Plant Physiology and Biochemistry 215 (2024) 108976

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Combined salt and low nitrate stress conditions lead to morphophysiological changes and tissue-specific transcriptome reprogramming in tomato

Giorgia Batelli [a], Alessandra Ruggiero [a], Salvatore Esposito [a], Accursio Venezia [b], Antonio Lupini [c], Roberta Nurcato [a], Antonello Costa [a], Samuela Palombieri [a,1], Antonella Vitiello [a], Antonio Mauceri [c], Maria Cammareri [a], Francesco Sunseri [c], Silvana Grandillo [a], Antonio Granell [d], Maria Rosa Abenavoli [c,**], Stefania Grillo [a,*]

[a] National Research Council of Italy, Institute of Biosciences and BioResources, Research Division Portici (CNR-IBBR), Portici, 80055, Italy
[b] Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics (CREA-OF), 84098, Pontecagnano Faiano, Italy
[c] Department of Agraria, University Mediterranea of Reggio Calabria, Reggio Calabria, Italy
[d] Instituto de Biología Molecular y Celular de Plantas (IBMCP). Consejo Superior de Investigaciones Científicas (CSIC), Universitat Politècnica de València, València, Spain

ARTICLE INFO

Keywords:
Solanum lycopersicum
Single and combined stress conditions
RNAseq
Roots
Leaves
Differentially expressed genes

ABSTRACT

Despite intense research towards the understanding of abiotic stress adaptation in tomato, the physiological adjustments and transcriptome modulation induced by combined salt and low nitrate (low N) conditions remain largely unknown. Here, three traditional tomato genotypes were grown under long-term single and combined stresses throughout a complete growth cycle. Physiological, molecular, and growth measurements showed extensive morphophysiological modifications under combined stress compared to the control, and single stress conditions, resulting in the highest penalty in yield and fruit size.

The mRNA sequencing performed on both roots and leaves of genotype TRPO0040 indicated that the transcriptomic signature in leaves under combined stress conditions largely overlapped that of the low N treatment, whereas root transcriptomes were highly sensitive to salt stress. Differentially expressed genes were functionally interpreted using GO and KEGG enrichment analysis, which confirmed the stress and the tissue-specific changes. We also disclosed a set of genes underlying the specific response to combined conditions, including ribosome components and nitrate transporters, in leaves, and several genes involved in transport and response to stress in roots. Altogether, our results provide a comprehensive understanding of above- and below-ground physiological and molecular responses of tomato to salt stress and low N treatment, alone or in combination.

1. Introduction

Salinity, drought, heat, and cold are among the most harmful abiotic stressors with detrimental effects on different aspects of plant physiology, including growth, biomass accumulation, productivity, seed production, and fruit quality (Wani, 2023). Among them, salt stress, commonly resulting from excess NaCl in soil, has become a major concern for plant growth and food production worldwide due to high surface evaporation and inadequate precipitation, which further exacerbates the productivity decline of agricultural lands (Hosseinifard et al., 2022; Wang et al., 2023). According to the Food and Agriculture Organization (FAO), more than 10% of global cropland is affected by salt, with an additional 1.5 million hectares of farmland rendered unproductive each year (FAO, 2022), posing a significant threat to food security. Salinity stress symptoms in plants are contingent on the genotype, growth stage, duration, and intensity of the stress imposed, making its understanding harder, especially under combined conditions (Holsteens et al., 2022; Bai et al., 2018). For example, following salinity

* Corresponding author.
** Corresponding author.
E-mail addresses: mrabenavoli@unirc.it (M.R. Abenavoli), stefania.grillo@ibbr.cnr.it (S. Grillo).
[1] Current affiliation: Department of Agriculture and Forest Science, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy.

https://doi.org/10.1016/j.plaphy.2024.108976
Received 23 May 2024; Received in revised form 23 July 2024; Accepted 25 July 2024
Available online 27 July 2024

stress, photosynthesis is inhibited due to the stomatal closure caused by the reduction of leaf turgor and the production of related hormones (Chaves et al., 2009; Hannachi et al., 2022). However, the low photosynthetic rate may also be due to the increased Na^+ levels, which are responsible for the inhibition of enzymatic activity related to photosynthesis, and consequently the chlorophyll content (Hannachi et al., 2022). Salt stress further results in ion imbalance, osmotic stress, and the accumulation of reactive oxygen species (ROS), leading to severe reductions in crop yield. Excessive NaCl diminishes soil water potential, curtails water import, and inhibits cell division and elongation (Guo et al., 2022), impacting both direct ion toxicity and competition for the Na^+ and Cl^- uptake against essential nutrients such as K^+, Ca^{2+}, and NO_3^- (Nazir et al., 2023).

In the case of tomato (*Solanum lycopersicum* L.), a globally significant horticultural crop, salt stress induces adverse effects on seed germination, plant growth, and fruit development. Although some tomato wild relatives display a higher tolerance to salt stress, this trait may have been lost during domestication (Pailles et al., 2020). However, a large germplasm collection of traditional tomatoes is available, in which high throughput efforts have uncovered genotypic and phenotypic differences (Blanca et al., 2022; Pons et al., 2022). Recently, a genome-wide association study in a large population including wild, domesticated and improved tomato genotypes identified a variation in the coding sequence of *SlHAK20* as responsible for differences in root Na^+/K^+ ratio and salt tolerance loss in domesticated tomato (Wang et al., 2020b). *SlHAK20* was reported as a Na^+ and K^+ transporter, involved in their homeostasis during salt stress (Wang et al., 2020b). Similarly, variations in the promoter sequence affecting recognition by *SlDREB2* of *SlSOS1*, a key antiporter responsible for Na^+ extrusion from the cytoplasm, were also found to be associated with a high root Na^+/K^+ ratio in domesticated tomatoes (Wang et al., 2021).

Plants, including tomato, respond to osmotic stress and excess ROS by accumulating compatible osmolytes like glycine betaine and proline (Claussen, 2005; De la Torre-González et al., 2018; Cappetta et al., 2020; Ruggiero et al., 2022). However, their accumulation is contingent upon the nitrogen (N) availability (Carillo et al., 2008). N is an essential macronutrient for plants, mainly high-demand crops such as tomatoes, that need consistent fertilizer rates for boosting yields, with high economic and environmental implications (West et al., 2014). Nitrate (NO_3^-) is the major inorganic N form used by plants in agricultural soil, and the most prone to leaching (West et al., 2014).

Nitrate uptake and translocation rely on proton-coupled importers of the NPF/NRT1 (Peptide Transporter/Nitrate Transporter 1) and NRT2 transporter families. NPF/NRT1s include mainly dual/low-affinity nitrate transporters, whereas NRT2 family members are usually high-affinity nitrate transporters. Nitrate reductase (NR) and nitrite reductase (NiR) operate conversion to NO_2^- and NH_4^+, respectively. Ammonium is then converted to glutamine by glutamine synthetase (GS) and to glutamate by glutamate synthase (GOGAT) (Debouba et al., 2007). Through the xylem, nitrate and N assimilate are transferred from roots to shoots through the leaf transpiration-driven flow (Tegeder and Masclaux-Daubresse, 2018).

In addition to the uptake, salt stress restricts nitrate reduction and assimilation by inhibiting the biosynthesis and activity of enzymes such as NR and GOGAT, and by interfering with translocation through reduced leaf transpiration (Debouba et al., 2007; Lopez-Delacalle et al., 2020). Thus, the perturbation of N dynamics is identified as a critical factor influencing the impact of salt stress on plants (Nazir et al., 2023).

Furthermore, as well reported by Murtaza et al. (2013) salt stress affects nitrogen use efficiency (NUE), which is a complex trait defined as the total biomass per unit N supplied (Moll et al., 1982), and it is controlled by gene networks involved in N uptake, assimilation, and remobilization. NUE is usually divided into two main components: nitrogen uptake efficiency (NUpE), defined as the ability of the plant to take up N from the soil, and nitrogen utilization efficiency (NUtE), which encompasses the ability of the plant to assimilate, transfer, and utilize N (Xu et al., 2012). Genetic variation for this trait in germplasm collections, including old landraces, has been explored also in tomato (Chardon et al., 2010; Hawkesford, 2012; Abenavoli et al., 2016; Mauceri et al., 2020; Aci et al., 2021; Sunseri et al., 2023).

Salt stress and N limitation induce partially overlapping responses, imposing limitations on photosynthesis and stomatal conductance, accumulation of ROS and components of the antioxidant machinery. However, despite several studies aimed at understanding the genetic mechanisms underlying salt stress and N limitation in tomato, the physiological adjustments and transcriptome modulation induced by combined salt stress and N limitation remain poorly characterized. Indeed, responses to concurrent multiple abiotic stressors may be radically different, and even contrasting depending on the specific nature of the applied stimuli, from those deployed under single stresses (Pandey et al., 2015).

In addition, several studies have aimed at elucidating early responses to stress in young plants, with a gap in the knowledge of how adult plants balance growth and stress responses when exposed to long-term stress conditions.

The main goal of our study was to investigate the impact of long-term single salt stress, low N, and combined stress on three selected traditional tomato ecotypes grown in a complete growth cycle. We show that distinctive physiological, molecular, growth, and yield responses are elicited depending on the nature of the stress applied, as reflected in leaf and root transcriptomic signatures to single and combined stress conditions.

2. Materials and methods

2.1. Plant material and growth conditions

Seventeen Italian tomato (*Solanum lycopersicum* L.) accessions (16 traditional landraces and San Marzano variety, Supplementary Table S1) were screened at the seedling stage for salt stress tolerance in hydroponics (Supplementary material). Then, three tomato accessions (Crovarese, TRPO0670; Acampora, TRPO0040; Linosa, TRPA0130) were selected and grown in soil in a semi-controlled greenhouse condition.

Seeds of the three selected genotypes were germinated in soil in a semi-controlled greenhouse for cultivation using standard agricultural practices in a soilless system. At two true leaves stage, the seedlings were transplanted in coconut coir dust grow bags (Jiffy-grow bag) previously imbibed with fertigation solution (Supplementary Table S2). At transplant, plants were disposed in eight blocks, each containing seven-eight replicates per genotype, for a total of 180 plants in the whole experiment. The plants were fertigated through a drip irrigation closed system. Two blocks per treatment were used as control (13.5 mM NO_3^-; 0 mM NaCl), low N (3.4 mM NO_3^-; 0 mM NaCl), salt stress (13.5 mM NO_3^-; 80 mM NaCl) and combined stress (3.4 mM NO_3^-, 80 mM NaCl). A recirculating solution was used to apply NaCl (80 mM) and low N (3.4 mM NO_3^-) stress, alone and in combination, whereas the control plants were fertigated using a solution containing 0 mM NaCl and 13.5 mM NO_3^-. Low N treatment started concurrently with the seedling transfer to the coconut coir dust growbags, while NaCl stress was applied after one week by the first 40 mM, until reaching a final NaCl concentration (80 mM) at 13 days after seedling transfer (DAT, Fig. 1A), with an electrical conductivity of 9.8 dS/m compared to the control (1.8 dS/m; Supplementary Table S2). Both conductivity and pH of solutions were checked daily. The composition of the growing solutions is reported in Supplementary Table S2. In the greenhouse, the average air temperature and humidity were 21 °C and 72%, while the daily global solar radiation was 76 Rs. Stress progression was monitored through measurements of Leaf Relative Water Content (LRWC) and SPAD units (details are below). Leaf samples for molecular and biochemical analyses were collected at different time points, as detailed below, using the fifth youngest leaf in all the analyses.

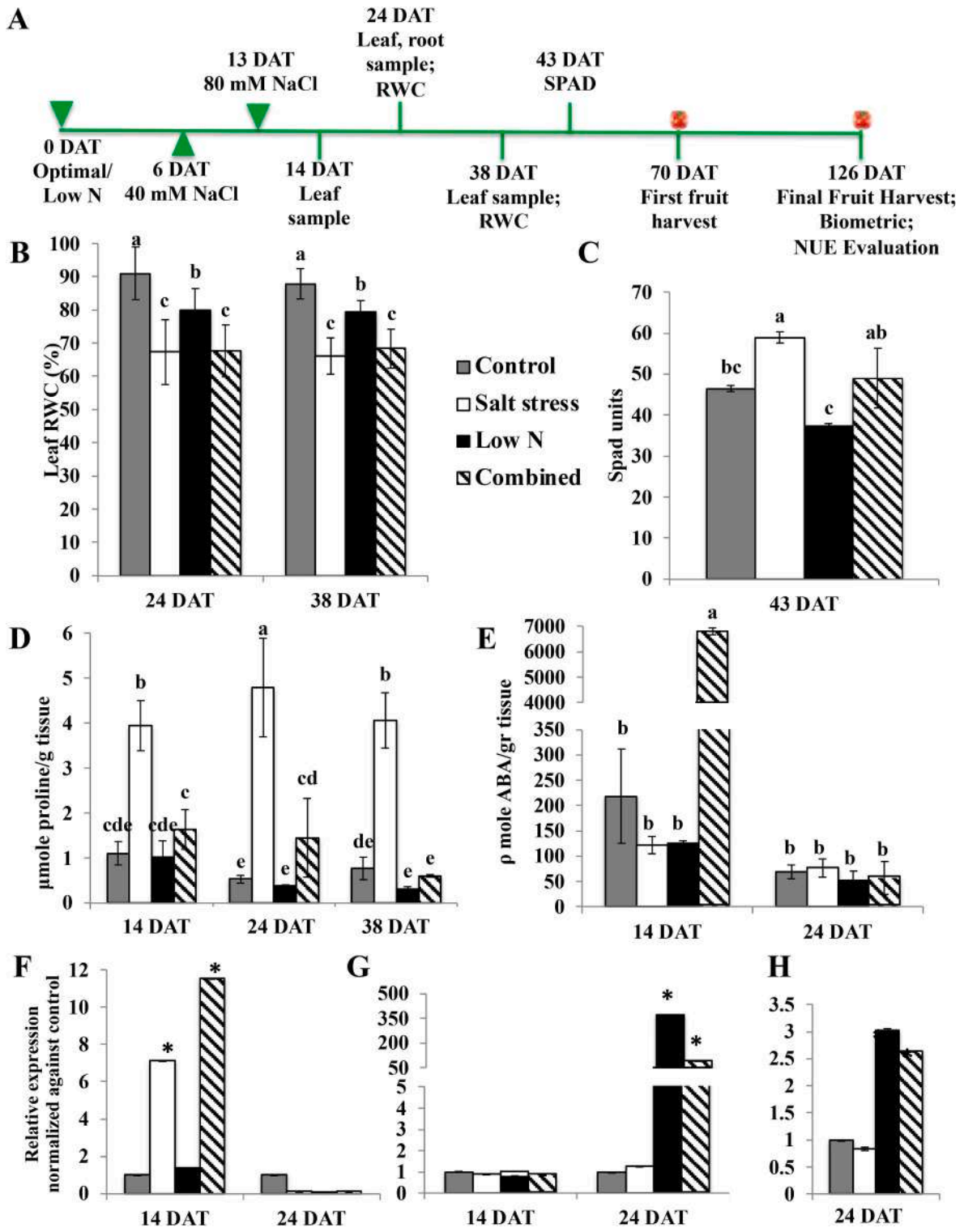


Fig. 1. Experimental outline and monitoring of stress progression. A) Experimental design highlighting significant time points; Arrowheads indicate treatments start points; DAT: day after transplant; RWC: relative water content; Leaf and root samples: tissue collection for gene expression analyses, proline and ABA content determination; B) Leaf RWC of TRPO0040 in the four treatments at 24 and 38 DAT. Values indicate mean ± SD (n = 6); C) Mean chlorophyll meter readings (SPAD units) in TRPO0040 in the four treatments at 43 DAT. Values indicate mean ± SD (n = 4); D) Leaf proline content in TRPO0040 at 14, 24 and 38 DAT. Values indicate mean ± SD (n = 6).; E) Leaf ABA content in TRPO0040 in the four treatments at 14 and 24 DAT. Values indicate mean ± SD (n = 3); F) Relative quantification of LEA gene expression measured by qRT-PCR in TRPO0040 in the four treatments at 14 and 24 DAT. All data are expressed as mean ± SD (n = 3) relative to control treatment; G-H) Relative quantification of Nrt2.1 gene expression measured by qRT-PCR in TRPO0040 in leaves in the four treatments at 14 and 24 DAT (G), and roots at 24 DAT (H). Different letters indicate significant differences at $p < 0.05$ according to the Duncan post hoc test.

