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Jasmonic acid is required for tomato acclimation to multifactorial stress combination

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Abstract

As a result of global warming and climate change, the number and intensity of weather events such as droughts, heat waves, and floods are increasing, resulting in major losses in crop yield worldwide. Combined with the accumulation of different pollutants, this situation is leading to a gradual increase in the complexity of environmental factors affecting plants. We recently used the term 'multifactorial stress combination' (MFSC) to describe the impact of three or more stressors occurring simultaneously or sequentially on plants. Here, we show that a MFSC of six different abiotic stressors (high light, heat, nitrogen deficiency, paraquat, cadmium, and salinity) has a negative impact on the growth, photosystem II function, and photosynthetic activity of mature tomato plants. We further reveal a negative correlation between proline accumulation and the increasing number of stress factors combined, suggesting that proline could have an adverse effect on plants during MFSC. Our findings further indicate that alterations in hormonal levels and stomatal responses are stress/stress combination-dependent, and that a tomato mutant deficient in jasmonic acid accumulation is more sensitive to high light and its combinations with salinity and/or paraquat. Taken together, our study reveals that the effects of MFSC on tomato plants are broad, that photosynthesis and proline accumulation are especially vulnerable to MFSC, and that jasmonic acid is required for tomato acclimation to MFSCs involving high light, salinity and paraquat.

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CRediT authorship contribution statement

L.S.P. performed experiments and analyzed the data. M.F.L-C., V.V-P., R.M., R.S., L.S.P., M.A.P-V., A.G-C., and S.I.Z. designed experiments, analyzed the data, provided funding and/or wrote the manuscript. All authors read and approved the final version of the manuscript.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2023.105425.

Keywords

Climate change; Multifactorial stress combination; Photosynthesis; Phytohormones; Jasmonic acid; Tomato

1. Introduction

Within the last decade, global warming, climate extremes, and/or industrial pollution have negatively impacted crop growth, development, and yield (Masson-Delmotte et al., 2021; Zandalinas et al., 2021a; Pascual et al., 2022). Climate extremes (e.g., heat waves, cold snaps, droughts, and/or floods) are sometimes combined with increasing levels of different soil contaminants (e.g., herbicides, pesticides, microplastics, and/or heavy metal), as well as with poor soil quality (e.g., nutrient deficiency, extreme pH, and/or salinity), creating different combinations of multiple stress conditions, occurring simultaneously. This phenomenon was recently termed “multifactorial stress combination” (MFSC), and defined as the co-occurrence of three or more stress conditions affecting plants (Rillig et al., 2019, 2021; Zandalinas et al., 2021b; a; Zandalinas and Mittler, 2022; Pascual et al., 2022). Multifactorial stress combination of several different low-level abiotic stressors applied to *Arabidopsis thaliana* plants was shown to cause a gradual and drastic decline in plant growth and survival (Zandalinas et al., 2021b; a; Zandalinas and Mittler, 2022). A recent study of the influence of an increasing number of global change factors including fungicide, light pollution, microplastics, eutrophication, salinity and warming on plant-community responses revealed that the number of simultaneously acting stress factors impact the species composition, productivity and diversity of these communities (Speißer et al., 2022). In addition, studies of the impact of up to ten different global change-associated stress factors on soils and their microbiomes demonstrated a gradual decline in microbial diversity and soil properties/processes, associated with the increased complexity of different stresses applied (Rillig et al., 2019, 2021). These findings suggest that the combined impact of climate change, global warming, and industrial pollution on crops growing in many different regions around the world could already be affecting food production (Zandalinas and Mittler, 2022; Pascual et al., 2022; Rivero et al., 2022). Recently, the effects of MFSC on commercial cultivars of rice (*Oryza sativa*) and maize (*Zea mays*) were reported (Sinha et al., 2022). This study demonstrated that a MFSC of up to five abiotic stresses, each applied at a low level (salinity, heat, the herbicide paraquat, phosphate deficiency, and the heavy metal Cd), negatively impacted the growth and biomass of rice and maize plants. In addition, the levels of different proteins involved in iron and reactive oxygen species (ROS) homeostasis were found to be specifically altered during MFSC in rice (Sinha et al., 2022). These findings agreed with the transcriptomic analysis of MFSC conducted in Arabidopsis by Zandalinas et al. (2021b), and suggested that ROS metabolism and/or signaling play a key role in plant resilience to MFSC.

To expand our knowledge of plant responses to MFSC, we studied the impact of a MFSC of up to six individual stresses on the physiology, hormonal, and metabolic responses of tomato (*Solanum lycopersicum*) plants; an important dicot crop cultivar. For this purpose, we subjected mature tomato plants to a combination of up to six different

abiotic stress conditions (heat, salinity, high light, nitrogen deficiency, Cd, and paraquat) imposed in an increasing level of complexity. Our findings show that MFSC has a gradual negative effect on growth, oxidative stress levels, photosystem II (PSII) efficiency, and photosynthetic rate of tomato, and that the accumulation of the osmo-protectant amino acid proline is suppressed with the increased complexity of MFSC. In addition, we reveal that changes in hormonal accumulation and stomatal responses are stress-combination specific, and that jasmonic acid (JA) is specifically accumulated in plants during high light and its combination with salinity and/or paraquat. Using a tomato mutant deficient in JA accumulation, we further reveal a key role for JA in the acclimation of tomato plants to a MFSC of light stress in combination with salinity and/or paraquat. Taken together, our findings highlight the negative effects of MFSC on photosynthesis, hormonal responses, and proline accumulation, and reveal that JA plays a key role in plant acclimation to MFSC.

