condition, a high number of GO terms were identified and particularly in SA_MYC_NS and CHI+SA_MYC_NS (Supplementary Fig. S5A, S6A). It is worth noting that 'ABA-8′-hydroxylase activity', correlated to ABA catabolic processes, was among the enriched terms in CHI+SA_MYC_WS (Supplementary Fig. S6B). This was in agreement with the lower ABA levels quantified in CHI+SA_MYC_WS leaves in comparison with CTRL_MYC_WS plants (Fig. 4C).

The impact of abiotic stress conditions on tomato leaf transcriptome

A core of stress marker genes was differentially regulated upon both stresses, independently of inoculation and seed priming. Particularly, 274 DEGs were up-regulated by WS (Dryad Dataset S27). Most of them encoded proteins related to stress response (Dryad Dataset S21), including two genes encoding dehydrins, i.e. *ERD14* and *TAS14*. In detail, *TAS14* was particularly expressed upon WS in NMYC plants (mean $\log_2$FC value of 6.03±0.44) compared with MYC samples (mean $\log_2$FC of 3.96±0.87), consistently with the higher ABA content in NMYC with respect to MYC plants (Fig. 4). Four genes were exclusively up-regulated upon WS, regardless of AMF inoculation and seed priming treatment (Fig. 6A): an abscisic stress-ripening protein 1 (*ASR1*), a serine/threonine-protein kinase SAPK10 (LOC101268620), a class I heat shock protein (LOC101256536), and a JA *O*-methyltransferase-like (LOC101265985; Dryad Dataset S20).

Considering SS, irrespective of the AMF inoculation and seed priming treatments, 517 DEGs were found to be up-regulated. Among them, genes involved in sugar transport and calcium signalling cascades, such as sodium/calcium exchangers, scarecrow-like transcription factors (TFs), calcium binding proteins, and heat shock proteins, were identified (Dryad Dataset S22). Additionally, four genes, encoding the transcription regulatory protein DOT6 (LOC104644498), a protein SUPPRESSOR OF MORE AXILLARY GROWTH (MAX) 2-like (LOC101262825), a probable serine/threonine-protein kinase PBL1 (LOC101264267), and an uncharacterized protein (LOC101245413), were exclusively regulated upon SS (Fig. 6A; Dryad Dataset S20).

The impact of arbuscular mycorrhizal symbiosis on tomato leaf transcriptome

Transcriptome reprogramming events dependent on AMF inoculation were also explored. Starting from CTRL_MYC_NS, 2026 genes (1153 up-regulated, 873 down-regulated) were differentially expressed. Among them, TFs, genes involved in defence, signal transduction, and proteolysis, and genes encoding transporters and heat shock proteins were detected. A comparison of this dataset with that obtained by Cervantes-Gámez *et al.* (2016) on the leaf transcriptome of tomato AMF-inoculated plants led to identification of 28 genes (18 up-regulated, 10 down-regulated) showing a similar expression trend (Dryad Dataset S23). The remaining 45 DEGs in common with the previous dataset showed a different expression profile (Dryad Dataset S23), likely due to the differences in the genotypes of the partners and in growth conditions. Looking at the 25 most up-regulated genes in CTRL_MYC_NS leaves, the most up-regulated of them (Dryad Dataset S3) encoded a nudix hydrolase 8-like (LOC101261537, $\log_2$FC=6.15), which is known to play a role in plant defence (Fonseca and Dong, 2014), two genes encoded a subtilisin-like protease SBT1.9 (sbt4d and LOC101257301), and two genes were involved in phosphorous homeostasis, one encoding an inorganic pyrophosphatase 1-like (psi14C) and one encoding purple acid phosphatase 17-like (LOC101249252). Among the up-regulated genes also a probable carotenoid cleavage dioxygenase 4 (LOC101250535) was detected, while a gene encoding a 9-*cis*-epoxycarotenoid dioxygenase (*LeNCED1*) was slightly down-regulated together with a carotenoid 9,10(9′,10′)-cleavage dioxygenase 1-like gene (*LeCCD1B*; Dryad Dataset S20). These data partially supported what was observed in terms of ABA accumulation patterns in these plants (Fig. 4C).

Looking at MYC plants subjected to WS (MYC_WS), 199 DEGs were identified as up-regulated. Forty-five out of these corresponded to pentatricopeptide repeat (PPR)-containing proteins (Dryad Dataset S24). Furthermore, a gene encoding a 4-coumarate-CoA ligase-like 5 (LOC101251665) was found slightly up-regulated, regardless of priming treatment