2.2. Nitrogen concentration and nitrogen use efficiency

Nitrogen content was determined by dry combustion as reported by Abenavoli et al. (2016). Briefly, plant material (0.25 g) was maintained in the oven at 72 °C for 4 days, to obtain a homogenized powder. Finally, N determination (mg kg^{-1} dry matter) was performed using the LECO CN628 instrument (LECO Corporation). N values were used to estimate NUE based on different formulas suggested for hydroponic and greenhouse experiments.

In the hydroponic experiment, the Nitrogen Use Efficiency (NUE) [SDW $N\%^{-1}$, where N% is the g N (100 g DW)$^{-1}$] and the Nitrogen Uptake Efficiency (NUpE) [total (shoot + root) dry weight (TDW) x N concentration (g N g TDW^{-1})] were calculated as reported by Chardon et al. (2010). The Nitrogen Utilization Efficiency (NUtE) as the square of SDW divided by N concentration (g^2 N^{-1}) (Siddiqi and Glass, 1981) was calculated.

In the greenhouse experiment, NUE and its components (NUpE and NUtE) were calculated according to Moll et al. (1982). In detail, the efficiency of N uptake was calculated as total N content at maturity (Nt) divided by the N rate supplied (Ns) (Nt/Ns); the efficiency of N utilization was calculated as fruit weight (Fw) divided for Nt (Fw/Nt). Finally, NUE was obtained as fruit and total dry weight (plant) at maturity divided for Ns. Of course, NUE was also calculated by multiplying NUpE with NUtE (Moll et al., 1982).

2.3. Ion content determinations

Ions were extracted from stems, leaves, and fruits and analyzed by using ion chromatography (DIONEX ICS-1100, Thermo Fisher Scientific Waltham, MA, USA). One g of dry material was transferred to 550 °C for 6 h in a porcelain capsule and the obtained ash was then acidified for 30 min at 100 °C using 1M HCl solution. Finally, it was filtered using Whatman 1 and measured using the ion chromatograph with 20 mM methane-sulfonic acid as eluent. The amount of each ion was calculated using standard curves.

2.4. Physiological, osmolyte and ABA measurements

Chlorophyll content was measured on leaves of four plants per treatment after 30 days of salt stress (43 DAT) using a Chlorophyll meter SPAD-502Plus (Konika Minolta), while the stomatal conductance was checked weekly through porometer AP4-UM3 (Delta-T Devices). Leaf relative water content (LRWC) was measured on six replicates per genotype and treatment at 24 and 38 DAT. The excised leaves were instantly weighed to obtain the fresh weight (FW) and after hydration with distilled water for 24 h to obtain the turgid weight (TW). Leaf samples were then oven-dried at 70 °C for 72 h and the dry weight (DW) was measured. The LRWC percentage was calculated using the following equation: LRWC (%) = (FW-DW)/(TW-DW) × 100.

Leaf samples were collected from six biological replicates per treatment after 24 h, 11 days, and 25 days of salt stress, corresponding to 14, 24, and 38 DAT, for ABA and leaf proline content measurements. Two technical replicates were performed for each sample. Proline content was determined according to Claussen (2005) in six biological replicates at 14, 24, and 38 DAT. Three replicates per treatment at 14 and 24 DAT were used for ABA measurements. ABA extraction and measurement were performed as described (Tamburino et al., 2017; Iovieno et al., 2016).

2.5. Growth and yield measurements

Shoot dry weight and plant leaf area were measured after 126 days of culture using eight replicates per treatment and genotype. The collected samples were oven-dried at 70 °C until a stable weight was reached, to obtain shoot dry weight. Plant leaf area was measured on excised leaves of four replicates per treatment using a scanning plan meter (LI – 3400 area meters, Licor). Red mature fruits were collected in four rounds starting from 70 DAT on each plant (16 replicates/genotype/treatment), each fruit was weighed and checked for the presence of blossom end rot. Afterward, total yield, divided into marketable and non-marketable (presence of blossom end rot), fruit number, and weight per plant were calculated. Total soluble solid (TSS) content was analyzed from tomato juice of 22 fruits per treatment using pocket refractometer PAL-1 (Atago).

2.6. RNA Isolation, cDNA Synthesis, qRT-PCR

Total RNA was extracted from leaf and root samples at 14 and 24 DAT as described in Ruggiero et al. (2019) and Tamburino et al. (2017). DNase-treated RNA was used for reverse transcription using QuantiTect Reverse Transcription kit (Qiagen, Germany), and qRT-PCR was performed as described in Ruggiero et al. (2019) and Tamburino et al. (2017). For the relative quantification of gene expression, elongation Factor EF1-α (*Solyc06g005060*) or ubiquitin (*Solyc07g064130*) was used as endogenous reference. Primers used are listed in Supplementary Table S3. Quantification of gene expression was carried out using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and reported as relative expression levels, compared to control conditions as internal calibrator.

2.7. RNAseq analysis

RNA sequencing was performed on 12 leaf samples and 12 root samples, corresponding to three biological replicates per treatment. Amount and quality of RNA were measured by Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). As previously described (Iovieno et al., 2016), the Illumina TruSeq RNA Sample Preparation Kit (Illumina, SanDiego, CA, USA) was used for library construction prior to sequencing using the Illumina platform HiSeq2500 with 100-bp paired-end reads in triplicate and ~30 million reads per replicate were obtained. The sequencing service was provided by Genomix4life (http://www.genomix4life.com) at the Laboratory of Molecular Medicine and Genomics (University of Salerno, Italy). Low-quality reads were removed from further analyses and the long high-quality NGS reads were further processed for adaptor trimming using the software BBDuk, setting the minimum length and the quality score to 35 bp and 1, respectively. The *Solanum lycopersicum* cv. Heinz reference genome sequence (SL3.0/ITAG3.10) together with STAR aligner (version 2.5.0c) were used for reads alignment. FeatureCounts (version 1.6.0) was used to calculate gene expression values as raw fragment counts (annotation ITAG3.10). Normalization was applied to the raw fragment counts by using the Trimmed Mean of M-values (TMM) normalization and Fragments Per Kilobase Million (FPKM) normalization. Statistical analyses were performed using packages HTSFilter (Rau et al., 2013) and edgeR (Robinson et al., 2010). Not expressed genes and highly variable ones for each comparison were removed using the HTSFilter package (Rau et al., 2013) and the Jaccard similarity index. The TMM normalization strategy was used. The experiment quality was assessed by a Principal Component Analysis (PCA) using the normalized gene expression values as input (Supplementary Fig. S1). The differential expression analysis was performed to identify significant differentially expressed genes (DEGs) based on FDR values (<0.05) and Fold change (FC) $\geq |1.5$. Gene Ontology Enrichment Analysis (GOEA) was performed on differentially expressed genes using in-house scripts based on the hypergeometric test (Du et al., 2010). The p-values were then corrected for multiple testing using the Benjamini–Hochberg method, obtaining the final FDR values (Galise et al., 2021).

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) was calculated by SPSS software package (SPSS 19, SPSS Inc., United States). Duncan's multiple range test was applied to significant differences identified by ANOVA.

Different letters indicate significant differences at $p < 0.05$.

3. Results

3.1. Tomato response to salt and low nitrate stresses at the seedling stage

Seventeen genotypes from Southern Italy (Supplementary Table S1) were cultivated in hydroponics and treated with 150 mM NaCl. After 7 days of exposure, the scoring of twenty morphological and physiological parameters in control and stress conditions revealed three different genotype clusters (Supplementary Fig. S2A). Cluster I included TRPO2330, TRPA0130, TRPO0040, TRPO0670, TRPA0240, and TRPO0660, in which leaf area and number, plant height, and biomass were least affected by salt stress. By contrast, Cluster II included TRPA0160, TRPO0020, TRPO0304, TRPO0280, TRPO0510 and TRPO0140, in which the same parameters were markedly reduced compared to control and thus classified as salt sensitive. Finally, genotypes in Cluster III exhibited an intermediate response.

The Principal Component Analysis of all morpho-physiological parameters confirmed the observed clustering (Supplementary Fig. S2B). Dim1, which explained 23.5% of the observed variance, appeared strongly correlated to root system performances, whereas Dim2 (16.7% of the variance) was correlated to shoot parameters.

TRPA0130, TRPO0670, and TRPO0040 were selected among members of Cluster I based on their different origins (Sicily, TRPA0130; Campania, TRPO0670, and TRPO0040), to analyze the interaction of salt stress and limited nitrate (low N) conditions in terms of their effect on growth parameters and Nitrogen Use Efficiency (NUE) and its components uptake (NUpE) and utilization (NUtE) (Supplementary Figs. S3–S4). As expected from the above results, salt stress conditions did not impact root and shoot dry weight in the three genotypes, whereas low N and combined stress conditions caused a reduction particularly in roots (Supplementary Fig. S3). Analysis of root architecture traits showed that total root length, specific root length, and root fineness were significantly increased under low N in all tested genotypes, whereas salt and combined stress had little to no effect on these parameters. The root length ratio increased under low N and combined stress in all the genotypes except for TRPO0040, whereas only TRPA0130 reduced the root mass ratio under low N (Supplementary Fig. S3).

Low N significantly impacted the NUE of TRPO0670, whereas TRPA0130 and TRPO0040 were not affected. Conversely, salt and combined conditions determined a NUE reduction in all genotypes. NUpE was not influenced by the treatments in TRPA0130 and TRPO0670, whereas a reduction was observed in TRPO0040 following salt and combined conditions. NUtE was not affected in TRPO0670, while TRPA0130 and TRPO0040 showed a significant reduction under combined stress (Supplementary Fig. S4). Taken together, these results show that, although causing a reduced NUE, salt stress had little impact on biomass accumulation, both above- and below-ground on genotypes TRPO0040, TRPO0670, and TRPA0130, whereas low N and combined stress conditions caused reductions in biomass particularly in roots.

3.2. Morphophysiological and molecular responses to single and combined stress in a complete production cycle

To verify salt and low N stress impact during a whole tomato production cycle, the selected three genotypes (TRPO0040, TRPA0130, and TRPO0670) were grown in a closed soilless culture system for approximately 4 months until fruit harvest (Fig. 1A). Leaf Relative Water Content (LRWC), measured at 24 and 38 DAT, was mainly reduced under salt and combined stress at both sampling times, but significant differences compared to the control were also observed under low N (Fig. 1B; Supplementary Figs. S5A–5B). As expected, SPAD units indicated a lower chlorophyll content under low N conditions compared to the control at 43 DAT. Interestingly, the salt stress condition resulted in a slight increase in SPAD Units, while the combined stress determined values comparable to the control (Fig. 1C; Supplementary Fig. S5C). Proline accumulation, a well-known salt stress-responsive compatible osmolyte, was measured at different time points. The leaf proline content under salt stress was significantly higher in TRPO0040 at 14, 24, and 38 DAT compared to the control. In low N plants, leaf proline content was comparable to the control at all measured time points. Interestingly, at 24 DAT proline accumulation was significantly higher under combined stress compared to the control, showing no significant difference at 38 DAT (Fig. 1D). In TRPA0130 and TRPO0670, proline levels followed the same trends described for TRPO0040 at control and under low N and combined stress treatments. By contrast, at 14 and 24 DAT, TRPA0130 and TRPO0670 accumulated lower proline levels compared to TRPO0040. Proline in NaCl-treated plants of these two genotypes increased over time, possibly indicating a different kinetic elicited by salt stress compared to TRPO0040 (Supplementary Figs. S6A–6C).

A striking accumulation of abscisic acid (ABA) was detected at 14 DAT in TRPO0040 leaves under combined stress, with 6808 pmol/g FW compared to the control (218 pmol/g FW), indicating the induction of downstream stress-related molecular responses. Interestingly, ABA did not increase under salt stress, suggesting that salt and combined stresses elicit responses with a different kinetic of induction. As expected, at 24 DAT ABA content in stressed plants was similar to control plants, indicating the weakening of the stress signal following the initial response (Fig. 1E, Supplementary Figs. S6D–6E).

The relative expression of *Solyc03g116390*, encoding a Late Embryogenesis Abundant protein (*LEA*, Gong et al., 2010; Iovieno et al., 2016) as a marker of osmotic stress, was quantified (Fig. 1F). As expected, a significant *LEA* up-regulation was observed in plants subjected to salt or combined stress for 24h (14 DAT) (Van Oosten et al., 2018), whereas transcript abundance did not significantly differ between stressed plants and the control at 24 DAT (Fig. 1F).

Finally, to monitor low N stress responses, the relative expression of the high-affinity nitrate transporter coding gene (*Solyc06g074990*, *NRT2.1*) was assessed at 14 and 24 DAT. At 14 DAT, TRPO0040 did not show significant responses to both stresses, alone and in combination (Fig. 1G). By contrast, at 24 DAT a marked *NRT2.1* up-regulation under low N and combined stress was observed, both in leaves and roots (Fig. 1G–H). The quantification of additional transcripts in the three genotypes is shown in Supplementary Figs. S7–S8.

Altogether, combined stress conditions elicited physiological responses shared with single stresses, including LRWC, and up-regulation of *LEA* osmotic stress marker gene, shared with the salt stress treatment, and induction of *NRT2.1* nitrate transporter, as observed under low N. However, differences in ABA accumulation as well as a lower level of proline in combined stress compared with single salt stress were also observed, indicating specific responses to the combined stress condition.