2. Materials and Methods

2.1. Plant material and growth conditions

Moneymaker and Castlemar tomato seeds, purchased from a commercial nursery (Clemente Viven, Semillas Clemente S.A., Vitoria, Álava, Spain), as well as *spr2* seeds (Li et al., 2003), were sown in seedling trays filled with a mixture of peat moss, perlite, and vermiculite (80:10:10) under greenhouse conditions (70% relative humidity with natural photoperiod, 200 μmol photons m^{-2} s^{-1} light intensity, and day and night temperature averaging 25.0 ± 3.0°C and 18.0 ± 3.0°C, respectively). After germination, seedlings were transplanted to 10-cm diameter pots filled with perlite, maintained under greenhouse conditions as described above, and watered three times a week with half-strength Hoagland solution. Temperature and relative humidity were recorded regularly with a portable USB datalogger (OM-EL-WIN-USB, Omega, NJ, United States).

2.2. Stress treatments and experimental design

To study MFSC in tomato plants, different combinations of up to six stress parameters including high light (700 μmol m^{-2} s^{-1}; HL), heat stress (37°C; HS), salinity (75 mM NaCl; S), nitrogen deficiency (Ca $(NO_3)_2$ concentration was reduced by 90%; N−), heavy metal stress (using Cd, 10 μM $CdSO_4$), and the herbicide paraquat (1 μM PQ) were imposed on 8 plants per stress treatment, and all experiments were repeated at least three times. HS, HL, S, and PQ stresses were conducted in all possible combinations, and N− and Cd were added as single stresses, as well as in combination with HL+HS+S+PQ to generate two different five-stress and one six-stress combinations, similarly to (Rillig et al., 2019; Zandalinas et al., 2021b). One week after transplanting, a group of tomato plants were subjected to N-deficiency by watering plants with half-strength Hoagland solution containing 10% of N ($Ca(NO_3)_2$) concentration. After one week, different groups of plants were subjected to the following stresses for 15 days (Fig. 1;Table S1), applying each stressor in the half-strength Hoagland solution: S (75 mM NaCl), PQ (1 μM PQ), Cd (10 μM $CdSO_4$), S+PQ (75 mM NaCl + 1 μM PQ), S+PQ+N− (75 mM NaCl + 1 μM PQ + 10% N), S+PQ+Cd (75 mM NaCl + 1 μM PQ + 10 μM $CdSO_4$) and S+PQ+Cd+N− (75 mM NaCl + 1 μM PQ + 10 μM $CdSO_4$ + 10% N). For stress combinations that included HS and/or HL, plants watered in the presence or absence of the stresses mentioned above, were subjected to a 9-h treatment

of HS (37°C) and/or HL (700 μmol m^{-2} s^{-1}) in growth chambers (Fig. 1; Table S1). Once all treatments were completed, leaf injury index, scored as the percentage of leaves with no symptoms of damage (Pascual et al., 2023), distance between nodes 2 and 3 (internode distance), and plant height (Sinha et al., 2022) were scored for all control and stressed plants, followed by sampling of mature fully expanded leaves that were flash frozen in liquid nitrogen. Samples were stored at − 80°C until further analysis. For each analysis described below, 5–8 independent technical repeats per bio-logical repeat and stress group were performed.

2.3. Photosynthetic parameters and PSII efficiency

Photosynthetic rates were measured simultaneously on plants of each treatment between 15:30 and 17:00 p.m. Leaf gas exchange parameters were measured by using a LICOR Portable Photosynthesis System (LI-6800, LICOR, Lincoln, NE, USA) under ambient CO_2 and moisture. After instrument stabilization, six measurements were taken on three different mature fully expanded leaves, in three replicate plants from each treatment. PSII efficiency was measured on the same leaves and plants using a portable fluorometer (FluorPen FP-MAX 100, Photon Systems Instruments, Czech Republic).

2.4. Hormone analysis

Hormone extraction and analysis were performed as described in Balfagón et al. (2019) with some modifications. A mixture containing 50 ng of [$^2H_6$]-ABA, [^{13}C]-SA, and dihydrojasmonic acid was added to 200 mg of grounded, frozen leaf tissue. The tissue was homogenized in 2 mL of ultrapure water in a ball mill (MillMix20, Domel, Železniki, Slovenija). After centrifugation at 10000 g at 4°C for 10 min, supernatants were recovered, and pH adjusted to 3 with 80% acetic acid. The water extract was partitioned twice against 2 mL of diethyl ether and the organic layer recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan, Saint Herblain Cedex, France). Then, samples were resuspended in a 90:10 (v/v) H_2O:MeOH solution by using a sonicator (Elma S30, Elmasonic, Singen, Germany). After filtering through 0.22 μm polytetrafluoroethylene membrane syringe filters (Albet S.A., Barcelona, Spain), extracts were directly injected into an ultra-performance UPLC system (Xevo TQ-S, Waters Corp., Milford, MA, USA). Chromatographic separations were performed on a reversed-phase C18 column (Gravity, 50 ×2.1 mm, 1.6-μm particle size, Luna Omega, Phenomenex, Torrance, CA, USA) using a H_2O:MeOH (both supplemented with 0.1% formic acid) gradient at a flow rate of 300 μL min^{-1}. Hormones were quantified with a triple quadrupole mass spectrometer connected online to the output of the column though an orthogonal Z-spray electrospray ion source. Results were processed using Masslynx v. 4.1 software, and the phytohormone content was quantified with a standard curve prepared with commercial standards as described in Balfagón et al. (2019) and expressed as percentage of control.