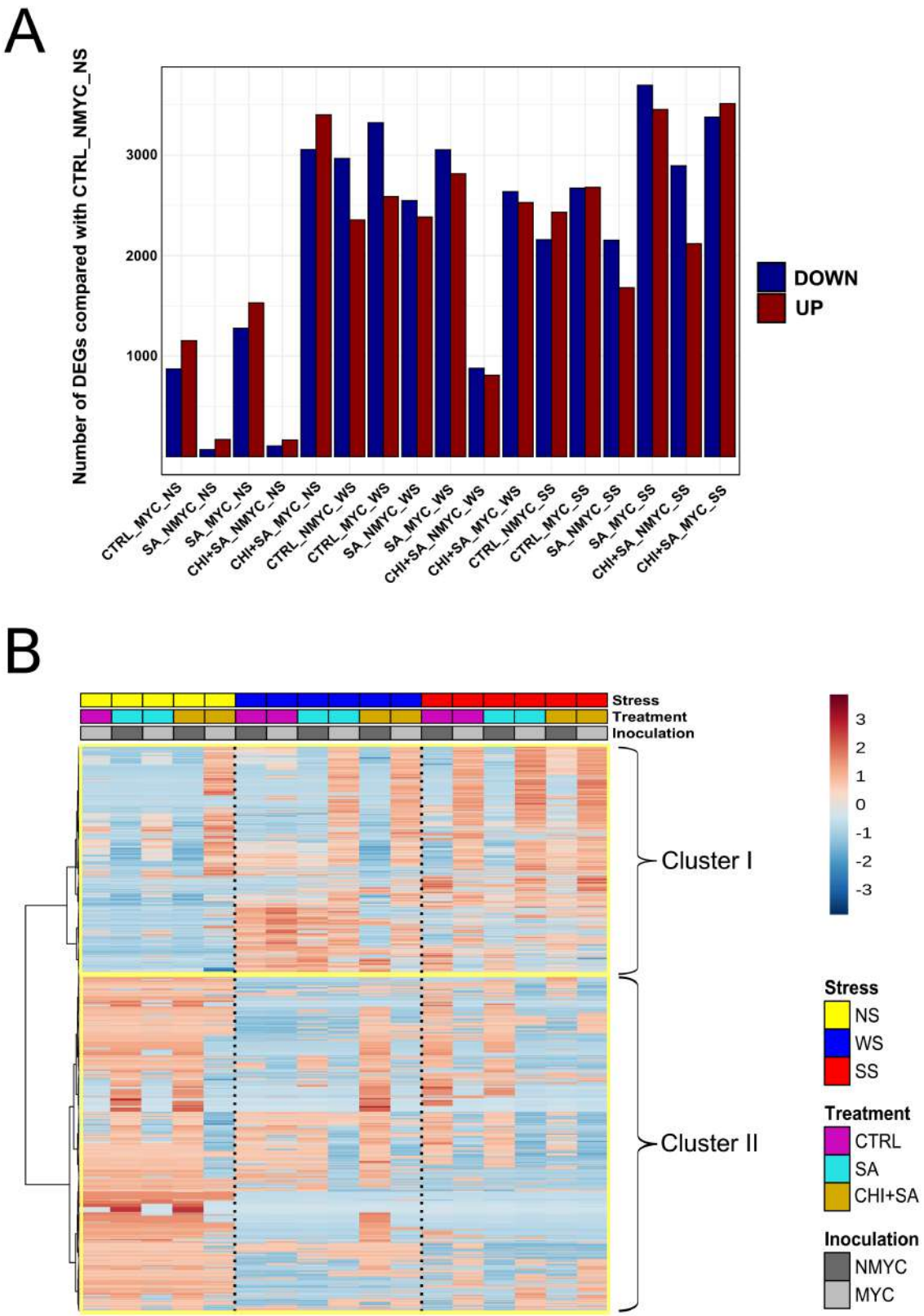

Fig. 5. Overview of differentially expressed genes (DEGs) compared with control (CTRL_NMYC_NS) between the 17 different conditions. (A) Total number of up- and down-regulated DEGs generated using the *ggplot2* R package (R version 4.1.1). (B) Heatmap and clustering (Pearson distance) of the normalized expression levels of the 14 316 DEGs among the different conditions, generated using the *pheatmap* R package (R version 4.1.1). Columns indicate different conditions, and rows indicate genes and their expression profiles. Colour intensity indicates different levels of expression (log_2 fold change). CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress; WS, water stress.

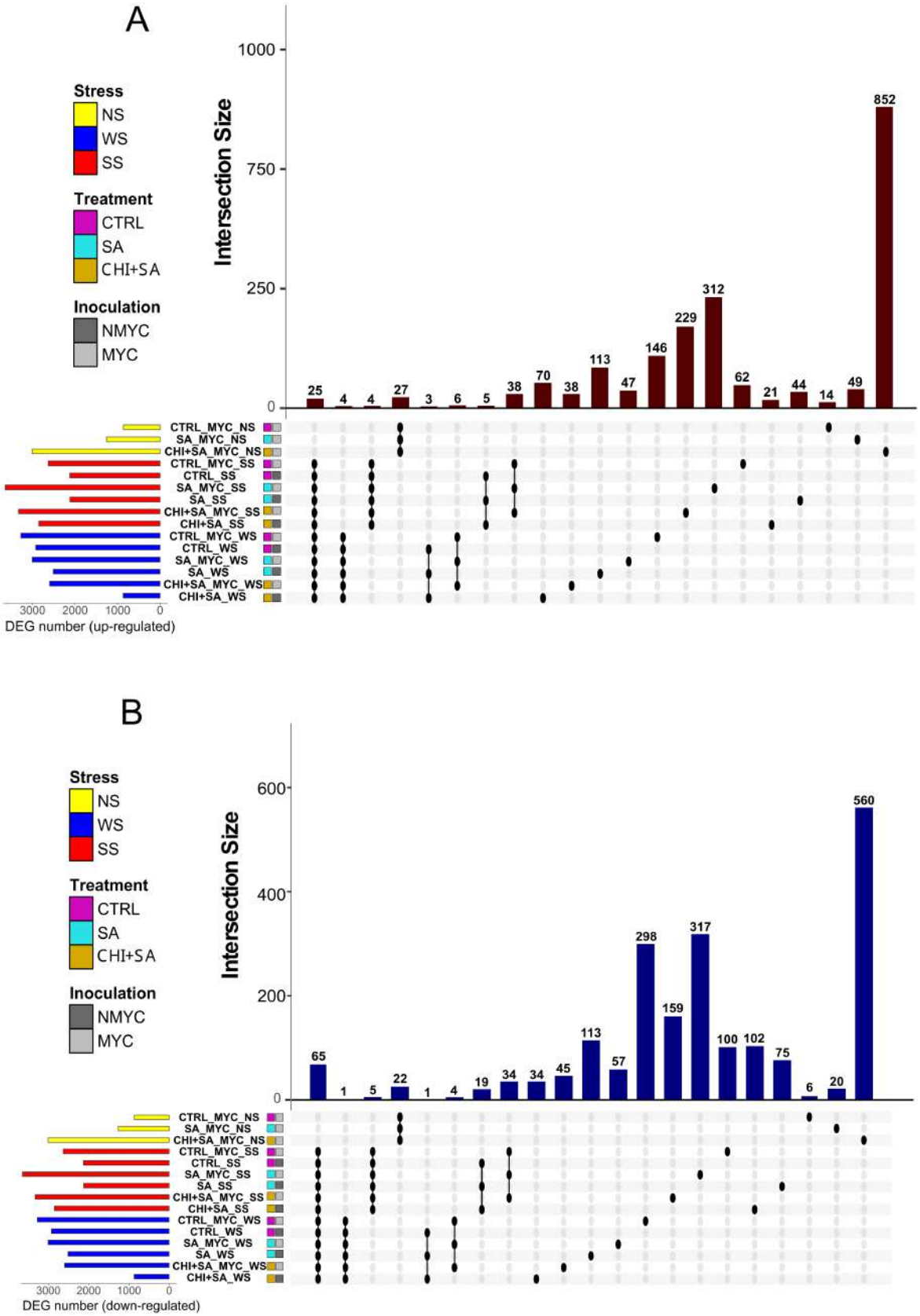

Fig. 6. UpSet plot to summarize up-regulated (A) and down-regulated (B) differentially expressed genes (DEGs) overlapping in each condition. Black dots in each panel matrix represent unique and overlapping DEGs. Connected circles indicate an intersection of DEGs among conditions. Bottom left horizontal bars show the number of total detected DEGs per condition, while the top bar graph in each panel summarizes the number of DEGs for each unique or overlapping combination generated using the upsetplot R package (R version 4.1.1). CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress; WS, water stress.

(Dryad Dataset S20). Six transcripts were exclusive to MYC_WS plants (Fig. 6A), including genes encoding an inorganic pyrophosphatase 1-like (LOC101246036), a serine/threonine-protein kinase (LOC101262118) and a DPH4 homologue (LOC104645200; Dryad Dataset S20).