3.3. Plant growth and crop yield performance under stress

Salt and low N stresses, alone and combined, had a macroscopic effect on plant growth, causing a significant reduction in above-ground biomass accumulation. The salt-stressed plants showed loss of basal leaves, while low N resulted in marked leaf chlorosis (Fig. 2A). These phenotypes were reflected in all the biometric measurements. Salt stress significantly affected leaf area only in TRPO0670 (57% reduction), while the limited N supply caused a dramatic reduction in leaf area in all genotypes, ranging from 70% (TRPA0130) to 83% (TRPO0670) compared to the control. Similar results on all the genotypes were observed under combined stress (Fig. 2B). The above-ground biomass of all the genotypes was severely and significantly reduced by stress treatments, alone and in combination, with the interesting exception of TRPA0130 in salt stress conditions (Fig. 2C). The fruit weight and related parameters were measured to assess crop yield under stress. The total yield was markedly and significantly reduced by all the treatments,

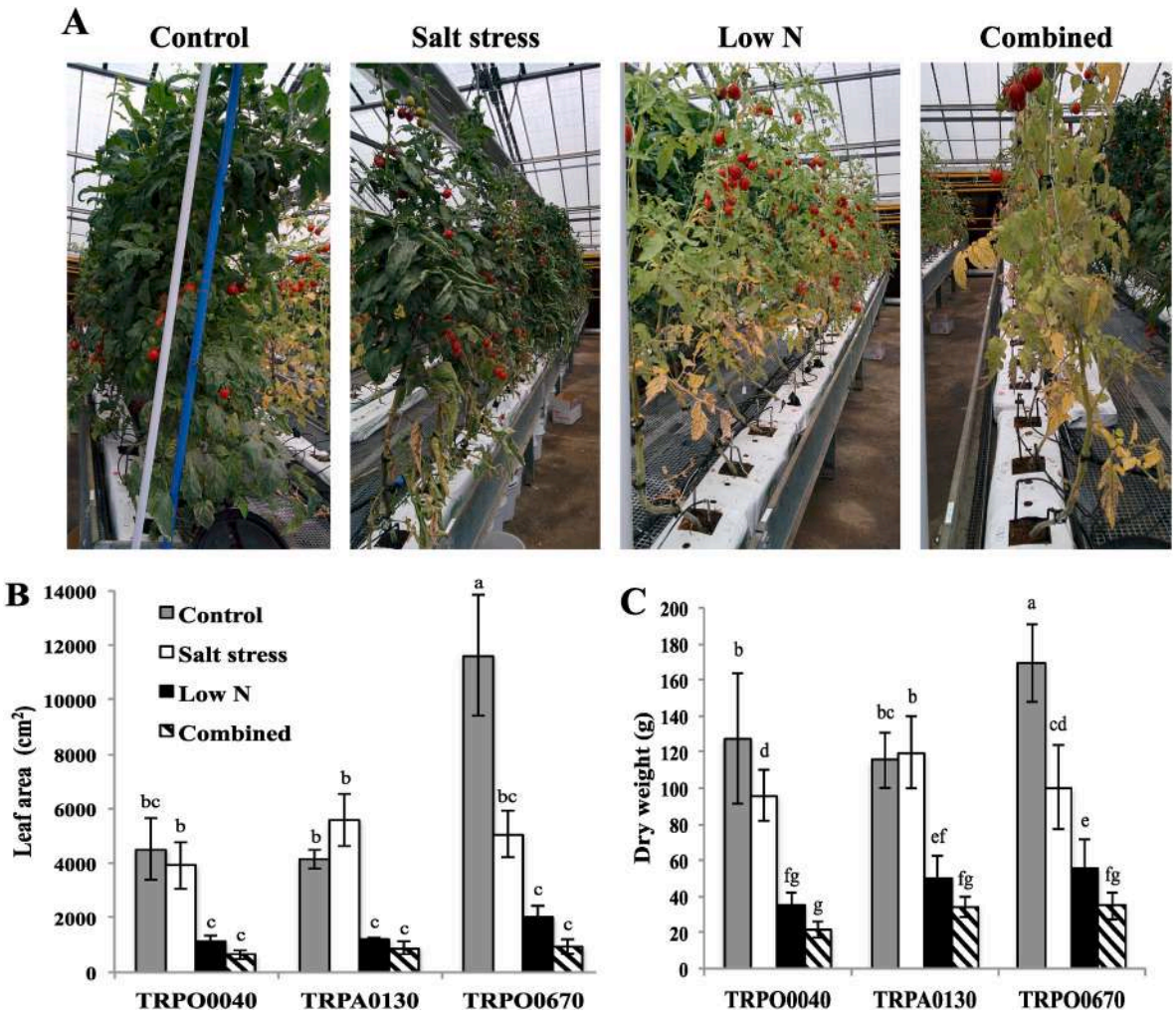


Fig. 2. Impact of salt and low N stresses on vegetative parameters of TRPO0040, TRPA0130 and TRPO0670. A) Overview of plants grown in Control, salt stress, low N and combined stress conditions at 120 DAT; B) Leaf area. Values indicate mean ± SD (n = 4); C) Shoot dry weight. Values indicate mean ± SD (n = 8). Different letters indicate significant differences at $p < 0.05$ according to the Duncan post hoc test.

with interesting differences among genotypes (Fig. 3A–D). TRPA0130 and TRPO0670 showed a 50% decrease in fruit weight per plant when both single stresses were applied, and it was further reduced under combined stress. Interestingly, TRPO0040 exhibited a slight and not significant reduction of fruit yield under salt stress, while limited N and combined stress significantly affected crop yield (Fig. 3B). Data related to marketable and non-marketable fruits showed that the N-limited supply reduced the number of fruits presenting blossom end rot (non-marketable), also under combined stress. By contrast, the non-marketable fruits showed a slight increase in TRPO0040 and TRPA0130 under salt stress, while TRPO0670 showed a better performance in the same condition (Fig. 3B). The yield components fruit number and average fruit weight showed results correlated with crop yield, as expected (Fig. 3C–D). Interestingly, both TRPO0040 and TRPO0670 did not show a significant reduction in fruit number under salt stress, however, a significant reduction in the average fruit weight was observed in TRPO0040 and TRPA0130 (Fig. 3C–D). Finally, total soluble solids (TSS) were quantified to measure the impact of salt and low N stresses on this quality marker. TSS in the control condition ranged from 5.03°Brix (TRPA0130) to 6.29 (TRPO0040) with significant increases in all the stress treatments and genotypes (Fig. 3E). As expected, salt stress treatment resulted in the highest TSS increase in all three genotypes, ranging from 53% (TRPO0040) to 59% (TRPO0670), whereas fruits from N-limited stress showed a lower but significant increase in TSS, ranging from 10% (TRPA0130 and TRPO0670) to 13% (TRPO0040) compared to the control. TSS content of all three genotypes under combined stress was intermediate between salt and low N stresses, indicating that low-N treatment limited the salt stress-induced increase in TSS (Fig. 3E). The results above showed an additive effect of single stresses on fruit yield reduction measured in the combined stress condition, mainly caused by a greatly reduced fruit weight. By contrast, measurements of Brix degrees showed intermediate values in the combined stress condition with respect to single salt and low N stresses.

3.4. Nitrogen use efficiency

The low N stress significantly increased NUE in all the genotypes compared to the control, as expected (Fig. 4A). More interestingly, TRPA0130 and TRPO0040 showed the best NUE performances under low N compared to TRPO0670. Moreover, TRPO0040 showed an increased NUE, although not statistically significant, also under combined stress compared to the control (Fig. 4A). The N uptake efficiency (NUpE) component was significantly affected by both the treatments (alone and in combination) and genotypes, with a lower reduction for TRPO0040 and TRPA0130 compared to TRPO0670 (Fig. 4B). Finally, all the genotypes under low N, alone or in combination with salt, significantly increased the N utilization efficiency (NUtE) component compared to the control and the salt stress treatment. Noticeably, TRPO0040 showed a very high NUtE when N was limited also under combined stress (Fig. 4C).

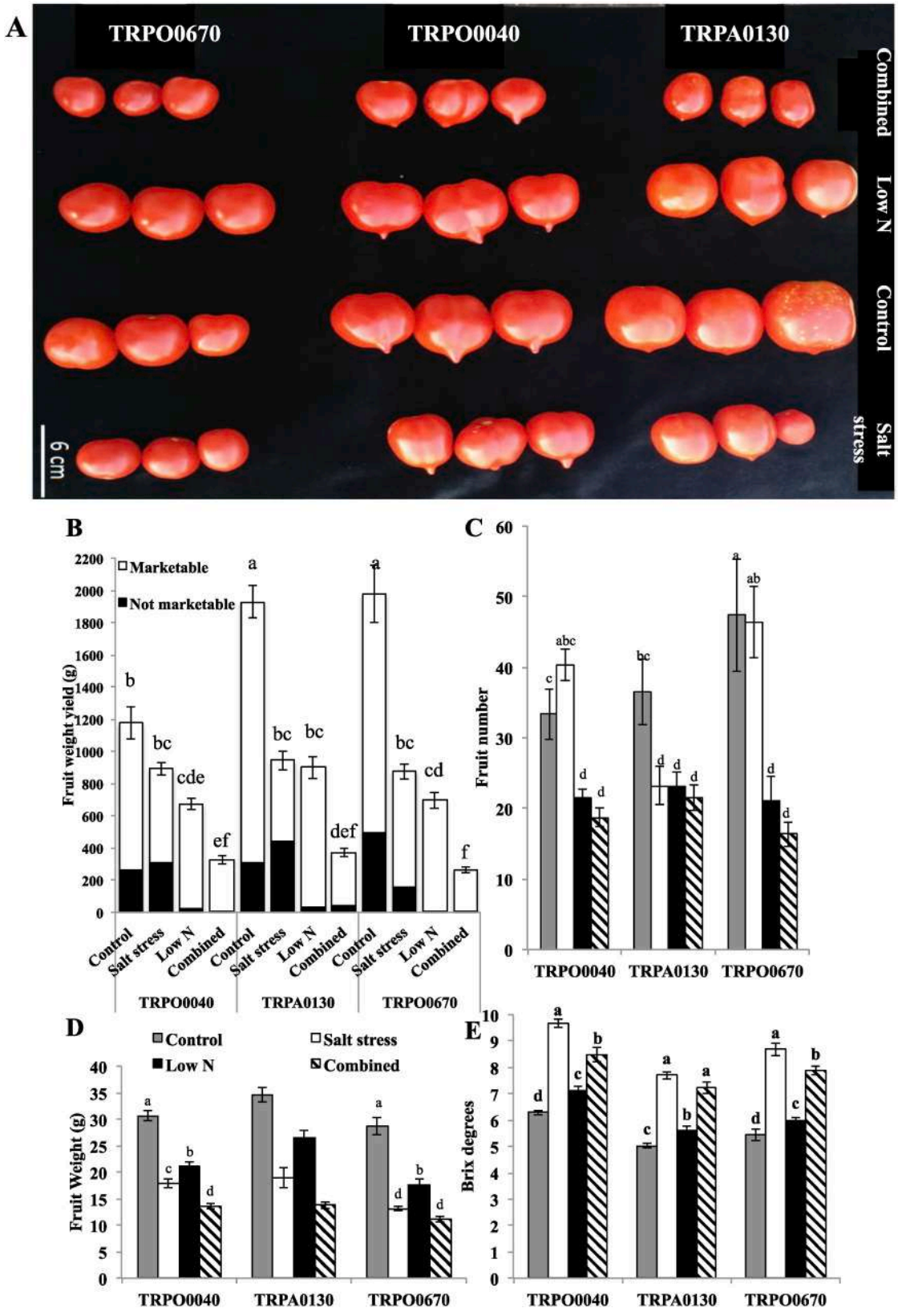


Fig. 3. Impact of salt and low N stresses on fruit yield of TRPO0040, TRPA0130 and TRPO0670. A) Representative red ripe fruits. Scale bar is indicated; B) Yield, divided in marketable (white) and not marketable (black, fruits presenting blossom end rot); C) Number of fruits; D) Single fruit fresh weight; E) Total soluble solid content from tomato juice of three genotypes in the four treatments. Values indicate mean ± SD (B-D, fruits harvested from 14 plants; E, n = 22). Different letters indicate significant differences at $p < 0.05$ according to Duncan post hoc test.

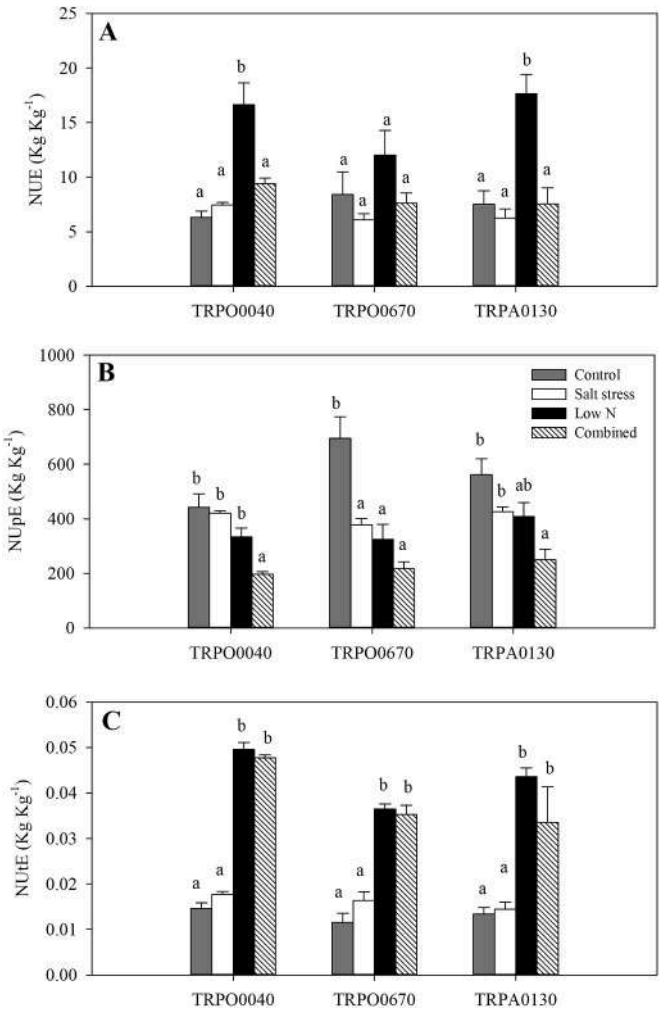


Fig. 4. Nitrogen Use Efficiency (A), uptake efficiency (B) and utilization efficiency (C) of tomato genotypes TRPO0040, TRPO0670, and TRPA0130 exposed to control, low N, salt stress and combined stress conditions for 120 days. Different letters indicate significant differences at $p < 0.05$ according to Duncan post hoc test.

3.5. Ion content

The Na^+ content of the three genotypes showed differences in the control condition in stems and fruit tissues, indicating genotype-specific differences in Na^+ distribution (Fig. 5A–F-K). Salt stress caused higher Na^+ accumulation in TRPO0040 and TRPA0130 genotypes in the leaves compared to TRPO0670. By contrast, under combined stress the total Na^+ content was comparable in all three genotypes in leaves and fruits, whereas in stems, a higher accumulation was observed in TRPO0040 (Fig. 5A–F-K). TRPO0670 exhibited a higher ammonium content in leaves, whereas TRPA0130 in fruits harvested from plants grown in control conditions. However, this pattern disappeared under N stress where all the genotypes did not show significant differences in stems and fruits. In salt stress conditions, TRPO0040 showed a higher ammonium content compared to the others in the stems, although no significant differences were observed in the same tissue under combined conditions (Fig. 5B–G-L).