2.5. Proline analysis

Around 50 mg ground, frozen leaf tissue was extracted in 5 mL of 3% sulfosalicylic acid (Panreac, Barcelona, Spain) by sonication for 30 min. Extracts were then centrifuged at 8000 g for 20 min at 4 °C. 1 mL of each recovered supernatants was mixed with 1 mL glacial acetic acid (Sigma–Aldrich, St. Louis, MO, USA) and 1 mL ninhydrin reagent (Panreac,

Barcelona, Spain) (1:1:1 ratio, v:v:v). The reaction mixture was incubated in a water bath at 100°C for 1 h, cooled down, and centrifuged 5 min at 8000 g at 4°C. Absorbance was measured at 520 nm with a spectrophotometer (Thermo Spectronic Genesys 10, Waltham, MA, USA). Proline quantification was performed with a standard curve made with a commercial standard of proline (Sigma–Aldrich, St. Louis, MO, USA,) and expressed as percentage of control.

2.6. MDA analysis

Approximately 200 mg of ground frozen leaf tissue was homogenized in 2 mL of 80% ethanol (Panreac, Barcelona, Spain) by sonication. Homogenates were then centrifuged at 10000 g for 10 min and different aliquots of the supernatant were mixed either with 20% trichloroacetic acid or with a mixture of 20% trichloroacetic acid and 0.5% thiobarbituric acid in a 1:1 (v:v) proportion. Both mixtures were incubated in a water bath at 90°C for 1 h. After cooling down in an ice bath, samples were centrifuged at 8000 g for 5 min at 4°C. The absorbance of the supernatant was read at 440, 534 and 600 nm against a blank, and MDA concentration was calculated as described in Zandalinas et al. (2017) and expressed as percentage of control.

2.7. Statistical analysis

Statistical analysis was performed with the Statgraphics Plus v.5.1. software (Statistical Graphics Corp., Herndon, VA, United States) by one- or two-way analysis of variance (ANOVA) followed by Tukey post hoc test (different letters denote statistical significance at $P < 0.05$) or by two-tailed Student's t-test (asterisks denote statistical significance at $P < 0.05$ with respect to control).

3. Results

3.1. Growth, leaf injury index, and malondialdehyde and proline accumulation of tomato plants subjected to multifactorial stress combination

Plant height, internode distance, leaf injury index, and malondialdehyde (MDA) and proline accumulation of tomato plants subjected to MFSC of up to six abiotic stresses including high light (HL), heat stress (HS), salinity (S), nitrogen deficiency (N−), cadmium, (Cd), and paraquat (PQ) were determined (Fig. 2; Table S2). The number of leaves with no symptoms of damage (leaf injury index; Pascual et al., 2023) decreased to a similar level when plants were exposed to 1-, 2-, 3- or 4-factor stresses. Adding one more factor (5-factor stress combination) and specially two more factors (6-factor stress combination) had a higher impact on leaf injury compared to control (CT) plants. Relative to CT, 5- and 6-factor stress combinations displayed a reduced number of healthy leaves by 65% and 85%, respectively (Fig. 2A). Plant height was calculated in plants subjected to up to 4-stress factor combination (only S, PQ, N− and Cd were considered due to the short 9-h period of HL and HS treatments applied; Fig. 1; Table S1). Plant height also decreased as the number of stress factors combined increased, showing the highest reduction in plant height when 3 and 4 factors were combined (about 50% of reduction compared to CT; Fig. 2B). Similarly, internode distance was reduced when 4 factors were combined compared to CT values (Fig. 2C).

The degree of lipid peroxidation in tomato plants subjected to MFSC was studied by monitoring changes in MDA levels (Taulavuori et al., 2001). As shown in Fig. 2D, MDA content in tomato leaves increased gradually as additional stress factors were combined, showing the highest MDA levels when plants were subjected to 5 and 6 stressors combined. In contrast to MDA results, proline levels gradually decreased with the increasing number and complexity of stress treatments added to the MFSC (Fig. 2E).

3.2. Photosynthetic and gas exchange parameters of tomato plants subjected to multifactorial stress combination

Photosynthetic parameters of tomato (PSII efficiency and photosynthetic rate) gradually decreased when plants were exposed to an increasing complexity of stress factors during MFSC (Fig. 3; Table S3). Compared to CT, PSII efficiency significantly decreased when tomato plants were exposed to combinations of 5 or 6 stresses (Fig. 3A), whereas the decline in photosynthetic rate was already evident when two stresses were combined and continued to gradually decrease with the addition of more stress treatments, reaching the lowest value when plants were subjected to 6-factor MFSC (Fig. 3B). These results suggest that PSII function and photosynthesis of tomato plants are negatively affected by the increasing number and complexity of stress treatments combined during MFSC, becoming more detrimental in response to the combination of all six abiotic stresses.