MYC plants upon salt stress (MYC_SS) showed the up-regulation of 464 DEGs (Dryad Dataset S25Among these genes, 63 encoded putative PPR-containing proteins, and it is worth noting that 38 DEGs were identified as exclusive for this condition (Fig. 6A; Dryad Dataset S20).

The combined effect of arbuscular mycorrhizal symbiosis and priming agents on leaf transcriptome

A relevant combined effect of AMF inoculation and seed priming in the reprogramming of the leaf transcriptome was observed under both NS and stressed conditions. Conversely, the presence of priming agents alone in NMYC plants only slightly perturbed the leaf transcriptome. Among the most up-regulated genes in the SA_NS treatments, a homologue of *LEA14* was detected (*ER5*, log2FC=5.26), already reported to protect pepper against desiccation (Zhang *et al.*, 2023). Considering the CHI+SA_NS treatment, one of the most up-regulated genes encoded a dehydration-responsive element-binding protein 1A-like (LOC104648613, $\log_2$FC=5.24) involved in oxidative stress responses (Zhang *et al.*, 2021). Looking at mycorrhizal plants, among the first 25 mostly up-regulated genes in SA_MYC_NS, 17 shared an expression profile similar to CTRL_MYC_NS (Dryad Dataset S26). In CHI+SA_MYC_NS plants a higher number of DEGs was identified (6455 DEGs; 3400 up-regulated, 3055 down-regulated) with respect to CTRL_MYC_NS and CHI+SA_NMYC_NS (Fig. 5A; Dryad Dataset S19). Transcripts involved in cell wall metabolism, defence, and transport were up-regulated in CHI+SA_MYC_NS (Dryad Dataset S19). The most up-regulated gene putatively encoded an *O*-acyltransferase WSD1-like (LOC101248922, $\log_2$FC=9.42) involved in wax metabolism.

Upon WS, the combined effect of SA priming and AMF inoculation on leaf transcriptome resulted in the up-regulation of several genes encoding heat shock proteins (36), abscisic acid 8′-hydroxylases (2), proteins putatively related to defence/resistance (23), ethylene-responsive TFs (8), and several PPR-containing proteins (198). Among the 47 genes exclusively up-regulated in SA_MYC_WS (Fig. 6A), the most expressed encoded a gibberellin 2-β-dioxygenase ($\log_2$FC=6.16) and a dehydration-responsive element-binding protein ($\log_2$FC=2.28; Dryad Dataset S27). Similarly, SA_MYC_SS plants showed up-regulation of transcripts putatively related to defence and resistance (23), PPR-containing proteins (218), heat shock proteins (13), and ethylene-responsive TFs (18). The most exclusively up-regulated gene encoded a feruloyl CoA ortho-hydroxylase ($\log_2$FC=7.44) linked to the phenylpropanoid pathway (Sun *et al.*, 2015; Dryad Dataset S28).

In CHI+SA_MYC_WS, in addition to the up-regulation of genes encoding several PPR-containing proteins (190) and heat shock proteins (27), two genes related to ethylene-responsive TFs were strongly up-regulated (LOC101268109, $\log_2$FC=11.57 and LOC101246484, $\log_2$FC=5.46) together with genes encoding an ABA hydroxylase (LOC101249565) and an abscisic-aldehyde oxidase (AO1). An ABA 8′-hydroxylase was found among the first 20 most up-regulated genes in this sample (LOC101254720, $\log_2$FC=6.45; Dryad Dataset S13). The strong up-regulation of a gene coding for an ascorbate-specific transmembrane electron transporter (LOC101259871, $\log_2$FC=6.25) in CHI+SA_MYC_WS, as well as in SA_MYC_WS ($\log_2$FC=5.69), was also observed. Compared with the other tested conditions, 38 up- and 45 down-regulated DEGs were exclusively identified in CHI+SA_MYC_WS plants (Fig. 6A; Dryad Dataset S29). Looking at CHI+SA_MYC_SS, the up-regulation of genes encoding heat shock proteins-related (35), ethylene-responsive TFs (22), and proteins associated to defence response (26) were detected. Among the genes exclusively up-regulated in CHI+SA_MYC_SS leaves was a glutathione-*S*-transferase gene (LOC101266443; Dryad Dataset S30).

A look into whole leaf metabolite profiling

Elaboration of metabolite profiling data revealed 14 516 chromatographic mass features differentially expressed in response to the various conditions (Drayd Datasets S31–S32). Among this mass feature dataset, 372 chromatographic masses were putatively annotated to chemically different compounds (Dryad Datasets S33, S34). Supervised bi-dimensional analysis (2D-PLSDs) of these 372 leaf chromatographic masses, which were potentially among the most significant, showed a clear separation among the different stress conditions (NS, WS, SS; Fig. 7A). Furthermore, AMF inoculation had an impact under both stressed and NS conditions, as demonstrated by output of 2D-PLSDs (Fig. 7B). Out of the 372 detected chromatographic masses, 230 individual metabolites were annotated (Dryad Dataset S35), and 64 of those were further selected as they exhibited the highest variability in the accumulation intensity ($\log_2$FC>1 compared with CTRL_NMYC_NS) in at least one condition (Dryad Dataset S36). Regardless of AMF inoculation and seed priming treatments, cluster analysis of the 64 selected metabolites highlighted that WS and SS had a significant impact on their modulation, leading to the identification of three distinct clusters (Fig. 7C). Cluster I included metabolites that were mainly accumulated under both stress conditions; cluster II grouped metabolites more abundant in response to salinity; and cluster III was characterized by metabolites showing higher concentrations under NS and WS conditions. During WS, MYC plants accumulated more of those compounds compared with NMYC plants (Fig. 7C).

A closer look at the distribution of single metabolites within each cluster revealed that in cluster I, in the absence