Concerning K^+, a higher content was observed in TRPO0670 leaves in control and combined conditions, followed by TRPO0040 and TRPA0130 (Fig. 5C–H-M). Low N and salt stress caused a drastic reduction in K^+ content in TRPO0670 in all tissues (Fig. 5C–H-M). Interestingly, TRPO0040 was the only genotype showing differences in K^+ content under low N conditions in leaves and stems. Finally, TRPA0130 showed the lowest K^+ content under combined stress conditions in leaves and stems (Fig. 5C–H-M).

As expected, Cl^- content was higher under salt treatment in all genotypes, with the exception of TRPO0040 fruits, and more accumulated in TRPO0670 leaves, TRPO0040 stems, and TRPA0130 fruits (Fig. 5D–I-N). Nitrate (NO_3^-) content was higher in the control compared to all treatments, which markedly affected its content in all the genotypes except in fruits (Fig. 5E–J-O). Under salt stress, TRPO0670 showed a higher NO_3^- content in leaves and stems compared to the other two genotypes.

Other ions such as PO_4^{3-}, Mg^{2+}, SO_4^{2-}, and Ca^{2+} were also analyzed and reported in Supplementary Fig. S9.

3.6. Transcriptomic differential profiles induced by stress in leaves and roots

To identify the differentially expressed genes (DEG) induced by low N and salt stress, alone or in combination in tomato, and responsible for phenotypic, physiological, and biochemical changes, transcriptomic analysis was performed on TRPO0040 roots and leaves collected after 11 days from stress application (24 DAT). The TRPO0040 genotype was chosen due to its best crop yield performance under stress compared to TRPO0670 and TRPA0130. The sampling time was selected as a long-term stress induction, to avoid short-term plant responses which may partially result from an osmotic shock, rather than identify responses leading to stress adaptation mechanisms. Combined stress and low N induced the highest number of DEGs in leaves (4262 and 3362), whereas salt stress conditions resulted in the lowest (188) (Fig. 6A; Supplementary Table S4). By contrast, roots were highly sensitive to salt stress (2740 DEGs) compared to combined and low N treatment (826 and 259) (Fig. 6B–Supplementary Table S4). In leaves, the majority of DEGs under combined conditions could be attributed to the low N treatment (2413 common DEGs), accounting for 56% of the DEGs, whereas salt stress contributed to a relatively small fraction (1.03% of DEGs, 44 common) (Fig. 6C–D). Similarly, despite roots being highly sensitive to salt stress (2740 DEGs), its impact on combined conditions accounted only for ~30% with 282 common DEGs, whereas more than 50% of DEGs were specific to combined conditions and only ~14% was attributed to low N conditions (123 common DEGs, Fig. 6E–F).

Accordingly, analysis of GO enriched categories showed overlap between low N and combined stress treatments in leaves, and salt and combined stress treatments in roots (Fig. 6G–H; Supplementary Table S5). Although the number of DEGs varied based on tissue or stress conditions, differentially expressed genes were widely distributed on all chromosomes (Supplementary Fig. S10). Results obtained by RNA-seq were validated through qRT-PCR (Supplementary Fig. S11).

3.7. Transcriptome reorganization induced by single and combined stress in leaves

Interestingly, 1805 DEGs (42%) were unique to the combined conditions, hinting at a distinctive response due to the synergic action of salt stress and low N treatment (Fig. 6C–D). Under this condition, ribosome pathways including ribosomal large and small subunits biogenesis, ribosome assembly, ribosome biogenesis, translation, and peptide biosynthetic process were the most enriched terms (Fig. 7A–B). All 99 genes belonging to these processes were down-regulated (Fig. 7C).

Four genes (*Solyc01g102670*, *Solyc07g041310*, *Solyc08g061850,* and *Solyc08g061960*) belonged to the oxidized DNA binding term. *Solyc01g102670* encodes for *DNA glycosylase,* and it was induced whereas *Solyc07g041310, Solyc08g061960 and Solyc08g061850,* all encoding Ribosomal S3 genes (*SRP3*) were repressed (Supplementary Table S4). It is noteworthy that recently Park et al. (2020) revealed that ribosomal proteins S3 are a novel negative regulator of non-homologous end joining repair of DNA double-strand breaks, explaining why these genes are repressed under combined conditions, whereas the *DNA glycosylase*

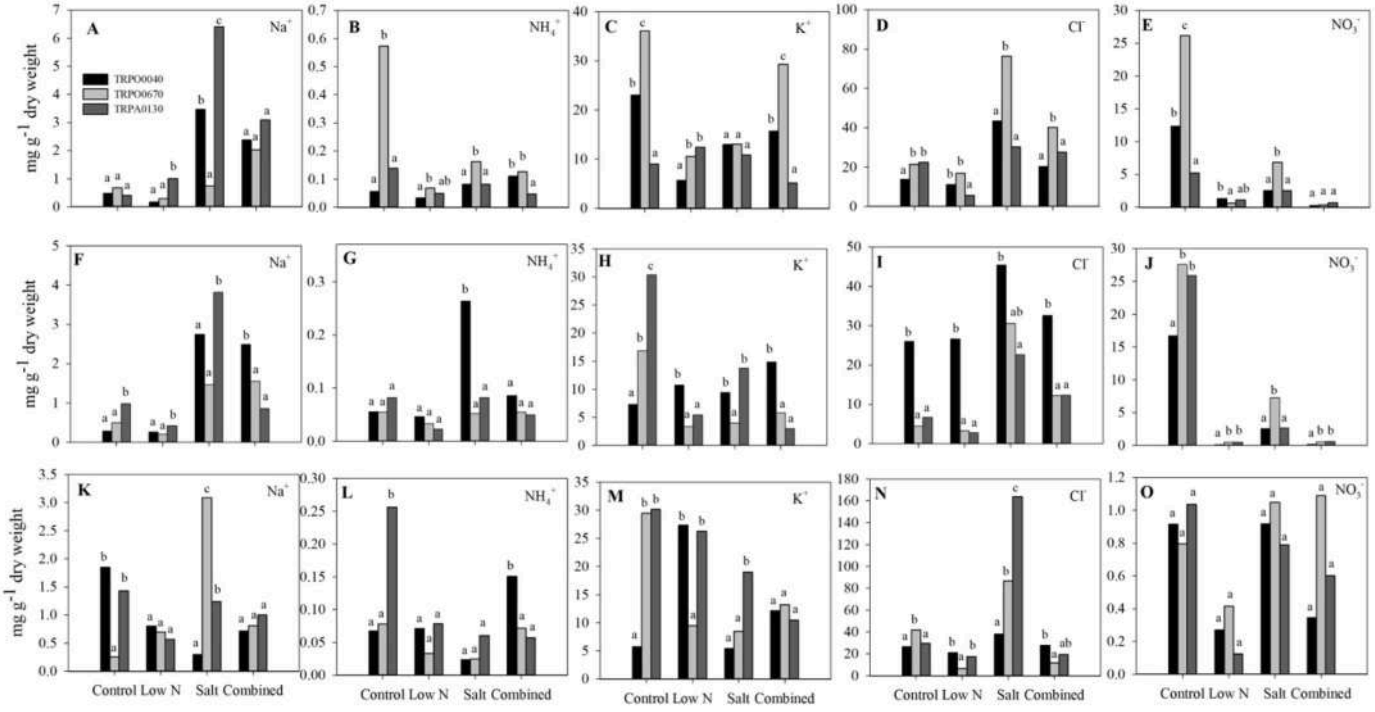


Fig. 5. Ion content (mg g −1 Dry Weight) in leaf (A–E), stem (F–J) and fruit (K–O) of three tomato genotypes exposed to control, low N, salt stress and combined stress conditions. Different letters within treatments for each ion indicate means that differ significantly, according to Tukey's HSD test at $p < 0.05$ ($n = 4$).

was induced.

The impact of low N on combined conditions (2413 DEGs shared between the two conditions) influenced all the main hormone pathways (auxin, cytokinin, gibberellin, and abscisic, jasmonic, and salicylic acids) (Supplementary Fig. S12). Indeed, genes encoding for *PHY* (*Solyc08g082180*), *PHL* (*Solyc01g095700*), *PP2C* (*Solyc05g052980*), *SnRK2* (*Solyc01g108280.3*), all involved in the abscisic acid pathway, were induced under low N and combined stress conditions, whereas within cytokinin pathway, genes such as *A-ARR* (*Solyc06g048600*) and *AHK* (*Solyc04g008110*) were repressed, confirming a fine-tuning of hormonal stress response (Supplementary Fig. S12).

Shared DEGs between low N and combined conditions in leaves were grouped in six clusters (Fig. 8). Clusters 1 and 2 contained DEGs whose expression was higher under combined stress conditions compared with low N. In particular, Cluster 1 grouped induced DEGs (e.g., *Tryptophan synthase*, *Ninja-family AFP1-like*, *ABC transporter*, *TIFY-8*), whereas Cluster 2 contained repressed DEGs (e.g., *Cinnamate-4-hydroxylases, Solyc06g082535* and *Solyc06g082530*; *4-coumarate-CoA ligase, Solyc03g117870; Phenylalanine ammonia-lyase, Solyc09g007910* and *Solyc05g056170*; *Flowering promoting factor-like 1, Solyc01g066957*; and *GATA transcription factor-like, Solyc08g074865*) (Fig. 8B). Clusters 3 and 4 instead grouped down-regulated (Cluster 3) and up-regulated DEGs (cluster 4) whose impact was greater in low N compared with combined conditions (Fig. 8B), whereas Clusters 5 and 6 instead contained genes similarly modulated by low N and combined conditions. ABA-related genes were grouped in cluster 1 and cluster 6, in line with the ABA accumulation detected at 14 DAT in TRPO0040 leaves under combined stress, confirming the induction of ABA-stress-related responses. ~~S~~several *glutaredoxins* (*Solyc04g011850*, *Solyc04g011810*, *Solyc04g011830*), *Glutathione S-transferases* (*Solyc12g006760*, *Solyc12g006770*, *Solyc12g006750*), and nine genes involved in fatty acid biosynthesis (*Fab* genes) and elongation (*Solyc01G006450*, *FabI*; *Solyc01G105060*, *FabZ; Solyc02G070790*, *FabF; Solyc06G053480*, *Fab2*; *Solyc06G069530*, *accB*; *Solyc06G071910*, *FabG; Solyc08G016170*, *FabF; Solyc09G013080, accA;* and *Solyc10G078740, FabI*) were all repressed under low N and combined conditions and grouped in Clusters 2 and 5. In addition, a proline dehydrogenase gene (*Solyc02g089620*), engaged in proline degradation, was suppressed in salt and combined conditions (Supplementary Table S4), possibly explaining the proline accumulation in the TRPO0040 accession observed at 24 DAT in these conditions.

3.8. Transcriptome reorganization induced by single and combined stress in roots

Despite roots being highly sensitive to salt stress (2740 DEGs), its impact on combined conditions accounted only for ~30% with 282 common DEGs, whereas more than 50% of DEGs were specific to combined conditions and only 14% was attributed to low N conditions (123 common DEGs) (Fig. 6E–F). Among DEGs specific to combined stress, GO terms related to regulation of photosynthesis (dark reactions), sulfate transport, response to cytokinin, regulation of growth, and many terms related to response to stress such as response to biotic stimulus, external biotic stimulus, abiotic stimulus, organic substance and response to stress were significantly enriched (p.value < 0.05) (Fig. 9A–B). In particular, several heat shock proteins (*Solyc03g082420*, *Solyc05g014280, Solyc03g007890, Solyc03g115230, Solyc11g020040*) were induced under combined stress (Fig. 9C; Supplementary Table S4). In addition, a cysteine protease similar to senescence-associated gene (*Solyc02g076910, SAG12*), Germin-like (*Solyc07g041720* and *Solyc09g090010*), an MLO-like (*Solyc03g095650*), all involved in stress response, were induced roughly three times more under combined conditions, whereas a defensin-like (*Solyc11g006950*) was repressed up to five-fold compared to control (Supplementary Table S4). Many transcriptions factors (TFs) were also differentially expressed only under combined conditions~~,~~ including a *LIM* TF (*Solyc01g094315*), which was induced sixfold, and a *MYB* (*Solyc08g082890,* similar to *AtMYB66*), which was repressed up to fivefold. Two hundred eighty-two shared genes between salt and combined conditions were analyzed (Fig. 6E–F; Fig. 8C). Among them, *Starch Synthase* (*Solyc07g042830*), Gibberellin *receptor GID1* (*Solyc07g040890*), *WAT1-related gene* (*Solyc12g035400),* and *Acyl-CoA N-acyltransferases* (*NAT*) (Solyc02g064690) were induced under both conditions, whereas *Trehalose 6-phosphate phosphatase*