A Principal Component Analysis (PCA) was performed on the parameters depicted in Figs. 2 and 3 (Fig. S1). The results of this analysis revealed that the primary source of variation in the data was attributed to changes associated with the increased number of combined factors. Principal Component 1 (PC1), which accounted for 60.03% of the total variance, effectively separated the effects of individual stresses from the majority of stress combinations involving three or more stress factors. Notably, under conditions of individual stresses, physiological measurements such as photosynthetic rate and PSII function, plant height, and proline accumulation exhibited higher values. However, when three or more stress conditions were combined, these parameters displayed an opposite trend. Conversely, levels of MDA were higher under combinations of three or more stresses compared to those observed under individual or control conditions (Fig. S1).

Gas exchange parameters (stomatal conductance [gsw] and transpiration rate [E]) and leaf temperature (Leaf T) were also determined in tomato plants subjected to MFSC (Fig. 4). Stomatal regulation and transpiration levels depended on each particular stress combination, showing reductions in response to the majority of stress combination treatments, but more prominently in response to certain stress combinations involving S (Fig. 4A, B). As expected, increments in leaf temperature were evident in response to any stress treatment involving HS, whereas individual or combined PQ, Cd, N−, S, and HL did not alter the foliar temperature of tomato plants (Fig. 4C). A PCA conducted for gsw, E and Leaf T of all treatments revealed that gas exchange parameters (gsw and E) as well as leaf temperature were affected differently among the different treatments. This analysis further highlighted the important effect of HL+HS on gsw and E (Fig. 4D).

3.3. Hormonal responses of tomato plants subjected to multifactorial stress combination

Abscisic acid (ABA), salicylic acid (SA), and JA, as well as the JA precursor 12-oxophytodienoic acid (OPDA) were determined in tomato plants subjected to MFSC (Fig. 5). ABA levels fluctuated depending on the type of stress and the specific stress combinations (Fig. 5A). ABA accumulated in response to S+PQ, the 3-factor stress combination HL+HS+S, as well as in response to different combinations that included HL+HS+S (the 4-factor stress combination HL+HS+S+PQ, the 5-factor stress combination HL+HS+S+PQ+N−, and the 6-factor stress combination HL+HS+S+PQ+Cd+N−). In contrast, individual S slightly decreased ABA content probably due to the low intensity of the stress applied. Similarly, ABA levels declined in response to PQ and N−, as well as PQ+HS, HL+HS, HL+S, HL+PQ, and HL+HS+PQ compared to CT (Fig. 5A). SA levels diminished in tomato plants subjected to all stress treatments, except in response to S+PQ and HL+PQ (SA content remained similar to CT), and in response to HL (increased SA content was observed; Fig. 5B). In addition to SA and ABA, JA levels were altered in tomato plants in response to MFSC (Fig. 5C, D). Interestingly, whereas OPDA levels (JA precursor) decreased in response to one-factor stresses (except in response to HL), as well as in response to most stress combinations involving 2 and 3 factors (except for HL+PQ, in which OPDA content increased), plants subjected to HL+HS+S+PQ and HL+HS+S+PQ+N− displayed increased OPDA levels compared to CT (Fig. 5C). Interestingly, JA accumulated only in response to HL, and when HL was combined with S and/or PQ (HL+S, HL+PQ and HL+S+PQ; Fig. 5D). A PCA conducted for the different hormones measured in all treatments revealed that the main source of variation in the data (PC1 explained a total of 33.59% of total variance) was due to hormonal changes associated with HL and its combinations with PQ and/or S (Fig. 5E).

3.4. Involvement of JA in plant acclimation to MFSCs of high light with salinity and/or paraquat

To further study the role of JA in tomato plants subjected to HL and its combination with S and/or PQ, we analyzed the response of the JA-deficient mutant *Suppressor of prosystemin-mediated responses 2* (*spr2*, that encodes SlFAD7, a chloroplast fatty acid desaturase required for JA biosynthesis; Li et al., 2003) to HL, S, PQ, and all their possible combinations of two and three factors (Fig. 6). As the *spr2* mutant is in the Castlemar (CSL) background, we used wild type CSL plants as controls for this study. As shown in Fig. 6A, JA levels were suppressed in the *spr2* mutant under control and all stress treatments studied. Leaf injury index measurements of wild type and the *spr2* mutant revealed that *spr2* plants subjected to HL, HL+PQ, and HL+S+PQ had a higher number of damaged leaves compared to wild type plants (Fig. 6B). In addition, MDA levels were significantly higher in the *spr2* mutant compared to CSL in response to all 2- and 3-factor combinations involving HL, S, and PQ (Fig. 6C), whereas no significant differences were observed in PSII efficiency between wild type and *spr2* plants subjected to the different stresses (Fig. 6D).