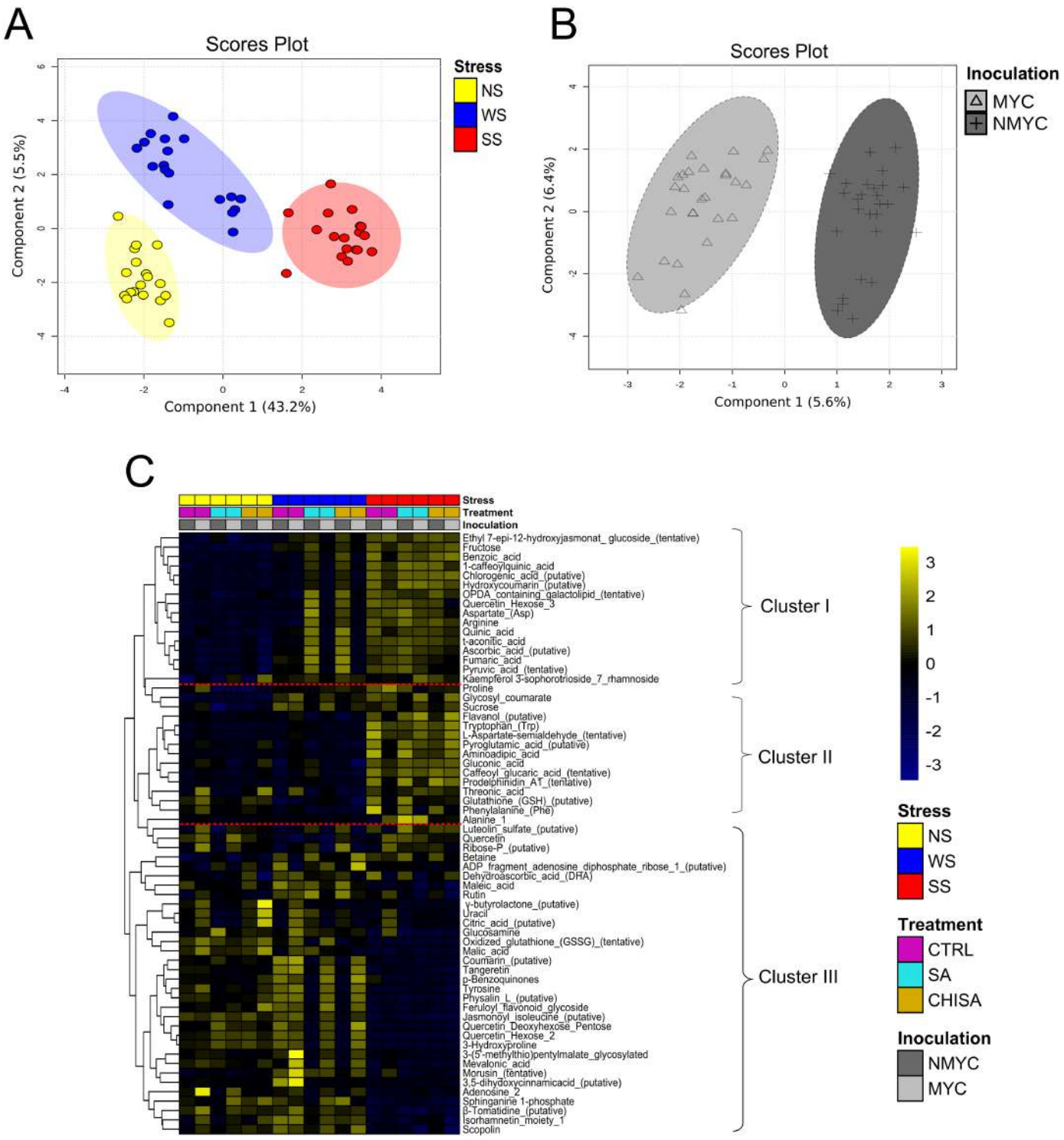

Fig. 7. Overview of metabolomic changes in tomato (*Solanum lycopersicum* cv. Moneymaker) leaves under the different tested conditions: inoculated (MYC), not inoculated (NMYC), seed-treated with chemical priming [salicylic acid (SA), chitosan (CHI)+SA], untreated (CTRL), not stressed (NS), water stressed (WS), or salt stressed (SS). (A) Two-dimensional partial least squares discriminant analysis (2D-PLSDAs) of the 372 most significant chromatographic masses in NS and stressed (WS, SS) conditions, regardless of priming treatment and inoculation. (B) 2D-PLSDAs of the 372 most significant chromatographic masses in NMYC and MYC plants, regardless of priming treatment and stress condition. 2D-PLSDA plots were generated with MetaboAnalyst software, considering the normalized dataset as $\log_{10}$. (C) Heatmap and clustering (Euclidean distance) of 64 differentially accumulated metabolites selected among those having the highest variability in accumulation intensity ($\log_2$ fold change >1 compared with CTRL_NMYC_NS) in at least one condition. The heatmap was generated using the *pheatmap* R package (R version 4.1.1). Data in cells are $\log_2$-transformed mean values of three biological replicates.