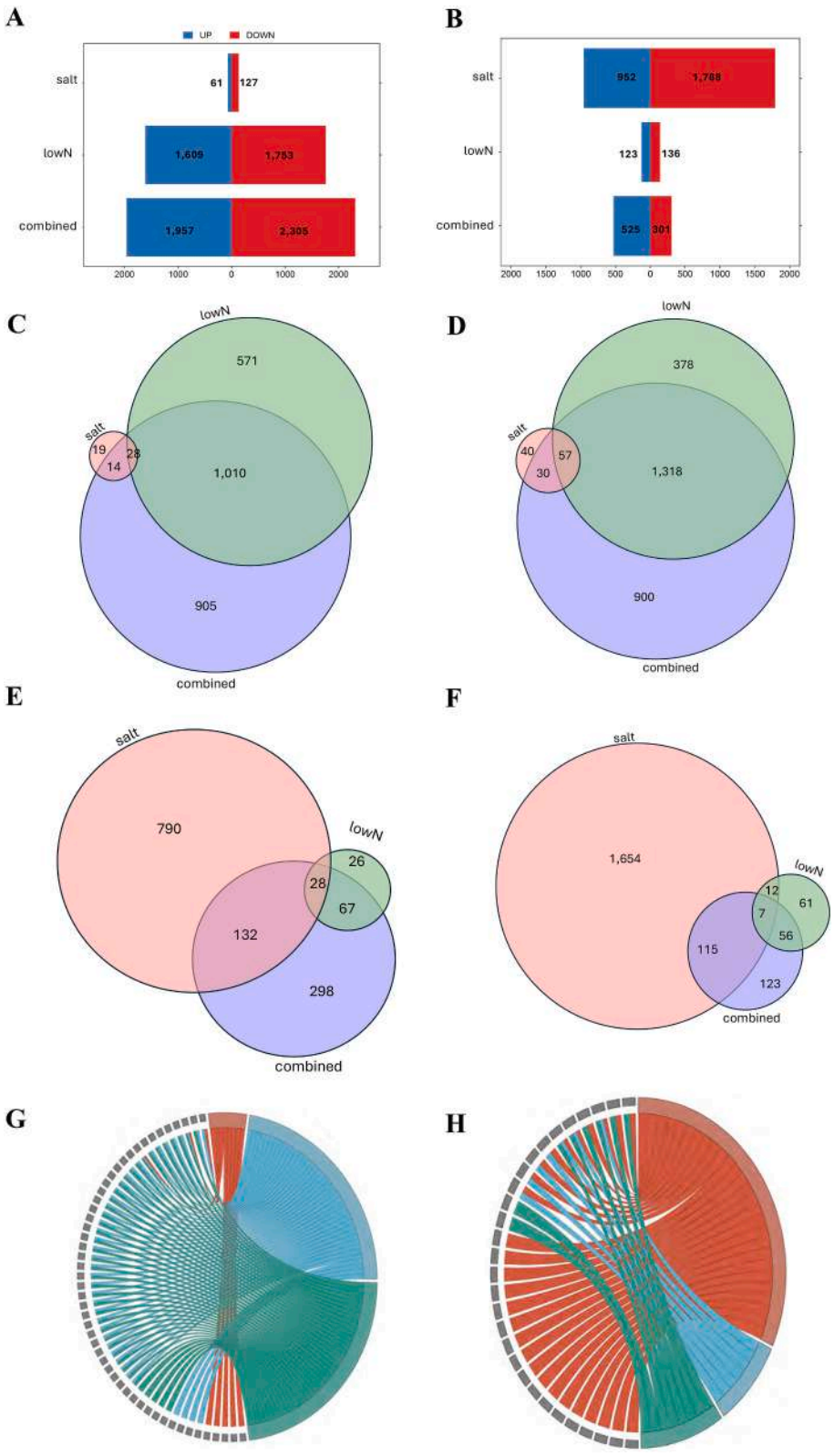
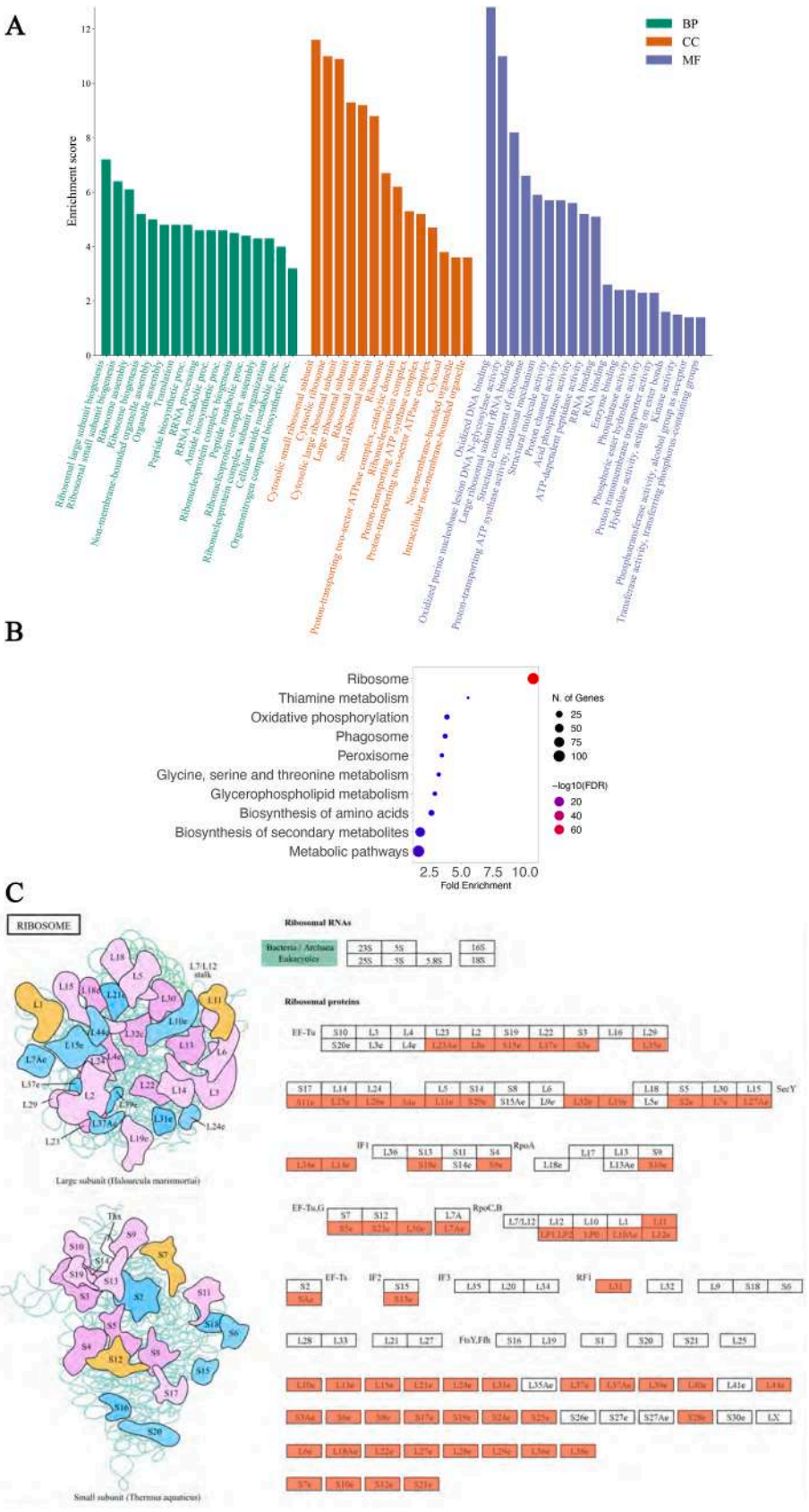


Fig. 6. Transcriptomic changes induced by salt, low N and combined stresses. A-B) Number of differentially expressed genes (DEGs) identified through pairwise comparison (salt vs control; low N vs control and combined vs control) in leaves (A) and roots (B); C-D) Venn Diagrams depicting number and overlap of up (C) and down (D) -regulated DEGs in leaves under salt, low N and combined stress conditions. E-F) Venn Diagrams depicting the number and overlap of up (E) and down (F) -regulated DEGs in roots under salt, low N and combined stress conditions. The diagrams were drawn using the online tool Venny2 (Oliveros, 2007–2015). G-H) GOchord plots showing the most enriched GO terms under salt, low N, and combined conditions in leaf (G) and roots (H). The categories shown were filtered based on FDR value ($\leq$0.05) and gene number ($\geq$2). The GO terms reported in G and H are reported in Supplementary Table S5.



(caption on next page)

Fig. 7. Transcriptomic changes uniquely induced by combined conditions in leaves. Gene ontology (GO) (A) and KEGG (B) enrichment analysis of differentially expressed genes (DEGs) unique in leaves under combined conditions. GO analysis was performed by assigning GO terms to DEGs in three categories: biological process (BP, top), and cellular component (CC, middle), molecular function (MF, bottom). The most enriched KEGG pathways of the target genes were reported. The categories were filtered based on False Discovery Rate (FDR) value (≤0.05) and gene number (≥2). The significance is indicated by -log10(FDR). Red values indicate higher values, whereas green values indicate lower values. The size of the circle indicates the number of enriched target genes. C) KEGG pathways related to ribosomes uniquely enriched under combined conditions in leaves. Red boxes indicate differentially expressed genes (DEGs).

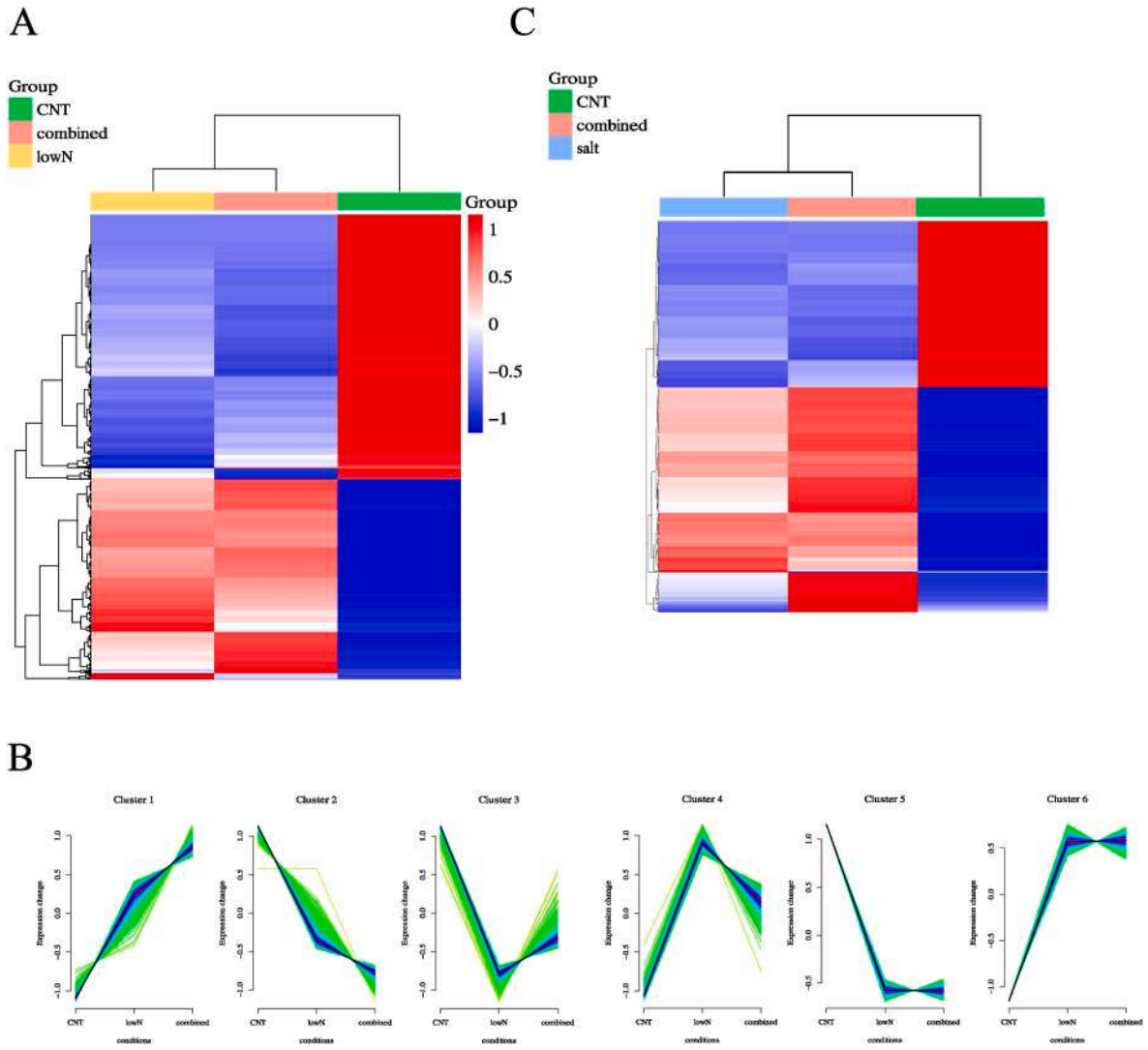


Fig. 8. Profiling of differentially expressed genes (DEGs) shared among low N and combined treatments in leaves (A-B) and salt and combined stress in roots (C). A) Heat map showing the expression pattern of shared genes among low N and combined stress conditions in leaves; B) Six gene expression patterns were identified by mfuzz c-means clustering using DEGs shared under low N and combined conditions in leaves. X-axis represents stress conditions, and the Y-axis shows log2 transformation and normalization intensity; C) Heat map showing the expression pattern of shared genes among salt and combined stress conditions in roots. A, C) Color code represents row Z score.

(Solyc04g054930), a *Terpene cyclase/mutase* (*Solyc07g042630*), *Heavy metal transport/detoxification* genes (*Solyc05g008300* and *Solyc03g080100*) were repressed. By contrast, when looking at DEGs shared between combined and low N stresses, several genes related to transport, including ammino-acid transporters (*Solyc09g098380*), potassium channel inhibitors (*KAT3*, *KC1*, *Solyc08g068000*), urea transporters (*Solyc08g075570*), Nodulin-like genes (*Solyc05g005870*), high-affinity nitrate transporters (*Solyc11g069750*), sugar transporters (*Solyc01g010350*, *Solyc03g097585*), as well as genes encoding regulatory proteins such as PP2C phosphatases (*Solyc08g065540*, *Solyc08g065670*) were identified (Supplementary Table S4).

4. Discussion

The balance between plant growth and stress responses is key in determining crop yield under non-optimal conditions (Zhang et al., 2020). Under stress, plants inhibit growth through both passive and active signaling mechanisms, aimed at shifting resources toward a new homeostasis (Zhang et al., 2020). In this view, we have set up a study aimed at deciphering physiological and molecular mechanisms underlying the interaction between salt stress response and nitrogen availability in tomato.

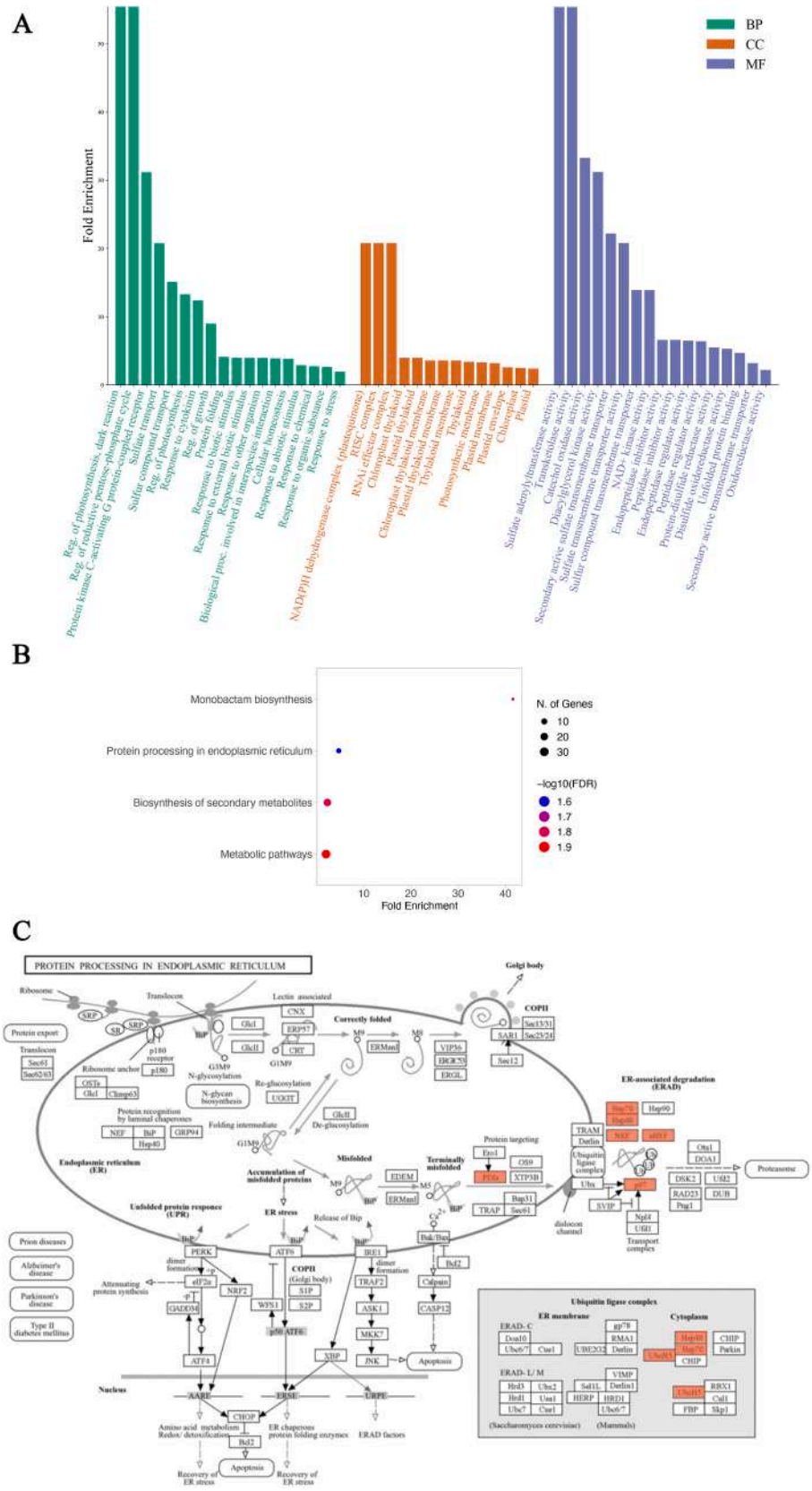


Fig. 9. Transcriptomic changes uniquely induced by combined conditions in roots. Gene ontology (GO) (A) and KEGG (B) enrichment analysis of differentially expressed genes (DEGs) unique in roots under combined conditions. GO analysis was performed by assigning GO terms to DEGs in three categories: biological process (BP, top), and cellular component (CC, middle), molecular function (MF, bottom). The most enriched KEGG pathways of the target genes were reported (B). The categories were filtered based on False Discovery Rate (FDR) value (≤0.05) and gene number (≥2). The significance is indicated by -log10(FDR). Red values indicate higher values, whereas green values indicate lower values. The size of the circle indicates the number of enriched target genes. C) KEGG pathways related to protein processing in endoplasmic reticulum uniquely enriched under combined conditions in roots. Red boxes indicate differentially expressed genes (DEGs).