4. Discussion

We recently demonstrated that MFSC has a negative impact on seedlings of the model plant *Arabidopsis thaliana* (Zandalinas et al., 2021b; a; Zandalinas and Mittler, 2022), as well

as two commercial monocot cultivars (rice and maize; Sinha et al., 2022). Here, we show that, in addition to rice and maize seedlings (Sinha et al., 2022), MFSC has a negative impact on mature tomato plants (a dicot); the second-most important cultivar in the world economically with more than 4.8 million ha cropland (http://fao.org/faostat/en, 2019; Figs. 2, 3; Tables S2, S3). In contrast to our previous studies with Arabidopsis, rice, and/or maize, that determined the effects of MFSC on plant growth, biomass, survival, and transcriptomics and proteomics responses (but did not address the effects of MFSC on plant physiology), our current analysis determined the effects of MFSC on photosynthesis, PSII function, stomatal function, transpiration, and leaf temperature. In addition, we studied the impact of MFSC on hormonal levels (ABA, SA, OPDA, and JA), MDA, and proline accumulation (Figs. 2D, E, 5). Interestingly, MFSC negatively affected almost all of these aspects of plant physiology, while increasing the levels of MDA (Fig. 2D, S1), a marker for increased ROS and lipid peroxidation (Taulavuori et al., 2001).

The important role ROS metabolism and/or signaling play in plant responses to MFSC was demonstrated in Arabidopsis plants using whole-plant ROS imaging and mutants lacking Ascorbate Peroxidase 1 (*apx1*) or Respiratory Burst Oxidase Homolog D (*rbohD*), that were more sensitive to MFSC compared to wild type plants (Zandalinas et al., 2021b). In addition, rice plants subjected to MFSC contained a higher abundance of many ROS scavenging proteins including APX1, APX4, glutathione reductase (GR), catalase B (CAT-B), and Cu/Zn superoxide dismutase 2 (Cu/ZnSOD2), compared to plants subjected to one factor or up to 3-factor combinations (Sinha et al., 2022). Taken together, these findings suggest that with the increasing number and complexity of stressors acting simultaneously on plants, oxidative stress increases, and active antioxidant mechanisms are activated to avoid excess cellular damage. In agreement with these reports, our data showed that a combination of 5- and 6-factor MFSC significantly increased MDA concentration (Fig. 2D), suggesting that an active process of lipid peroxidation may occur under these stress combinations. Altogether, the different reports on MFSC in Arabidopsis (Zandalinas et al., 2021b), rice (Sinha et al., 2022) and the results presented here in tomato plants (Fig. 2D) indicate that scavenging of ROS could represent a potential strategy to increase the tolerance of plants and crops to MFSC.

Proline is a crucial osmo-protectant in plant acclimation to drought, salinity or cold, but not to high temperatures (e.g., Rizhsky et al., 2004; Szabados and Savouré, 2010; Lugan et al., 2010; Lv et al., 2011; Per et al., 2017; Fu et al., 2018). Previous reports suggest that plants that over-accumulate proline are more resilient to different abiotic stresses (Kishor et al., 1995; Nanjo et al., 1999; Hong et al., 2000; Rontein et al., 2002). However, excess of proline might also be toxic to plants (Deuschle et al., 2001; Mani et al., 2002; Nanjo et al., 2003), and it was shown that during a combination of drought and heat stress, Arabidopsis plants accumulated sucrose instead of proline as a major osmo-protectant (Rizhsky et al., 2004). These findings suggest that under a combination of heat stress and drought, proline could be toxic to plants (Rizhsky et al., 2004). The gradual decline in proline levels observed in tomato plants under increasing complexity of MFSC (Fig. 2E) indicates that proline might not act as an osmo-protectant under MFSC and that engineering tomato plants to over-accumulate proline might not be a successful strategy to increase the tolerance of

tomato to different field growth conditions. Further studies are needed to determine whether other key molecules such as sucrose may act as osmo-protectants in tomato under MFSC.

Hormones play a key role in plant responses to different stresses and their combinations (reviewed in Devireddy et al., 2020; Zandalinas et al., 2022). During MFSC, different hormonal signaling and/or biosynthetic pathways might integrate or collide, fine-tuning the plant response to different co-occurring stress conditions (Suzuki, 2016; Devireddy et al., 2020; Zandalinas et al., 2022). Our data show that hormonal responses were specifically altered depending on the particular stress, or stress combination, impacting the plant (Fig. 5). Interestingly, combinations that included HL, combined with PQ and/or S had a pronounce impact on hormonal responses, and JA specifically accumulated under these stress combinations (Fig. 5D, E), demonstrating that different stresses and their combinations could have a specific impact on hormones to elicit defined molecular and/or physiological responses. Interestingly, a tomato mutant deficient in JA accumulation showed a significant increase in leaf injury in response to HL and its combination to PQ and S+PQ (Fig. 5B), as well as increased levels of MDA accumulation, a sign of oxidative damage, in response to the different 2- and 3-factor stress combinations involving HL, S and PQ (Fig. 5C), revealing that JA is required for tomato acclimation to these specific stress conditions.