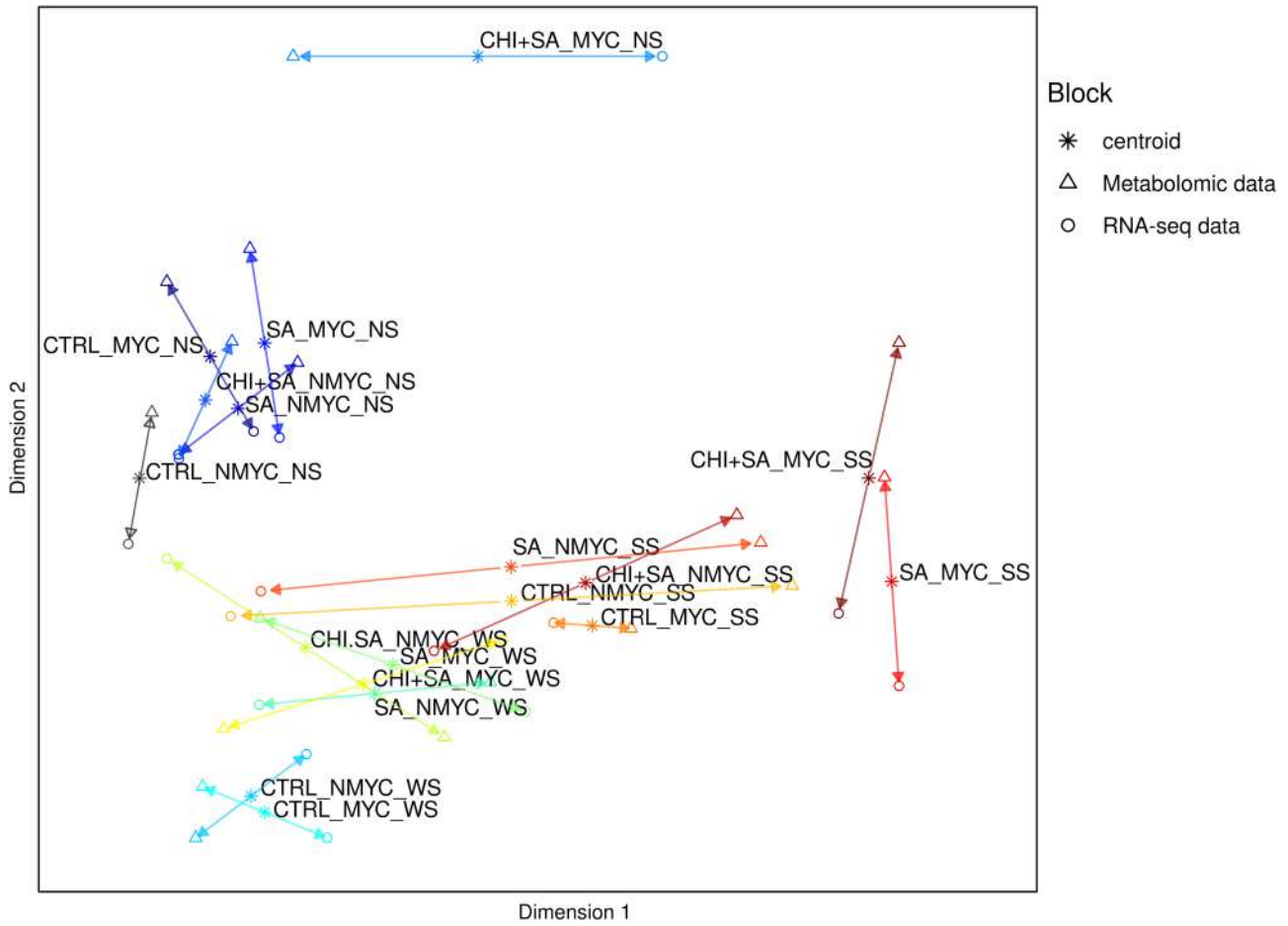

Fig. 8. Arrow plot from multiblock sPLS-DA performed on the metabolomic and RNA-seq data of all tested conditions. Start of the arrow indicates the centroid between all data sets for each condition, while tips of the arrows indicate their location in each block. CHI, chitosan; CTRL, control, untreated; MYC, inoculated; NMYC, not inoculated; NS, not stressed; SA, salicylic acid; SS, salt stress; WS, water stress.

of AMF inoculation, the combined impact of seed priming (SA and CHI+SA) and WS resulted in a higher accumulation of primary metabolites like fructose, ascorbic acid, pyruvic acid, arginine, and aspartate, and of secondary metabolites, including fumaric acid, *trans*-aconitic acid, quinic acid, benzoic acid, quercetin hexose, 12-oxo-phytodienoic acid (12-OPDA), and 1-caffeoylquinoic acid. Metabolites included in cluster II were mainly influenced by SS conditions, and among the most abundant there were prodelfinidin A1, glutathione (GSH), aminoadipic acid, pyroglutamic acid, L-aspartate semialdehyde, caffeoyl-glucaric acid, and proline. Notably, proline displayed accumulation patterns consistent with those obtained through the target analytical approach (Fig. 7C). The interaction between AMF inoculation and WS positively influenced most of the metabolites grouped in cluster III, which comprised coumarin, mevalonic acid, physalin L, jasmonyl isoleucine, tyrosine, tangeretin, *p*-benzoquinones, quercetin deoxyhexose pentose, 3-hydroxyproline, quercetin hexose, feruloyl flavonoid glycoside, and β-tomatidine (Fig. 7C).

Integration between transcriptomic and metabolomic data reveals overlapping and distinct metabolic response to water stress and salt stress in tomato plants in the presence of seed priming agents and arbuscular mycorrhizal fungal inoculation

The DIABLO framework allowed the identification of potential interactions between transcripts and metabolites in the different tested conditions. The arrow plot from multiblock sPLS-DA revealed distinct clusters for NS, WS, and SS conditions, with the exception of CHI+SA_MYC_NS (Fig. 8). Short arrows under NS conditions indicated a close correlation between RNA-seq and metabolomic data for both MYC and NMYC, while longer arrows were observed for the CTLR_NMYC_SS, SA_NMYC_SS, and CHI+SA_NMYC_SS conditions, suggesting less consistency between the two datasets (Fig. 8). Overall, the obtained output showed that, upon SS, the combination of AMF inoculation and chemical seed priming treatments functioned distinctly from all other conditions in regulating the plant metabolic response. Conversely, under WS

the main distinction was noticed between untreated (CTRL) and treated (CHI+SA and SA) plants, independent of AMF inoculation (Fig. 8). Moreover, a network analysis conducted on the same datasets highlighted that overlapping and specific metabolic routes were regulated in AMF-inoculated plants treated with priming agents depending on WS or SS (Supplementary Figs S7, S8). Particularly, the activation of transcripts and corresponding secondary metabolites involved in defence processes (such as tomatine, tomatidine, and flavonols) and in lipid signalling (e.g. diacylglycerol and phosphatidylglycerol) represented a response common to the two abiotic stresses (Supplementary Figs S7, S8). Nonetheless, signals mainly dependent on either WS or SS were also observed: this was the case for the activation of genes/metabolites with a strong antioxidant role (e.g. tangeretin, kaempferol, physalin) or involved in cell homeostasis regulation and signal transduction, including hormone-signalling, upon WS (Supplementary Fig. S7), and of specific sugar signalling routes as the basis of osmotic response upon SS (such as genes encoding sugar transporters, sugar biosynthetic enzymes, photosynthesis-related proteins, in association with osmoprotectant solutes, like proline; Supplementary Fig. S8).

Discussion

Priming of plants with chemical or biological agents has received increasing attention in the last years (Savvides *et al.*, 2016; Gohari *et al.*, 2024). It has already been shown that the combined use of different agents, potentially acting synergistically, is a promising strategy for stimulating stress tolerance in plants (Balestrini *et al.*, 2018; González Guzmán *et al.*, 2022; Gohari *et al.*, 2024). In this framework, we investigated the effectiveness of the potential beneficial effects of chemical seed treatments (i.e. SA or CHI+SA) alone or in combination with post-germination AMF inoculation. The evaluation of the effect of seed priming on the ability of AMF to colonize roots highlighted that the mycorrhization varied based on the interaction between stress conditions and treatments. Arbuscule abundance was not significantly affected by the tested variables, suggesting that the seed priming treatments did not deeply affect the ability of AMF to colonize the root, at least considering these tomato and fungal genotypes.

Chemical seed priming and arbuscular mycorrhizal fungal inoculation modify the tomato plant response to water deficit and salinity

Overall, our results showed that the interplay between seed priming and AMF inoculation positively modulated plant responses to the imposed stresses (Fig. 9). During WS, primed AMF-inoculated plants were able to maintain higher stomatal conductance and transpiration rates in comparison with plants subjected to either seed priming or inoculation alone. These plants also exhibited a lower water loss over the experimental trial, in agreement with previous studies demonstrating that SA and CHI improved water stress tolerance in crop species (Hidangmayum *et al.*, 2023; Hidangmayum and Dwivedi, 2023; Das *et al.*, 2023). The impact of AM symbiosis on plant performance in conditions of water limitation has been extensively studied (Balestrini *et al.*, 2017), showing an improvement of tolerance for different plant species, including tomato (Chitarra *et al.*, 2016; Ruiz-Lozano *et al.*, 2016; Rivero *et al.*, 2018; Volpe *et al.*, 2018). The novel aspect here is that the synergistic effect of seed priming and subsequent AMF colonization further boosts the plant's ability to endure water deprivation. The collected data further suggested that the combination of CHI+SA and AMF inoculation was the most effective one in providing a long-term protective effect against water deficit (as attested by measurement of g_s and Ψ_{stem} values at the end of the stress imposition), whereas chemical priming alone could ultimately lead to detrimental effects (at least for some physiological parameters, as it was the case for Ψ_{stem} in SA_NMYC_WS plants). Although the effect of exogenous application of CHI and SA on stomatal closure and photosynthetic processes upon water stress is well documented (Janda *et al.*, 2014; Hidangmayum *et al.*, 2019; Hidangmayum *et al.*, 2023; Hidangmayum and Dwivedi, 2023; Gao *et al.*, 2023; Silva *et al.*, 2023), available literature investigated short-term (over days) effects of these compounds in either seedlings or leaves of treated plants. Moreover, most information deals with foliar application rather than seed priming treatment, the latter representing a more recent research field (Gohari *et al.*, 2024). Our findings provide novel insights into this subject, showing that long-lasting (2 months after sowing the primed seeds) priming defence responses could also be established, resulting in improved physiological adaptation to water shortage conditions.