4.1. Morphological and physiological signature under single and combined stress conditions

By taking advantage of the rich tomato germplasm cultivated in Southern Italy (Landi et al., 2023; Ruggiero et al., 2022), we screened genotypes exposed to salt stress and low nitrate (low N), alone or in combination at the seedling stage. Given that growth maintenance is tightly correlated to salinity tolerance, especially in seedlings where yield cannot be measured (Negrão et al., 2017; Pailles et al., 2020), several growth and physiological parameters were used to identify salt-tolerant genotypes. Interestingly, tolerant genotypes showed a lower root mass ratio (RMR) and specific root length (SRL) under salt stress compared to control and sensitive genotypes, indicating that a preferential biomass allocation to roots under salt stress did not take place in tolerant genotypes. Similar results were also recently confirmed by Fu et al., 2023, who reported that drought and combined salt stress significantly reduced the root length, root surface area, and root volume in wheat seedlings.

Exposure of three genotypes to salt stress, low N, and combined salt/low N conditions allowed us to dissect the effects of single and combinatorial conditions on physiological, biochemical, and molecular responses in a complete growth cycle. As expected, salt stress reduced leaf relative water content (LRWC), indicating the occurrence of osmotic stress under this condition (Parvin et al., 2019). Low N also impacted LRWC, although no additive effect was observed under combined conditions. Previous studies showed that a high nitrate supply increases root water uptake in tomato (Gorska et al., 2008; Górska et al., 2010), suggesting that a reduced root water flow may be responsible for the observed lower LRWC. SPAD measurements further confirmed that low N conditions negatively impacted the physiology, resulting in a lower chlorophyll content, a parameter expected for a high nitrate-demanding plant such as tomato (Górska et al., 2010). These observations were also reflected in reduced biomass accumulation and leaf chlorosis. Higher SPAD units measured in salt-stressed plants may be the result of a smaller leaf area and thickness, which might reflect a higher chlorophyll density, as also reported by Negrao et al. (2017).

Proline is a compatible osmolyte, useful for osmotic adjustment as well as for protection from oxidative stress (Negrao, 2017; De la Torre-González et al., 2018). Monitoring its content in leaves over time indicated an early accumulation following salt stress, consistent with previous reports (Carillo et al., 2008; Annunziata et al., 2019). In addition, our results highlight its dependency on nitrate availability, as indicated by the reduction of proline accumulation in combined stress, confirming the theory that a reduced osmotic adjustment capability is possible in plants subjected to combined stress, or exists a shift towards other osmolytes, whose accumulation is not dependent on nitrate availability (Carillo et al., 2008).

Hormones are also important under stress conditions (Waadt et al., 2022). Mounting evidence in the literature shows that ABA is involved in the optimization of nitrate uptake both under high and low nitrate availability (Wang et al., 2020a). Indeed, consistent with the observation made by Landi et al. (2023), in our study, we observed a high ABA accumulation under combined stress conditions in TRPO0040, which was correlated to the higher expression levels of a *Late embryogenesis abundant (LEA, Solyc03g116390)* gene, a well-known drought and salt stress-responsive gene in tomato (Iovieno et al., 2016).

Plant growth and fruit production parameters were dramatically reduced in stress conditions, particularly under combined stress. This may result from direct toxicity and competition for uptake of Na^+ and Cl^- with K^+, $NH4^+$ and NO_3^-, respectively (Nazir et al., 2023) as observed from TRPO0670 and TRPA0130 leaf ion measurements, further reducing N availability in the combined stress condition. Consistent with previous results (Hernandez et al., 2020), fruit number, average fruit weight, and total yield decreased in low N treatments. Interestingly, whereas salt stress increased total soluble sugars, a decrease in fruit number caused by salt stress was only observed in genotype TRPA0130, indicating a genotype-dependent response, and no salt-stress-dependent decline in fruit number under combined stress was observed. Similarly, Flores et al. (2003), did not observe a decrease in fruit number in tomato plants exposed to moderate salt stress, indicating that this parameter is not highly sensitive to salt stress. Fruits harvested under low N treatment showed a lower incidence of blossom end rot (BER), virtually absent in fruit harvested from the combined stress treatment. Since BER appears to be correlated to the fruit growth rate (Aslani et al., 2020) and cell expansion, a lower fruit cell expansion rate in combined stress conditions may be responsible for the observed phenotype (Ho and White, 2005).

4.2. Transcriptomic signature in leaves and roots under single and combined stress conditions

The modulation of the tomato transcriptome induced by combined salt and low N conditions remains largely unknown, especially in the root system. Therefore, here, we identified the transcriptome landscape of TRPO0040, showing a higher yield performance under single and combined stress conditions, in both leaves and roots, highlighting that the transcriptomic signature was tissue- and stress-specific. Previous studies in different plant species such as barley, tomato, *Arundo donax,* and *Arachis* also reported similar results (Dossa et al., 2019; Jan et al., 2019; Docimo et al., 2019; Khan et al., 2020; Mota et al., 2021; Morales-Merida et al., 2023), suggesting that the response to multiple stresses is the result of synergistic and antagonistic effects of the individual stresses, as well as of tissue-specific transcriptomic alterations (Ramegowda et al., 2015). In line with the above reports, Kissoudis et al. (2016) and Davila Olivas et al. (2016) indicated that adaptive responses are the result of fine tuning of different hormone signalling cascades elicited by the specific single or combined stresses applied (Kissoudis et al., 2016). In our scenario, the majority of DEGs under combined conditions in leaves were attributed to the low N treatment, whereas salt stress contributed to a relatively small fraction. Evidently, under combined conditions, plants prioritize the response strategy to the more severe stress or whose effect impacts first, as also suggested by Pandey et al. (2015). Similarly, Rizhsky et al. (2004) reported in *Arabidopsis* that the transcriptomic response to combined drought and heat stress responses was mainly due to drought rather than heat since roughly half the transcripts were in common between drought and combined conditions. The authors, thus, concluded that plant responses to multiple stressors might be a means of shared and unique transcriptomic changes, and their proportions depended on several factors, including intensity and timing. Another theory might be related to the idiosyncrasy plant response, often reported by researchers as the main driver following combined conditions in different plant species (Prasch and Sonnewald, 2015). For example, a unique molecular response to simultaneous drought and heat compared to single stress was observed in *Arabidopsis* (Rasmussen et al., 2013), sorghum (Johnson et al., 2014), and wheat (Rampino et al., 2012). Consistent with the above observations, 1805 DEGs in leaves were unique to the combined conditions, hinting at a distinctive response due to the synergic action of salt stress and low N treatment. Interestingly, under this condition, ribosome pathways (including large and small subunits biogenesis, ribosome assembly, ribosome biogenesis, translation, and peptide biosynthetic processes) were all repressed. Several studies demonstrated that canonical protein translation is significantly suppressed under stress conditions to help plants adapt and survive (Bailey-Serres and Voesenek, 2008; Browning and Bailey-Serres, 2015; Son and Park, 2023). For example, the ribosome activity in the cytoplasm is directly and dynamically fine-tuned by dehydration stress (Kawaguchi et al., 2004), hypoxia (Branco-Price et al., 2008; Juntawong et al., 2014) and heat stress (Zhang et al., 2017). Previous studies in *Arabidopsis* following short-term exposure also reported different evidence that translation is greatly inhibited (Merret et al., 2015; Lukoszek et al., 2016). However, Lukoszek et al. (2016) showed that under prolonged heat exposure, translation is fully active,

suggesting that despite the global repression at a translational level under short-term heat stress, some transcripts might be selectively translated, including those involved in transcriptional regulation, chromatin structure rearrangements, mRNA degradation and protein phosphorylation (Lukoszek et al., 2016). It will be intriguing to determine the molecular mechanism of ribosome sensing in tomatoes in the future to explore the possibility of developing improved plant tolerance to various stress conditions through the modulation of protein translation.

As reported above, the majority of DEGs under combined conditions in leaves were attributed to the low N treatment rather than salt stress, and different KEGG pathways related to plant hormone signal transduction, fatty acid biosynthesis, carbon and glutathione metabolisms, and DNA replication were enriched in both low N and combined conditions. For example, genes involved in the ABA pathway such as *PHY* (*Solyc08g082180*), *PHL* (*Solyc01g095700*), *PP2C* (*Solyc05g052980*), *SnRK2* (Solyc01g108280.3) were induced under low N conditions, whereas *A-ARR* (*Solyc06g048600*) and *AHK* (*Solyc04g008110*), both acting in the cytokinin pathway, were repressed, confirming the fine-tuning of hormonal stress response and the antagonistic action between ABA and cytokinin signaling. Huang et al. (2018) reported the tight link between ABA and cytokinin during seed germination, development, and abiotic stress responses, where ABA-dependent kinases such as SnRK2.2/2.3/2.6 phosphorylate ARR5, enhancing its stability. Consistent with previous reports that *Arabidopsis ahk2*, *ahk3*, and *ahk2-ahk3* mutants were associated with the increase in tolerance levels (Wohlbach et al., 2008; Kumar et al., 2013), the downregulation of *AHK* in tomato leaves might contribute to withstand adverse stress conditions. In addition, nine genes involved in fatty acid biosynthesis (*Fab* genes) and elongation were repressed under low N and combined conditions, suggesting that fatty acid elongation and biosynthesis are potentially reduced to adjust energy consumption and primary metabolism following stress conditions (He et al., 2020).

Genes commonly regulated in low N and combined stress conditions were also identified in leaves, that were associated with different processes, including responses to stress, photosynthesis, lipid transport, LEA, and HSP proteins, which were differentially modulated by stress treatments.

In contrast with what we observed in leaves, roots were highly sensitive to salt stress rather than low N and combined stress conditions, suggesting that the stress response is tissue-specific (Ruggiero et al., 2022). However, despite their sensitivity, the impact of salt stress on combined conditions accounted in roots only for ~30%, whereas the majority of DEGs were specific to combined stress, confirming that the response to combined conditions is not predictable by single-stress responses. Similar results were also reported by Osthoff et al. (2019) in barley and Rasmussen et al. (2013) in *Arabidopsis* ecotypes, where both species exhibited non-additive responses to combinatorial conditions. Interestingly, among the DEGs unique under combined conditions many GO terms related to response to stress including response to biotic stimulus, abiotic stimulus, organic substance, and response to stress were significantly enriched. Among them, many transcription factors (TFs) belonging to *MYB* and *MYB*-related, *bHLH*, *TIFY, NAC*, *ARF/B3* and *LIM* were differentially expressed, confirming their important regulatory role in response to abiotic stress in plants, as already reported by many authors (Fowler and Thomashow, 2002; Knight and Knight, 2012; Ng et al., 2018; Nie et al., 2018; Esposito et al., 2021).

Five different *MYBs* were also identified, of which three were induced and two repressed. Functional studies have shown that *MYBs* are involved in plant secondary metabolism (Uimari and Strommer, 1997), hormone (Khadem et al., 2023), and environmental responses (Li et al., 2016), and play an important regulatory role in cell differentiation, cell cycle, and leaf morphogenesis (Chen et al., 2003, 2022; Okushima et al., 2007; Jiang and Rao, 2020). Among the *MYBs* identified in our study, *Solyc08g082890,* reported by Li et al. (2016) as *SlMYB66* and direct ortholog of *AtMYB66,* was five-fold repressed under combined conditions. *MYB66* was reported to contribute to root hair cell fate determination in *Arabidopsis* (Bernhardt et al., 2005; Dubos et al., 2010) and promote drought tolerance via stomatal movement regulation (Chang et al., 2008). The modified expression of *SlMYB66* may reflect modifications in root architecture and a slower root growth rate under combined stress.

5. Conclusions

Our study provided an integrated view of morpho-physiological and molecular responses in tomato exposed to low nitrate, salt stress and combined conditions in a complete growth cycle. Our data revealed that the stress conditions impacted plant growth and physiology in a genotype- and tissue-specific manner, as well as dependent on the nature of the stress applied. Combined stress resulted in the lowest yield, since fruit weight and fruit number, highly affected by the presence of NaCl and nitrate availability were both compromised in this condition. By contrast, total soluble solids content in fruits harvested under combined stress was lower compared to the salt stress treatment in two of the three tested genotypes, indicating a non-additive effect of the two stress components on this quality parameter. A tissue-specific response was also confirmed by transcriptomics, since the identified DEGs were different between leaves and roots. Leaves were highly sensitive to nitrate availability, and showed a general down-regulation of ribosome component-encoding genes, which may contribute to explain a growth and production halt in the combined stress condition. By contrast, abiotic stress-dependent and defence related genes were regulated in roots, indicating a higher sensitivity of roots to salt stress. Altogether, our results contribute to the elucidation of mechanisms of response to combined salt stress and low nitrate availability in tomato, providing candidate genes fur further studies aimed to increase plant tolerance under combined stress conditions.

Funding

This work was supported by European Commission H2020 research and innovation program through TRADITOM grant agreement No.634561. This study was carried out within the Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 June 17, 2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data has been deposited to NCBI. The PRJNA number is specified in the manuscript

Acknowledgments

The authors are grateful to Mr. Mario Ruggiero, Mr. Gaetano Guarino and Mr. Carlo De Cesare for their excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108976.