Taken together, the current and recent studies of MFSC in Arabidopsis (Zandalinas et al., 2021b), rice and maize (Sinha et al., 2022), and tomato plants (this study) highlight the specificity of plant responses to MFSC in terms of transcriptomic (Zandalinas et al., 2021b), proteomic (Sinha et al., 2022), hormonal responses (Figs. 5, 6), physiological adaptations (Figs. 3, 4, S1), and plant growth and overall health (Fig. 2; Zandalinas et al., 2021b; Sinha et al., 2022), and emphasize the potential devastating effects of climate change, global warming, and human-made pollution on agriculture and food security. In addition, they highlight the underlying principle of MFSC, demonstrating the combined and severe impact of multiple low-level stress conditions (each with a minimal effect on plants) on plant health, growth, and survival (Fig. 2; Zandalinas et al., 2021b; a; Zandalinas and Mittler, 2022). This principle should act as a dire warning to our society indicating that if the current trend of increasing the number and complexity of different stressors in our environment will not slow down or reverse, our food supplies might severely dwindle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data Availability

Data will be made available on request.

Abbreviations:

ABA	abscisic acid
HL	high light
HS	heat stress
JA	jasmonic acid
MFSC	multifactorial stress combination
N−	nitrogen deficiency
OPDA	12-oxo-phytodienoic acid
PQ	paraquat
PSII	photosystem II
ROS	reactive oxygen species
SA	salicylic acid
S	salinity
Φ_{PSII}	photosystem II efficiency

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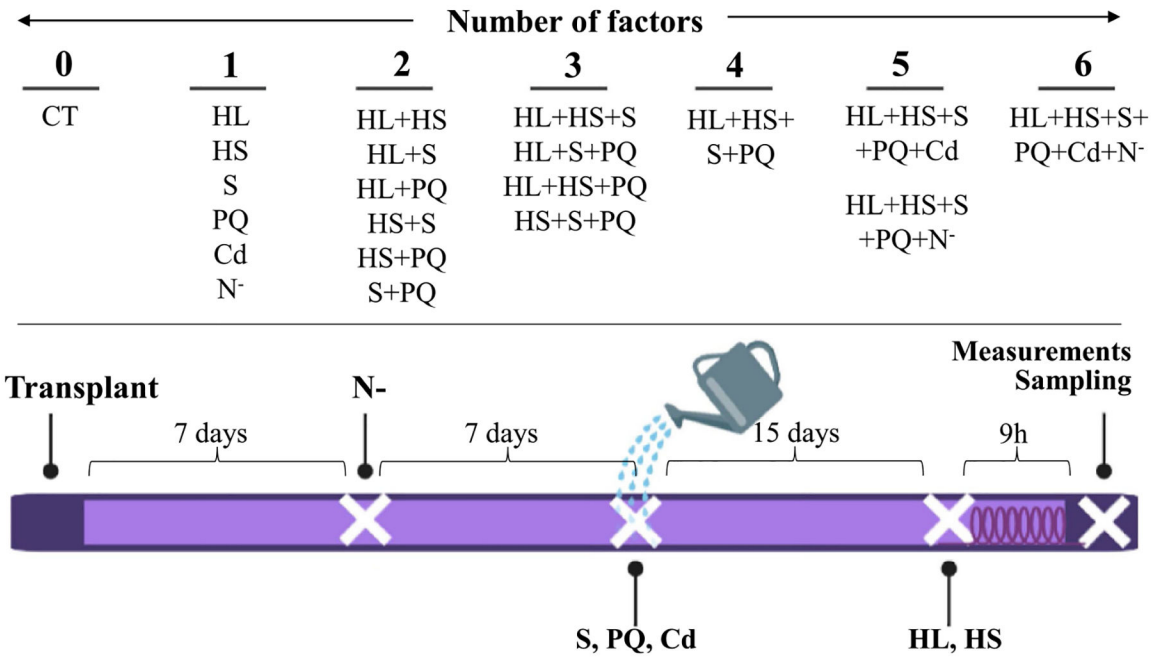

Fig. 1.

The experimental design used for the study of tomato responses to multifactorial stress combination. Nitrogen deficiency (N−), heat stress (HS), salinity (S), high light (HL), heavy metal as cadmium treatment (Cd), and the herbicide paraquat (PQ) were applied up to a combination of all six factors. Nitrogen deficiency was applied by watering plants with a half strength Hoagland solution with 10% of N ($Ca(NO_3)_2$) content one week after transplanting the plants. One week after the starting of the nitrogen-deficiency stress, plants with and without N deficiency were watered with half strength Hoagland solution containing each stressor(s): S (75 mM NaCl), PQ (1 μM PQ), Cd (10 μM $CdSO_4$), S+PQ (75 mM NaCl + 1 μM PQ), S+PQ+N− (75 mM NaCl + 1 μM PQ + 10% N), S+PQ+Cd (75 mM NaCl + 1 μM PQ + 10 μM $CdSO_4$) and S+PQ+Cd+N− (75 mM NaCl + 1 μM PQ + 10 μM $CdSO_4$ + 10% N). Following 15 days of stress treatments, a group of CT plants and a group of plants subjected to each of the individual and combined stresses were transferred to growth chambers and were subjected to HL (700 μmol $m^{-2}s^{-1}$) and/or HS (37°C). All experiments were repeated three times with at least 8 plants per stress treatment. Abbreviations: Cd, cadmium; CT, control; HL, high light; HS, heat stress; N−, nitrogen deficiency; PQ, paraquat; S, salinity.