Notably, physiological adjustments also occurred when tomato plants were exposed to salinity (SS). AM symbiosis alone improved the plant's capacity to counteract SS effects in terms of stomatal limitation, as CTRL_MYC plants did not experience the same steep drop in g_s and E as CTRL_NMYC. This is in agreement with the key role of AMF in the regulation of tolerance mechanisms to salt and osmotic stress reported in several plant species (Porcel *et al.*, 2012; Pollastri *et al.* 2018; Chandra *et al.*, 2023). Unlike WS, during SS exposure, the most important effect of the combined treatment resulted in a balance of the plant's physiological responses, with gas exchange rates similar for NS and SS conditions. Already in the absence of stress application, stomatal opening of CTR_MYC and SA and CHI+SA_MYC plants was significantly reduced with respect to NMYC plants, in turn resulting in a slowdown of photosynthesis. Additionally, seed primed NMYC and MYC plants were smaller than the untreated NMYC ones, indicating that the improved adaptation to stress encompassed phenotypic modifications able to alter the physiological performances (He *et al.*, 2022). Our results also pointed to an AMF-mediated balancing effect in the growth-defence trade-off mechanisms (Nerva *et al.*, 2023), which persisted with both seed priming treatments.

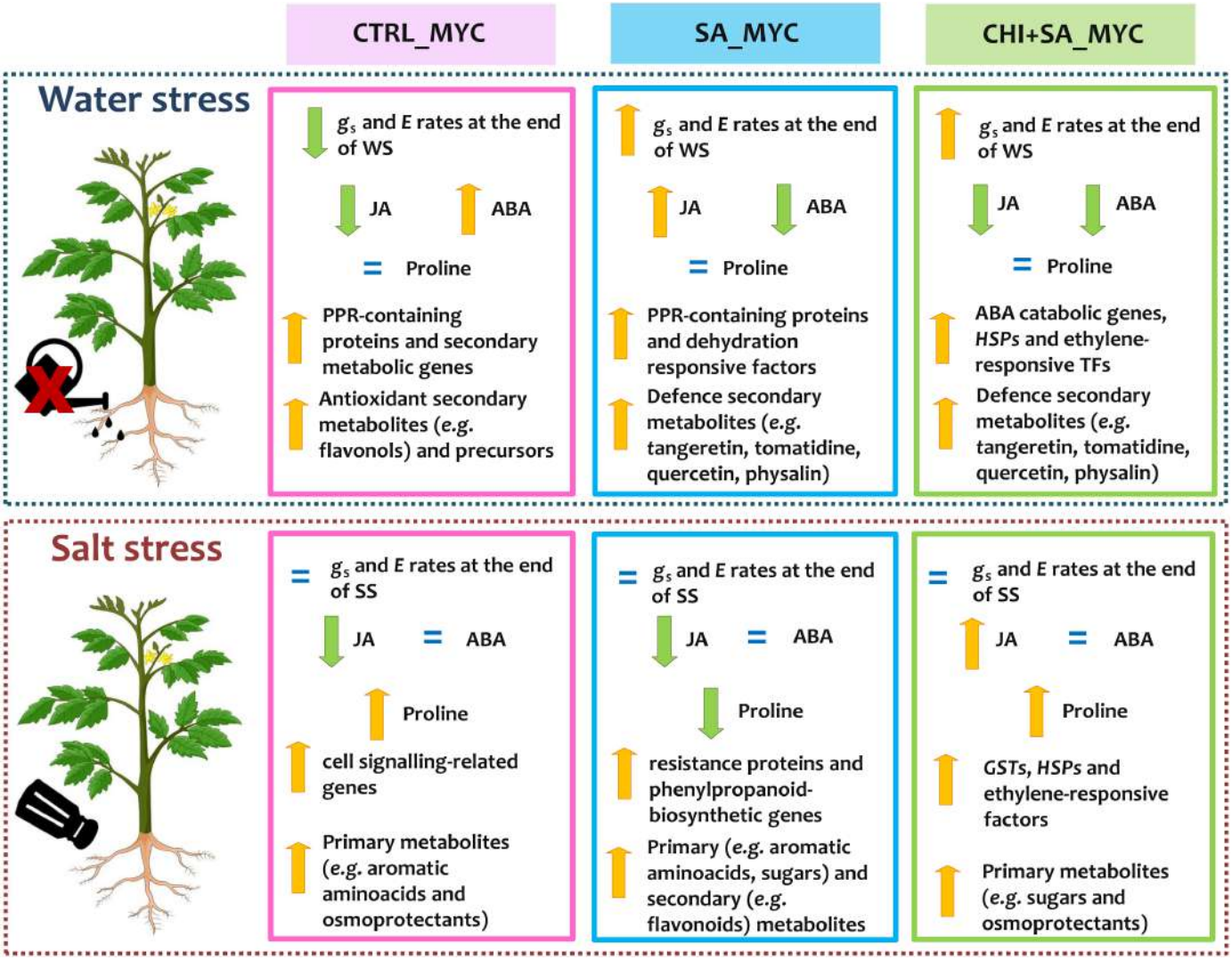

Fig. 9. Overview of long-lasting physiological, metabolic and molecular responses occurring in AMF-inoculated (MYC) tomato plants (*Solanum lycopersicum* cv. Moneymaker) either subjected to chemical seed priming (i.e. SA_MYC and CHI+SA_MYC) or not (CTRL_MYC). Green and yellow arrows respectively indicate decrease and increase in the measured ecophysiological parameters/gene expression/metabolite content, while the '=' symbol refers to absence of changes. ABA, abscisic acid; CHI, chitosan; JA, jasmonic acid; CTRL, control, untreated; SA, salicylic acid; MYC, inoculated; WS, water stress; SS, salt stress.