References

Abenavoli, M.R., Longo, C., Lupini, A., Miller, A.J., Araniti, F., Mercati, F., Princi, M.P., Sunseri, F., 2016. Phenotyping two tomato genotypes with different nitrogen use efficiency. Plant Physiol. Biochem. (Issy les Moulineaux, Fr.) 107, 21–32. https://doi.org/10.1016/j.plaphy.2016.04.021.

Aci, M.M., Lupini, A., Mauceri, A., Sunseri, F., Abenavoli, M.R., 2021. New insights into N-utilization efficiency in tomato (*Solanum lycopersicum* L.) under N limiting condition. Plant Physiol. Biochem. 166, 634–644. https://doi.org/10.1016/j.plaphy.2021.06.046.

Annunziata, M.G., Ciarmiello, L.F., Woodrow, P., Dell'Aversana, E., Carillo, P., 2019. Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Front. Plant Sci. 10, 230. https://doi.org/10.3389/fpls.2019.00230.

Aslani, L., Gholami, M., Mobli, M., 2020. The relationship between tomato fruit growth, incidence of blossom-end rot and phytohormone content as affected by sink/source ratio. Ann. Appl. Biol. 177, 211–222. https://doi.org/10.1111/aab.12611.

Bai, Y., Kissoudis, C., Yan, Z., Visser, R.G.F., van der Linden, G., 2018. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. Plant J. 93 (4), 781–793. https://doi.org/10.1111/tpj.13800.

Bailey-Serres, J., Voesenek, L.A.C.J., 2008. Flooding stress: acclimations and genetic diversity. Annu. Rev. Plant Biol. 59, 313–339. https://doi.org/10.1146/annurev.arplant.59.032607.092752.

Bernhardt, C., Zhao, M., Gonzalez, A., Lloyd, A., Schiefelbein, J., 2005. The *bHLH* genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. Development 132 (2), 291–298. https://doi.org/10.1242/dev.01565.

Blanca, J., Pons, C., Montero-Pau, J., Sanchez-Matarredona, D., Ziarsolo, P., Fontanet, L., Fisher, J., Plazas, M., Casals, J., Rambla, J.L., Riccini, A., Pombarella, S., Ruggiero, A., Sulli, M., Grillo, S., Kanellis, A., Giuliano, G., Finkers, R., Cammareri, M., Grandillo, S., Mazzucato, A., Causse, M., Díez, M.J., Prohens, J., Zamir, D., Cañizares, J., Monforte, A.J., Granell, A., 2022. European traditional tomatoes galore: a result of farmers' selection of a few diversity-rich loci. J. Exp. Bot. 73 (11), 3431–3445. https://doi.org/10.1093/jxb/erac072.

Branco-Price, C., Kaiser, K.A., Jang, C.J., Larive, C.K., Bailey-Serres, J., 2008. Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. Plant J. 56 (5), 743–755. https://doi.org/10.1111/j.1365-313X.2008.03642.x.

Browning, K.S., Bailey-Serres, J., 2015. Mechanism of cytoplasmic mRNA translation. Arabidopsis Book 13, e0176. https://doi.org/10.1199/tab.0176.

Cappetta, E., Andolfo, G., Di Matteo, A., Ercolano, M.R., 2020. Empowering crop resilience to environmental multiple stress through the modulation of key response components. J. Plant Physiol. 246–247, 153134 https://doi.org/10.1016/j.jplph.2020.153134.

Carillo, P., Mastrolonardo, G., Nacca, F., Parisi, D., Verlotta, A., Fuggi, A., 2008. Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine. Funct. Plant Biol. 35 (5), 412–426. https://doi.org/10.1071/FP08108.

Chang, C.S., Li, Y.H., Chen, L.-T., Chen, W.-C., Hsieh, W.-P., Shin, J., Jane, W.-N., Chou, S.-J., Choi, G., Hu, J.-M., Somerville, S., Wu, S.-H., 2008. LZF1, a HY5-regulated transcriptional factor, functions in *Arabidopsis* de-etiolation. Plant J. 54 (2), 205–219. https://doi.org/10.1111/j.1365-313X.2008.03401.x.

Chardon, F., Barthélémy, J., Daniel-Vedele, F., Masclaux-Daubresse, C., 2010. Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. J. Exp. Bot. 61 (9), 2293–2302. https://doi.org/10.1093/jxb/erq059.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103 (4), 551–560. https://doi.org/10.1093/aob/mcn125.

Chen, S., Peng, S., Huang, G., Wu, K., Fu, X., Chen, Z., 2003. Association of decreased expression of a Myb transcription factor with the TPD (tapping panel dryness) syndrome in *Hevea brasiliensis*. J. Plant Mol. Biol. 51, 51–58. https://doi.org/10.1023/A:1020719420867.

Chen, Z., Wu, Z., Dong, W., Liu, S., Tian, L., Li, J., Du, H., 2022. *MYB* Transcription factors becoming mainstream in plant roots. Int. J. Mol. Sci. 23 (16), 9262. https://doi.org/10.3390/ijms23169262.

Claussen, W., 2005. Proline as a measure of stress in tomato plants. Plant Sci. 168 (1), 241–248. https://doi.org/10.1016/j.plantsci.2004.07.039.

Davila Olivas, N.H., Coolen, S., Huang, P., Severing, E., van Verk, M.C., Hickman, R., Wittenberg, A.H., de Vos, M., Prins, M., van Loon, J.J., Aarts, M.G., van Wees, S.C., Pieterse, C.M., Dicke, M., 2016. Effect of prior drought and pathogen stress on *Arabidopsis* transcriptome changes to caterpillar herbivory. New Phytol. 210 (4), 1344–1356. https://doi.org/10.1111/nph.13847.

Debouba, M., Maâroufi-Dghimi, H., Suzuki, A., Ghorbel, M.H., Gouia, H., 2007. Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress. Ann. Bot. 99 (6), 1143–1151. https://doi.org/10.1093/aob/mcm050.

De la Torre-González, A., Montesinos-pereira, D., Blasco, B., Ruiz, J.M., 2018. Influence of the proline metabolism and glycine betaine on tolerance to salt stress in tomato (*Solanum lycopersicum* L.) commercial genotypes. J. Plant Physiol. 231, 329–336. https://doi.org/10.1016/j.jplph.2018.10.013.

Docimo, T., De Stefano, R., De Palma, M., Cappetta, E., Villano, C., Aversano, R., Tucci, M., 2019. Transcriptional, metabolic and DNA methylation changes underpinning the response of *Arundo donax* ecotypes to NaCl excess. Planta 251. https://doi.org/10.1007/s00425-019-03325-w.

Dossa, K., Mmadi, M.A., Zhou, R., Zhang, T., Su, R., Zhang, Y., Wang, L., You, J., Zhang, X., 2019. Depicting the core transcriptome modulating multiple abiotic stresses responses in sesame (*Sesamum indicum* L.). Int. J. Mol. Sci. 20 (16), 3930. https://doi.org/10.3390/ijms20163930.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., Su, Z., 2010. agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res. 38, W64–W70. https://doi.org/10.1093/nar/gkq310.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., Lepiniec, L., 2010. MYB transcription factors in *Arabidopsis*. Trends Plant Sci. 15 (10), 573–581. https://doi.org/10.1016/j.tplants.2010.06.005.

Esposito, S., Aversano, R., Bradeen, J., D'Amelia, V., Villano, C., Carputo, D., 2021. Coexpression gene network analysis of cold-tolerant *Solanum commersonii* reveals new insights in response to low temperatures. Crop Sci. 61, 3538–3550. https://doi.org/10.1002/csc2.20473.

FAO, 2022. Global soil partnership. soil salinity. Available at: https://www.fao.org/global-soil-partnership/areas-of-work/soil-salinity/en/.

Flores, P., Navarro, J., Carvajal, M., Cerdá, A., Martínez, V., 2003. Tomato yield and quality as affected by nitrogen source and salinity. Agronomie 23 (3), 249–256. https://doi.org/10.1051/agro:2002088ff ffhal-00886177f.

Fowler, S., Thomashow, M.F., 2002. *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14 (8), 1675–1690. https://doi.org/10.1105/tpc.003483.

Fu, Y., Li, P., Mounkaila Hamani, A.K., Wan, S., Gao, Y., Wang, X., 2023. Effects of single and combined drought and salinity stress on the root morphological characteristics and root hydraulic conductivity of different winter wheat varieties. Plants 12 (14), 2694. https://doi.org/10.3390/plants12142694.

Galise, T.R., Esposito, S., D'Agostino, N., 2021. Guidelines for setting up a mRNA sequencing experiment and best practices for bioinformatic data analysis. Methods Mol. Biol. 2264, 137–162. https://doi.org/10.1007/978-1-0716-1201-9_10.

Gong, P., Zhang, J., Li, H., Yang, C., Zhang, C., Zhang, X., Khurram, Z., Zhang, Y., Wang, T., Fei, Z., Ye, Z., 2010. Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. J. Exp. Bot. 61 (13), 3563–3575. https://doi.org/10.1093/jxb/erq167.

Górska, A., Lazor, J.W., Zwieniecka, A.K., Benway, C., Zwieniecki, M.A., 2010. The capacity for nitrate regulation of root hydraulic properties correlates with species' nitrate uptake rates. Plant Soil 337, 447–455. https://doi.org/10.1007/s11104-010-0540-x.

Gorska, A., Ye, Q., Holbrook, N.M., Zwienieck, i M.A., 2008. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. Plant Physiol. 148 (2), 1159–1167. https://doi.org/10.1104/pp.108.122499.

Guo, M., Wang, X.-S., Guo, H.-D., Bai, S.-Y., Khan, A., Wang, X.-M., Gao, Y.-M., Li, J.-S., 2022. Tomato salt tolerance mechanisms and their potential applications for fighting salinity: a review. Front. Plant Sci. 13, 949541 https://doi.org/10.3389/fpls.2022.949541.

Hannachi, S., Steppe, K., Eloudi, M., Mechi, L., Bahrini, I., Van Labeke, M.C., 2022. Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant ('Bonica') and one sensitive ('Black beauty') eggplant cultivars (*Solanum melongena* L.). Plants 11 (5), 590. https://doi.org/10.3390/plants11050590.

Hawkesford, M.J., 2012. The diversity of nitrogen use efficiency for wheat varieties and the potential for crop improvement. Better Crops 96, 10–12.

He, M., Qin, C.-X., Wang, X., Ding, N.-Z., 2020. Plant unsaturated fatty acids: biosynthesis and regulation. Front. Plant Sci. 11, 390. https://doi.org/10.3389/fpls.2020.00390.

Hernandez, V., Hellín, P., Fenoll, J., Flores, P., 2020. Impact of nitrogen supply limitation on tomato fruit composition. Sci. Hortic. 264, 1091739 https://doi.org/10.1016/j.scienta.2020.109173.

Holsteens, K., De Jaegere, I., Wynants, A., Prinsen, E.L.J., Van de Poel, B., 2022. Mild and severe salt stress responses are age-dependently regulated by abscisic acid in tomato. Front. Plant Sci. 13, 982622 https://doi.org/10.3389/fpls.2022.982622.

Hosseinifard, M., Stefaniak, S., Ghorbani Javid, M., Soltani, E., Wojtyla, Ł., Garnczarska, M., 2022. Contribution of exogenous proline to abiotic stresses tolerance in plants: a review. Int. J. Mol. Sci. 23 (9), 5186. https://doi.org/10.3390/ijms23095186.

Ho, L.C., White, P.J., 2005. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. Ann. Bot. 95 (4), 571–581.

Huang, X., Hou, L., Meng, J., You, H., Li, Z., Gong, Z., Yang, S., Shi, Y., 2018. The antagonistic action of abscisic acid and cytokinin signaling mediates drought stress response in *Arabidopsis*. Mol. Plant 11 (7), 970–982. https://doi.org/10.1016/j.molp.2018.05.001.

Iovieno, P., Punzo, P., Guida, G., Mistretta, C., Van Oosten, M.J., Nurcato, R., Bostan, H., Colantuono, C., Costa, A., Bagnaresi, P., Chiusano, M.L., Albrizio, R., Giorio, P., Batelli, G., Grillo, S., 2016. Transcriptomic changes drive physiological responses to progressive drought stress and rehydration in tomato. Front. Plant Sci. 7, 371. https://doi.org/10.3389/fpls.2016.00371.

Jan, S., Abbas, N., Ashraf, M., Ahmad, P., 2019. Roles of potential plant hormones and transcription factors in controlling leaf senescence and drought tolerance. Protoplasma 256 (2), 313–329. https://doi.org/10.1007/s00709-018-1310-5.

Jiang, C.-K., Rao, G.-Y., 2020. Insights into the diversification and evolution of r2r3-myb transcription factors in plants. Plant Physiol. 183 (2), 637–655. https://doi.org/10.1104/pp.19.01082.

Johnson, S.M., Lim, F.-L., Finkler, A., Fromm, H., Slabas, A.R., Knight, M.R., 2014. Transcriptomic analysis of sorghum bicolor responding to combined heat and drought stress. BMC Genom. 15 (1), 456. https://doi.org/10.1186/1471-2164-15-456.

Juntawong, P., Girke, T., Bazin, J., Bailey-Serres, J., 2014. Translational dynamics revealed by genome-wide profiling of ribosome footprints in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 111 (1), E203–E212. https://doi.org/10.1073/pnas.1317811111.

Kawaguchi, R., Girke, T., Bray, E.A., Bailey-Serres, J., 2004. Differential mRNA translation contributes to gene regulation under non-stress and dehydration stress conditions in *Arabidopsis thaliana*. Plant J. 38 (5), 823–839. https://doi.org/10.1111/j.1365-313X.2004.02090.x.

Khadem, A., Moshtaghi, N., Bagheri, A., 2023. Regulatory networks of hormone-involved transcription factors and their downstream pathways during somatic embryogenesis of *Arabidopsis thaliana*. 3 Biotech. 13 (5), 132. https://doi.org/10.1007/s13205-023-03546-7.

Khan, N., Bano, A., Ali, S., Babar, M.A., 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regul. 90, 189–203. https://doi.org/10.1007/s10725-020-00571-x.

Kissoudis, C., Sunarti, S., van de Wiel, C., Visser, R.G.F., van der Linden, G., Bai, Y., 2016. Responses to combined abiotic and biotic stress in tomato are governed by stress intensity and resistance mechanism. J. Exp. Bot. 67 (17), 5119–5132. https://doi.org/10.1093/jxb/erw285.

Knight, M.R., Knight, H., 2012. Low-temperature perception leading to gene expression and cold tolerance in higher plants. New Phytol. 195 (4), 737–751. https://doi.org/10.1111/j.1469-8137.2012.04239.x.

Kumar, M.N., Jane, W.N., Verslues, P.E., 2013. Role of the putative osmosensor *Arabidopsis* histidine kinase1 in dehydration avoidance and low-water-potential response. Plant Physiol. 161 (2), 942–953. https://doi.org/10.1104/pp.112.209791.