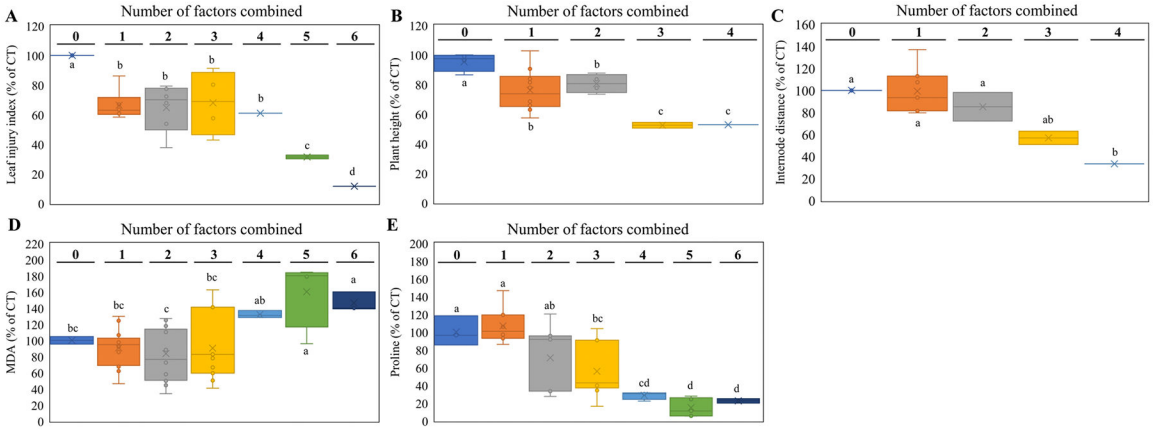

Fig. 2.

The impact of multifactorial stress combination on tomato growth, leaf injury, and MDA and proline accumulation. (A-E) The effects of multifactorial stress conditions (nitrogen deficiency, heat stress, salinity, high light, heavy metal as cadmium treatment, and the herbicide paraquat) applied up to a combination of all six factors on leaf injury index (A), plant height (B), internode distance (C), and MDA (D) and proline (E) accumulation in tomato plants. Box plots represent the median (horizontal line), the lower and upper bounds of each box plot represent the first and third quartiles (the 25th and 75th percentiles, respectively), and whiskers above and below the box plot indicate 1.5 times the interquartile range. Numbers on the top (0–6) depict the number of stress factors applied simultaneously. 0 represents measurements under CT conditions; 1 represents measurements under all individual stresses (PQ, Cd, HS, N−, S and HL); 2 represents measurements under all possible combinations of 2 different stresses involving PQ, HS, S and HL; 3 represents measurements under all possible combinations of 3 different stresses involving PQ, HS, S and HL; 4 represents measurements under the combination of 4 different stresses (PQ+HS+S+HL); 5 represents measurements under combinations of 5 different stresses (PQ+HS+S+HL+N− and PQ+HS+S+HL+Cd); 6 represents measurements under the combination of 6 different stresses (PQ+HS+S+HL+Cd+N−). See Fig. 1 and Table S1 for further details. Statistical analysis was performed by one-way ANOVA followed by a Tukey post hoc test (different letters denote statistical significance at $P < 0.05$). Abbreviations: Cd, cadmium; CT, control; HL, high light; HS, heat stress; MDA, malondialdehyde; N−, nitrogen deficiency; PQ, paraquat; S, salinity.

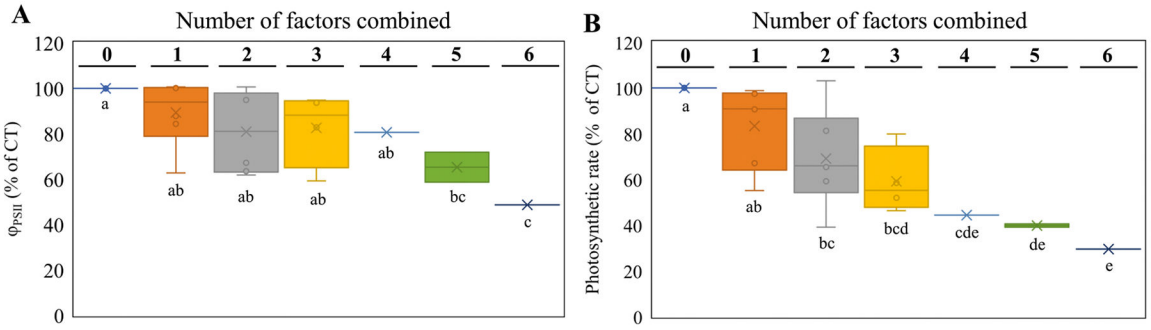

Fig. 3.