Overlapping and distinct transcriptomic and metabolic signatures are induced in stressed plants following treatments with seed priming agents and arbuscular mycorrhizal fungal inoculation

It has been long-known that plant tolerance to water deficit and salt stress involves profound changes in hormone metabolism and related signalling routes (Yu *et al.*, 2020; Salvi *et al.*, 2021). Here, besides a stress-dependent impact on the accumulation profiles of the main phytohormones, a significant effect of AM symbiosis, alone or in combination with both SA and CHI+SA treatments, revealed a reversed response with respect to untreated NMYC plants. Both SA and JA levels showed an increasing trend in SA and CHI+SA_MYC plants upon both WS and SS. It was already reported that seed priming with SA increased endogenous hormone content, including SA and ABA in cotton (Fang *et al.*, 2018). Additionally, proline accumulation was more accentuated in NMYC plants following both stress conditions, reflecting the well-known role of this solute in protecting cells from oxidative damages caused by osmotic stresses (Yoshiba *et al.*, 1997; Forlani *et al.*, 2019). Proline concentration patterns agreed with expression changes of genes involved in proline metabolism. Particularly, *P5CS* (LOC101244293), a gene encoding Δ^1-pyrroline-5-carboxylate synthetase, catalysing the first step of proline biosynthesis (Yoshiba *et al.*, 1997), was up-regulated in NMYC plants. CHI+SA combined with AMF inoculation led to significantly higher levels of the metabolite already in NS conditions, suggesting the establishment

of specific basal defence mechanisms shaping the plant stress perception. This result, coupled with the physiological modifications, could support a prompter ability of these plants in limiting cell water loss during stress through osmotic adjustment (Bartlett *et al.*, 2014; Forlani *et al.*, 2019).

At the transcriptomic level, regardless of inoculation or priming treatment, water deprivation caused a reprogramming of genes typically associated with stress responses, such as the dehydrin-encoding genes *ERD14* and *TAS14*, and several heat shock protein-related genes, as previously described (Iovieno *et al.*, 2016). ERD14 was reported in Arabidopsis to help plants coping with oxidative stress under dehydration (Nguyen *et al.*, 2020), while TAS14 was already associated with WS response in tomato (Chitarra *et al.*, 2016) and it is considered one of the main tomato drought markers, transcriptionally activated by ABA signalling (Muñoz-Mayor *et al.*, 2012). Among the genes exclusively up-regulated upon WS, two genes encoding an abscisic stress-ripening protein 1 (*ASR1*) and a jasmonate *O*-methyltransferase-like were found. Tomato plants overexpressing *ASR1* showed enhanced survival rates under WS (Golan *et al.*, 2014), while up-regulation of a jasmonate *O*-methyltransferase in rice allowed the plants to produce MeJA in drought conditions (Kim *et al.*, 2009). The mean expression value of *ASR1* in MYC plants subjected to WS was lower than in the NMYC ones, suggesting a role of AMF in mediating the ABA production, as previously reported (Begum *et al.*, 2020).

Salinity also caused a profound leaf transcriptomic remodelling, independent of priming treatments. The significant up-regulation of genes related to ion transport and stress response likely played a role in maintaining cell homeostasis and preventing sodium toxicity. Among the transcripts exclusively regulated under SS, a gene encoding a suppressor of max2-like protein (*SMXL2*) was identified. Genes from the SMXL family, which are recognized as downstream components of the strigolactone (SL) signalling pathway, were proven to control salt stress responses in soybean and chickpea (Fu *et al.*, 2022; Basso *et al.*, 2024).

Overall, under the same growth conditions (i.e. not stressed, water deficit, or high salinity), and irrespective of the seed priming treatment, AMF-inoculated tomato plants showed a distinct transcriptomic profile compared with uninoculated plants. In NS conditions, the systemic foliar response to AMF inoculation was comparable to that reported by Cervantes-Gámez *et al.* (2016), revealing the expression of genes already reported as a key signature of AMF colonization (Liu *et al.*, 2007; López-Ráez *et al.*, 2010; Takeda *et al.*, 2011). In stress conditions, MYC plants showed up-regulation of a large set of PPR-containing protein genes, involved in RNA processing and in various biological processes, including response to ABA signals under stress conditions (Yuan and Liu, 2012). In *Populus alba*, transcription of *PPR* genes was induced following AMF inoculation in plants grown both on heavy metal-contaminated soils and under optimal conditions (Cicatelli *et al.*, 2014); in *Citrus sinensis*, one of the most highly expressed genes in mycorrhizal plants encoded a PPR protein (Gao *et al.*, 2016). Our results further confirm a role for these proteins in the regulation of response to abiotic stresses in mycorrhizal plants.

In CHI+SA_MYC_WS plants, the transcriptome analysis highlighted a significant up-regulation of genes involved in ABA metabolism, such as an abscisic acid hydroxylase and an abscisic-aldehyde oxidase, which are considered integral to the ABA signalling pathway (Merilo *et al.*, 2015). A reduction in the expression level of an abscisic stress-ripening protein 1 (*ASR1*) gene in CHI+SA_MYC_WS was detected. This gene was previously found to be up-regulated in tomato leaves following WS treatment (Maskin *et al.*, 2001), so it could be argued that the decreased expression in the presence of AMF inoculation and priming agents might be correlated with a less stressed status of the plants. In parallel, upon SS, CHI+SA_MYC plants increased the expression of a glutathione-*S*-transferase-encoding gene, involved in cell detoxification processes (Xu *et al.*, 2015). Together, these findings might indicate that the interaction between AMF inoculation and the CHI+SA-seed treatment not only triggers general stress response mechanisms, but also activates specific defence pathways. These results also imply that effects of the seed priming treatment were still effective after 2 months from the sowing.

In agreement with the observed transcriptomic changes, analysis of whole leaf metabolic signatures outlined an extensive remodelling of primary and secondary metabolite profiles primarily controlled by the stress condition or the AMF inoculation. Hierarchical clustering of metabolomic data attested that soluble sugars (like fructose and sucrose), osmoprotectant solutes (including proline), primary and secondary metabolites with a known antioxidant and defence role (such as glutathione, ascorbic acid, flavonols, flavanols, and several precursors of the phenylpropanoid biosynthetic pathway) were the metabolic classes most differentially accumulated based on the three tested variables (stress, AM symbiosis, and seed priming treatments). The increased accumulation of antioxidant metabolites, such as ascorbic acid (Zhou *et al.*, 2016), was supported at the molecular level by the positive modulation of specific genes, such as an ascorbate-specific transmembrane electron transporter and a glutathione-*S*-transferase, which were induced in the same samples. Secondary metabolites were mainly affected by presence of AMF. In leaves, metabolomic data demonstrated an enhanced content of the alkaloid β-tomatidine in plants inoculated with AMF, irrespective of seed priming, especially under WS conditions. It has been observed that the concentration of tomatidine in tomato leaves substantially increases under water scarcity and when NaCl is added, suggesting that this compound is involved in chemical defence mechanisms (Bailly, 2021). The systemic accumulation of alkaloid compounds in tomato after AMF inoculation has also been documented, and it has been proposed that this class of metabolites may elicit plant metabolic responses to herbivore attack (Rivero *et al.*, 2021). This suggests that AMF may mediate the