Landi, S., Punzo, P., Nurcato, R., Albrizio, R., Sanseverino, W., Aiese Cigliano, R., Giorio, P., Fratianni, F., Batelli, G., Esposito, S., Grillo, S., 2023. Transcriptomic landscape of tomato traditional long shelf-life landraces under low water regimes. Plant Physiol. Biochem. 201, 107877 https://doi.org/10.1016/j.plaphy.2023.107877.

Li, X.D., Zhuang, K.Y., Liu, Z.M., Yang, D.Y., Ma, N.N., Meng, Q.W., 2016. Overexpression of a novel NAC-type tomato transcription factor, *SlNAM1*, enhances the chilling stress tolerance of transgenic tobacco. J. Plant Physiol. 204, 54–65. https://doi.org/10.1016/j.jplph.2016.06.024.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Lopez-Delacalle, M., Camejo, D.M., García-Martí, M., Nortes, P.A., Nieves-Cordones, M., Martínez, V., Rubio, F., Mittler, R., Rivero, R.M., 2020. Using tomato recombinant lines to improve plant tolerance to stress combination through a more efficient nitrogen metabolism. Front. Plant Sci. 10, 1702. https://doi.org/10.3389/fpls.2019.01702.

Lukoszek, R., Feist, P., Ignatova, Z., 2016. Insights into the adaptive response of *Arabidopsis thaliana* to prolonged thermal stress by ribosomal profiling and RNA-Seq. BMC Plant Biol. 16 (1), 221. https://doi.org/10.1186/s12870-016-0915-0.

Mauceri, A., Bassolino, L., Lupini, A., Badeck, F., Rizza, F., Schiavi, M., Toppino, L., Abenavoli, M.R., Rotino, G.L., Sunseri, F., 2020. Genetic variation in eggplant for nitrogen use efficiency under contrasting NO_3^- supply. J. Integr. Plant Biol. 62, 487–508. https://doi.org/10.1111/jipb.12823.

Merret, R., Nagarajan, V.K., Carpentier, M.-C., Park, S., Favory, J.-J., Descombin, J., Picart, C., Charng, Y.Y., Green, P.J., Deragon, J.M., Bousquet-Antonelli, C., 2015. Heat-induced ribosome pausing triggers mRNA co-translational decay in *Arabidopsis thaliana*. Nucleic Acids Res. 43 (8), 4121–4132. https://doi.org/10.1093/nar/gkv234.

Moll, R.H., Kamprath, E.J., Jackson, W.A., 1982. Analysis and interpretation of factors which contribute to efficiency to nitrogen utilization. Agron. J. 74, 562–564. https://doi.org/10.2134/agronj1982.00021962007400030037x.

Morales-Merida, B.E., Grimaldi-Olivas, J.C., Cruz-Mendívil, A., Villicaña, C., Valdez-Torres, J.B., Heredia, J.B., León-Chan, R., Lightbourn-Rojas, L.A., León-Félix, J., 2023. *RVE1*, *DBB1b*, and *COL2* transcription factors are responsive to combined stress by uv-b radiation and cold in bell pepper (*Capsicum annuum*). Horticulturae 9, 699. https://doi.org/10.3390/horticulturae9060699.

Mota, A.P.Z., Brasileiro, A.C.M., Vidigal, B., Oliveira, T.N., da Cunha Quintana Martins, A., Saraiva, M.A.P., de Araújo, A.C.G., Togawa, R.C., Grossi-de-Sá, M.F., Guimaraes, P.M., 2021. Defining the combined stress response in wild *Arachis*. Sci. Rep. 11 (1), 11097 https://doi.org/10.1038/s41598-021-90607-7.

Murtaza, G., Azooz, M.M., Murtaza, B., Usman, Y., Saqib, M., 2013. Nitrogen-use-efficiency (NUE) in plants under NaCl stress. In: Ahmad, P., Azooz, M.M., Prasad, M. N.V. (Eds.), Salt Stress in Plants. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-6108-1_16.

Nazir, F., Mahajan, M., Khatoon, S., Albaqami, M., Ashfaque, F., Chhillar, H., Chopra, P., Khan, M.I.R., 2023. Sustaining nitrogen dynamics: a critical aspect for improving salt tolerance in plants. Front. Plant Sci. 14, 1087946 https://doi.org/10.3389/fpls.2023.1087946.

Negrão, S., Schmöckel, S.M., Tester, M., 2017. Evaluating physiological responses of plants to salinity stress. Ann. Bot. 119 (1), 1–11. https://doi.org/10.1093/aob/mcw191.

Ng, D.W., Abeysinghe, J.K., Kamali, M., 2018. Regulating the regulators: the control of transcription factors in plant defense signaling. Int. J. Mol. Sci. 19 (12), 3737. https://doi.org/10.3390/ijms19123737.

Nie, J., Wen, C., Xi, L., Lv, S., Zhao, Q., Kou, Y., Ma, N., Zhao, L., Zhou, X., 2018. The *AP2/ERF* transcription factor *CmERF053* of chrysanthemum positively regulates shoot branching, lateral root, and drought tolerance. Plant Cell Rep. 37, 1049–1060. https://doi.org/10.1007/s00299-018-2290-9.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A., Tasaka, M., 2007. *ARF7* and *ARF19* regulate lateral root formation via direct activation of LBD/ASL genes in *Arabidopsis*. Plant Cell 19 (1), 118–130. https://doi.org/10.1105/tpc.106.047761.

Osthoff, A., Donà Dalle Rose, P., Baldauf, J.A., Piepho, H.P., Hochholdinger, F., 2019. Transcriptomic reprogramming of barley seminal roots by combined water deficit and salt stress. BMC Genom. 20 (1), 325. https://doi.org/10.1186/s12864-019-5634-0.

Pailles, Y., Awlia, M., Julkowska, M., Passone, L., Zemmouri, K., Negrao, S., Schmockel, S.M., Tester, M., 2020. Diverse traits contribute to salinity tolerance of wild tomato seedlings from the Galapagos Islands. Plant Physiol. 182 (1), 534–546. https://doi.org/10.1104/pp.19.00700.

Pandey, P., Ramegowda, V., Senthil-Kumar, M., 2015. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. Front. Plant Sci. 6, 723. https://doi.org/10.3389/fpls.2015.00723.

Park, Y.J., Kim, T.S., Kim, E.H., Kim, H.D., Kim, J., 2020. Ribosomal protein S3 is a novel negative regulator of non-homologous end joining repair of DNA double-strand breaks. Faseb. J. 34 (6), 8102–8113. https://doi.org/10.1096/fj.201903245R.

Parvin, K., Hasanuzzaman, M., Bhuyan, M.H.M.B., Mohsin, S.M., Fujita, M., 2019. Quercetin mediated salt tolerance in tomato through the enhancement of plant antioxidant defense and glyoxalase systems. Plants 8 (8), 247. https://doi.org/10.3390/plants8080247.

Pons, C., Casals, J., Palombieri, S., Fontanet, L., Riccini, A., Rambla, J.L., Ruggiero, A., Figás, M.D.R., Plazas, M., Koukounaras, A., Picarella, M.E., Sulli, M., Fisher, J., Ziarsolo, P., Blanca, J., Cañizares, J., Cammareri, M., Vitiello, A., Batelli, G., Kanellis, A., Brouwer, M., Finkers, R., Nikoloudis, K., Soler, S., Giuliano, G., Grillo, S., Grandillo, S., Zamir, D., Mazzucato, A., Causse, M., Díez, M.J., Prohens, J., Monforte, A.J., Granell, A., 2022. Atlas of phenotypic, genotypic and geographical diversity present in the European traditional tomato. Hortic. Res. 9, uhac112 https://doi.org/10.1093/hr/uhac112.

Prasch, C.M., Sonnewald, U., 2015. Signaling events in plants: stress factors in combination change the picture. Environ. Exp. Bot. 114, 4–14. https://doi.org/10.1016/J.ENVEXPBOT.2014.06.020.

Ramegowda, V., Senthil-Kumar, M., 2015. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J. Plant Physiol. 176, 47–54. https://doi.org/10.1016/j.jplph.2014.11.008.

Rampino, P., Mita, G., Fasano, P., Borrelli, G.M., Aprile, A., Dalessandro, G., De Bellis, L., Perrotta, C., 2012. Novel durum wheat genes up-regulated in response to a combination of heat and drought stress. Plant Physiol. Biochem. 56, 72–78. https://doi.org/10.1016/J.PLAPHY.2012.04.006.

Rasmussen, S., Barah, P., Suarez-Rodriguez, M.C., Bressendorff, S., Friis, P., Costantino, P., Bones, A.M., Nielsen, H.B., Mundy, J., 2013. Transcriptome responses to combinations of stresses in *Arabidopsis*. Plant Physiol. 161 (4), 1783–1794. https://doi.org/10.1104/pp.112.210773.

Rau, A., Gallopin, M., Celeux, G., Jaffrézic, F., 2013. Data-based filtering for replicated high-throughput transcriptome sequencing experiments. Bioinformatics 29, 2146–2152. https://doi.org/10.1093/bioinformatics/btt350.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., Mittler, R., 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. Plant Physiol. 134 (4), 1683–1696. https://doi.org/10.1104/pp.103.033431.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26 (1), 139–140. https://doi.org/10.1093/bioinformatics/btp616.

Ruggiero, A., Landi, S., Punzo, P., Possenti, M., Van Oosten, M.J., Costa, A., Morelli, G., Maggio, A., Grillo, S., Batelli, G., 2019. Salinity and ABA seed responses in pepper: expression and interaction of aba core signaling components. Front. Plant Sci. 10, 304. https://doi.org/10.3389/fpls.2019.00304.

Ruggiero, A., Punzo, P., Van Oosten, M.J., Cirillo, V., Esposito, S., Costa, A., Maggio, A., Grillo, S., Batelli, G., 2022. Transcriptomic and splicing changes underlying tomato responses to combined water and nutrient stress. Front. Plant Sci. 13, 974048 https://doi.org/10.3389/fpls.2022.974048.

Siddiqi, M.Y., Glass, A.D.M., 1981. Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4 (3), 289–302. https://doi.org/10.1080/01904168109362919.

Son, S., Park, S.R., 2023. Plant translational reprogramming for stress resilience. Front. Plant Sci. 14, 1151587 https://doi.org/10.3389/fpls.2023.1151587.

Sunseri, F., Aci, M.M., Mauceri, A., Caldiero, C., Puccio, G., Mercati, F., Abenavoli, M.R., 2023. Short-term transcriptomic analysis at organ scale reveals candidate genes involved in low N responses in NUE-contrasting tomato genotypes. Front. Plant Sci. 14, 1125378 https://doi.org/10.3389/fpls.2023.1125378.

Tamburino, R., Vitale, M., Ruggiero, A., Sassi, M., Sannino, L., Arena, S., Costa, A., Batelli, G., Zambrano, L., Scaloni, A., Grillo, S., Scotti, N., 2017. Chloroplast proteome response to drought stress and recovery in tomato (*Solanum lycopersicum* L.). BMC Plant Biol. 17, 40. https://doi.org/10.1186/s12870-017-0971-0.

Tegeder, M., Masclaux-Daubresse, C., 2018. Source and sink mechanisms of nitrogen transport and use. New Phytol. 217 (1), 35–53. https://doi.org/10.1111/nph.14876.

Uimari, A., Strommer, J., 1997. Myb26: a MYB-like protein of pea flowers with affinity for promoters of phenylpropanoid genes. Plant J. 12 (6), 1273–1284. https://doi.org/10.1046/j.1365-313x.1997.12061273.x.

Van Oosten, M.J., Di Stasio, E., Cirillo, V., Silletti, S., Ventorino, V., Pepe, O., Raimondi, G., Maggio, A., 2018. Root inoculation with *Azotobacter chroococcum* 76A enhances tomato plants adaptation to salt stress under low N conditions. BMC Plant Biol. 18 (1), 205. https://doi.org/10.1186/s12870-018-1411-5.

Waadt, R., Seller, C.A., Hsu, P.K., Takahashi, Y., Munemasa, S., Schroeder, J.I., 2022. Plant hormone regulation of abiotic stress responses. Nat. Rev. Mol. Cell Biol. 23 (10), 680–694. https://doi.org/10.1038/s41580-022-00479-6.

Wang, M., Zhang, P., Liu, Q., Li, G., Di, D., Xia, G., Kronzucker, H., Fang, S., Chu, J., Shi, W.M., 2020a. *TaANR1-TaBG1* and *TaWabi5-TaNRT2s/NARs* link ABA

metabolism and nitrate acquisition in wheat roots. Plant Physiol. 182 (3), 1440–1453. https://doi.org/10.1104/pp.19.01482.

Wang, X., Li, C., Shi, Y., Zhang, Z., Chi, Q., Wang, P., 2023. Improvements in saline soil and the law of water-salt transport based on salt inhibition using MICP technology. Biogeotechnics, 100055. https://doi.org/10.1016/j.bgtech.2023.100055.

Wang, Z., Hong, Y., Li, Y., Shi, H., Yao, J., Liu, X., Wang, F., Huang, S., Zhu, G., Zhu, J.K., 2021. Natural variations in *SlSOS1* contribute to the loss of salt tolerance during tomato domestication. Plant Biotechnol. J. 19 (1), 20–22. https://doi.org/10.1111/pbi.13443.

Wang, Z., Hong, Y., Zhu, G., Li, Y., Niu, Q., Yao, J., Hua, K., Bai, J., Zhu, Y., Shi, H., Huang, S., Zhu, J.K., 2020b. Loss of salt tolerance during tomato domestication conferred by variation in a Na^+/K+ transporter. EMBO J. 39 (10), e103256 https://doi.org/10.15252/embj.2019103256.

Wani, S.H., 2023. Editorial: mechanisms of abiotic stress responses and tolerance in plants: physiological, biochemical and molecular interventions, volume II. Front. Plant Sci. 14, 1272255 https://doi.org/10.3389/fpls.2023.1272255, 2023.

West, P.C., Gerber, J.S., Engstrom, P.M., Mueller, N.D., Brauman, K.A., Carlson, K.M., Cassidy, E.S., Johnston, M., MacDonald, G.K., Ray, D.K., Siebert, S., 2014. Leverage points for improving global food security and the environment. Science 345, 325–328. https://doi.org/10.1126/science.1246067.

Wohlbach, D.J., Quirino, B.F., Sussman, M.R., 2008. Analysis of the *Arabidopsis* histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20 (4), 1101–1117. https://doi.org/10.1105/tpc.107.055871.

Xu, G., Fan, X., Miller, A.J., 2012. Plant nitrogen assimilation and use efficiency. Annu. Rev. Plant Biol. 63, 153–182. https://doi.org/10.1146/annurev-arplant-042811-105532.

Zhang, H., Zhao, Y., Zhu, J.K., 2020. Thriving under stress: how plants balance growth and the stress response. Dev. Cell 55 (5), 529–543. https://doi.org/10.1016/j.devcel.2020.10.012.

Zhang, L., Liu, X., Gaikwad, K., Kou, X., Wang, F., Tian, X., Xin, M., Ni, Z., Sun, Q., Peng, H., Vierling, E., 2017. Mutations in eIF5B confer thermosensitive and pleiotropic phenotypes via translation defects in *Arabidopsis thaliana*. Plant Cell 29 (8), 1952–1969. https://doi.org/10.1105/tpc.16.00808.