systemic accumulation of tomatidine in plants subjected to WS and modulate the related stress responses. Furthermore, several flavonoids and related compounds, including tangeretin, feruloyl flavonoid glycoside, and quercetin, were also accumulated in AMF-inoculated plants under water deficit. Flavonoids are well-known antioxidant compounds that play a protective role against a plethora of abiotic stresses, such as heat stress, salinity, and drought (Hichri *et al.*, 2011; Martinez *et al.* 2016). High concentration of flavonoids in MYC WS plants has already been documented (Xie *et al.*, 2023), including tomato (Aseel *et al.*, 2019). Both AMF presence and water deficit affected the flavonoid content in tomato, suggesting that AMF may regulate the biosynthesis of these antioxidant molecules under stress conditions. A similar pattern was observed for coumarin, another antioxidant compound exerting a role in the activation of tomato tolerance to environmental stresses (Bourgaud *et al.*, 2006). Notably, recent studies have reported that beneficial microorganisms such as AMF can enhance coumarin production in tomato leaves (Iula *et al.*, 2021). Our transcriptomic data indicated an up-regulation of a 4-coumarate-CoA ligase-like 5 related gene in AMF-inoculated plants (both treated and untreated with priming agents) under water stress. This gene family includes multiple members and encodes several enzymes involved in the biosynthesis of hydroxycinnamate CoA esters, a critical step in diverting general phenylpropanoid metabolism toward coumarin biosynthesis (Hamberger and Halbrock, 2004). Particularly, the 4-coumarate-CoA ligase-like 5 gene was up-regulated in roots of soybean colonized by AMF, suggesting an involvement of AMF in fine tuning the expression of this gene (Marquez *et al.*, 2018). Here, a link between the accumulation of coumarin metabolites and the induction of this gene in AMF-inoculated plants was observed under water deprivation, likely indicating that an AMF-mediated systemic response occurred in tomato leaves.

The effect of priming agents on leaf metabolomic profiles, some secondary metabolites including a flavonoid-related one (i.e. quercetin-hexose) and an oxylipin (i.e. 12-OPDA) were more abundant in SA- and CHI+SA-primed plants, particularly under water deficit. The observed accumulation of quercetin-hexose, also observed in WS AMF-inoculated plants, further supports the hypothesis that priming agents may enhance the accumulation of antioxidant compounds (Król *et al.*, 2015). Concerning 12-OPDA, in Syrian bean capper plants (*Zygophyllum fabago*) pretreated with SA and exposed to different levels of lead, an increased accumulation of 12-OPDA was observed in the leaves, accompanied by an inhibition of the accumulation of the JA derivative jasmonoyl isoleucine (López-Orenes *et al.*, 2020). Plants with elevated levels of 12-OPDA were demonstrated to display an improved drought resistance associated with a reduction in stomatal aperture; moreover, it has been reported that drought interrupts the conversion of 12-OPDA to JA (Savchenko *et al.*, 2014). Similarly, in our study, tomato plants subjected to WS and primed with SA and CHI+SA showed an increased amount of 12-OPDA, whereas JA accumulated less in stressed than not-stressed plants. Interestingly, it has also been suggested that 12-OPDA acts in combination with ABA to elicit stomatal closure in response to drought (Savchenko *et al.*, 2014). Given the higher ABA levels in primed plants under WS compared with their untreated counterparts, it could be hypothesized that stomatal closure was driven by a priming elicitation of an interplay between ABA and 12-OPDA, as previously suggested (Savchenko *et al.*, 2014).

Collectively, our achievements suggest that seed treatments and AMF inoculation, alone or in combination, stimulate the tomato antioxidant and osmoprotective machinery, leading to an overall improvement of plants physiological performance upon different abiotic stress conditions. It was furthermore shown that, besides overlapping defence responses (e.g. lipid signalling pathways), tomato plants subjected to chemical seed priming and AMF inoculation were able to activate molecular and metabolic responses specific to the type of stress. In fact, while defence signals were primarily represented by differential expression of genes linked to antioxidant secondary metabolites upon WS, the transcriptional reprogramming of defence processes is shifted towards osmotic stress responses upon SS. Elicitation of multiple defence-associated metabolic routes is therefore at the basis of priming, confirming the success of the adopted approaches in enhancing plant stress tolerance.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Effects of priming treatment and arbuscular mycorrhizal fungal inoculation on stem water potential (Ψ_{stem}) under water stress.

Fig. S2. Effects of priming treatment and arbuscular mycorrhizal fungal inoculation on leaf transpiration (*E*) under water stress.

Fig. S3. Effects of priming treatment with chitosan on arbuscular mycorrhizal fungal colonization in comparison with the selected priming treatments (SA and CHI+SA).

Fig. S4. Effects of priming treatment with chitosan and arbuscular mycorrhizal fungal inoculation on stomatal conductance (g_s) upon water stress and salt stress.

Fig. S5. Functional analysis of DEGs in SA and CTRL under different conditions.

Fig. S6. Functional analysis of DEGs in CHI+SA and CTRL under different conditions.

Fig. S7. Network analysis of the interactions between metabolites and DEGs in AMF-inoculated tomato plants treated with priming agents and subjected to water stress, and list of the identified DEGs with their associated functional descriptions.

Fig. S8. Network analysis of the interactions between metabolites and DEGs in AMF-inoculated tomato plants treated with priming agents and subjected to salt stress.

Table S1. Statistical analysis of data on arbuscular mycorrhizal fungal colonization assessment.

Table S2. Statistical analysis of data on soil relative water content in tomato leaves.

Table S3. Statistical analysis of ecophysiological parameters of tomato leaves under water stress.

Table S4. Statistical analysis of ecophysiological parameters of tomato leaves under salt stress.

Table S5. Statistical analysis of plant biometric and chlorophyll content index.

Table S6. Statistical analysis of data on phytohormone (SA, JA, ABA, and IAA) and proline content in tomato leaves.

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Author contributions

VA, MGG, VF, and RB conceived and designed the research. LG, CP, EC, FS, SDR, EZ, WC, AI, AS, and FV conducted all measurements and wet lab experiments. LG, CP, FS, and WC conducted data analyses. LG, CP, FS, and RB wrote the first draft of the manuscript. All authors read and approved the manuscript.

Conflict of interest

Authors have no conflict of interest declared.

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Data availability

The RNA-seq raw data underlying this article are available in the NCBI Sequence Read Archive (SRA) at https://www.ncbi.nlm.nih.gov/sra and can be accessed with the BioProject accession number PRJNA1108677. The statistics and elaboration of RNAseq and untargeted metabolomics data (named as Dryad Datasets S1 to S36) that support the findings of this study are openly available in the Dryad digital repository at https://doi.org/10.5061/dryad.dbrv15fb9 (Giovannini *et al.*, 2025).

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