The impact of multifactorial stress combination on photosynthesis of tomato. The effect of multifactorial stress conditions (nitrogen deficiency, heat stress, salinity, high light, heavy metal as cadmium treatment, and the herbicide paraquat) applied up to a combination of all six factors on PSII efficiency (A) and photosynthetic rate (B). Box plots represent the median (horizontal line), the lower and upper bounds of each box plot represent the first and third quartiles (the 25th and 75th percentiles, respectively), and whiskers above and below the box plot indicate 1.5 times the interquartile range. Numbers on the top (0–6) depict the number of stress factors applied simultaneously. 0 represents measurements under CT conditions; 1 represents measurements under all individual stresses (PQ, Cd, HS, N−, S and HL); 2 represents measurements under all possible combinations of 2 different stresses involving PQ, HS, S and HL; 3 represents measurements under all possible combinations of 3 different stresses involving PQ, HS, S and HL; 4 represents measurements under the combination of 4 different stresses (PQ+HS+S+HL); 5 represents measurements under combinations of 5 different stresses (PQ+HS+S+HL+N− and PQ+HS+S+HL+Cd); 6 represents measurements under the combination of 6 different stresses (PQ+HS+S+HL+Cd+N−). See Fig. 1 and Table S1 for further details. Statistical analysis was performed by one-way ANOVA followed by a Tukey post hoc test (different letters denote statistical significance at $P < 0.05$). Abbreviations: Cd, cadmium; CT, control; HL, high light; HS, heat stress; N−, nitrogen deficiency; PQ, paraquat; PSII, photosystem II; S, salinity; φ_{PSII}, photosystem II efficiency.

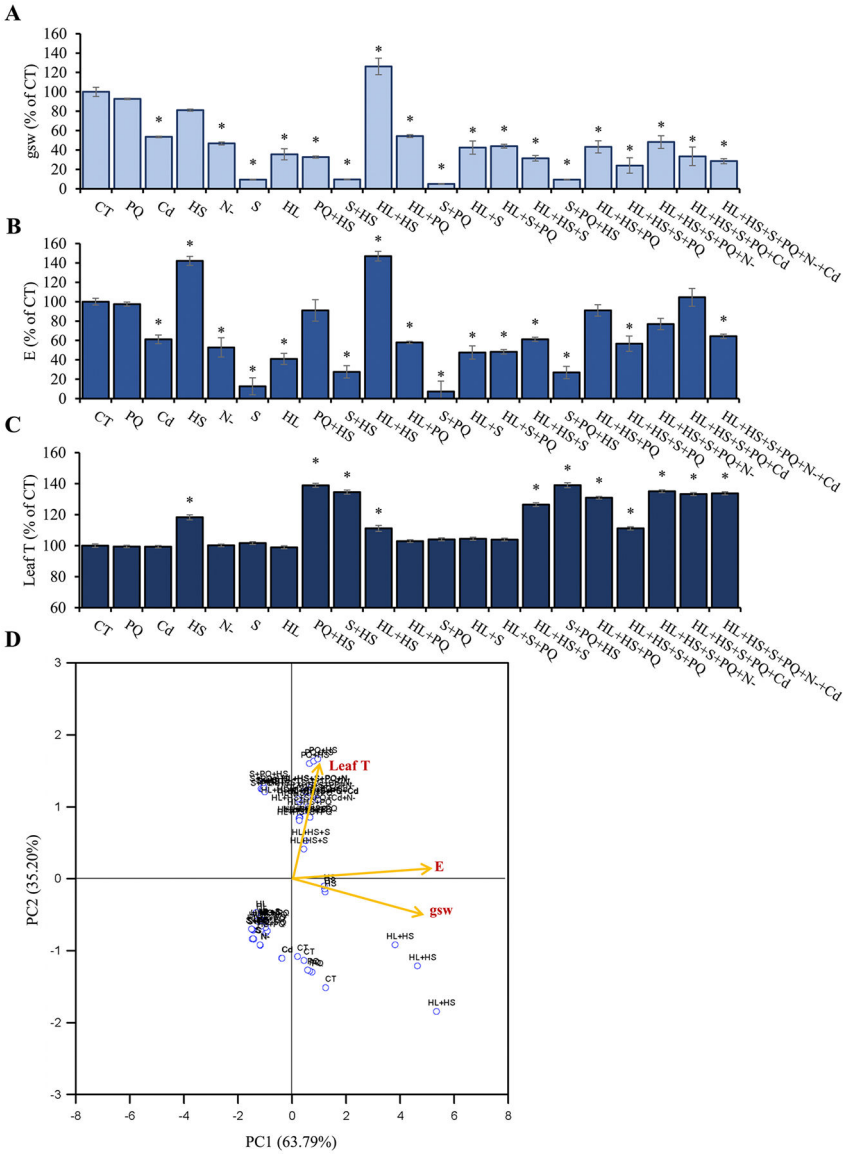

Fig. 4.

The impact of multifactorial stress combination on gas exchange parameters and leaf temperature in tomato. (A-C) The effect of multifactorial stress conditions (nitrogen deficiency, heat stress, salinity, high light, heavy metal as cadmium treatment, and the herbicide paraquat) applied up to a combination of all six factors on stomatal conductance (gsw; A), transpiration (E; B) and leaf temperature (Leaf T; C) in tomato plants. (D) PCA plot showing differences on gsw, E and leaf temperature between the different MFSCs. Loading plot is shown as arrows representing the influence of MFSC on gsw, E and leaf temperature. Statistical analysis was performed by Student's *t*-test (asterisks denote statistical significance at $P < 0.05$ compared to CT). Abbreviations: CT, control; E, transpiration; gsw, stomatal conductance; HL, high light; HS, heat stress; N−, nitrogen deficiency; PC, principal component; PCA, principal component analysis; PQ, paraquat; S, salinity; T, temperature.

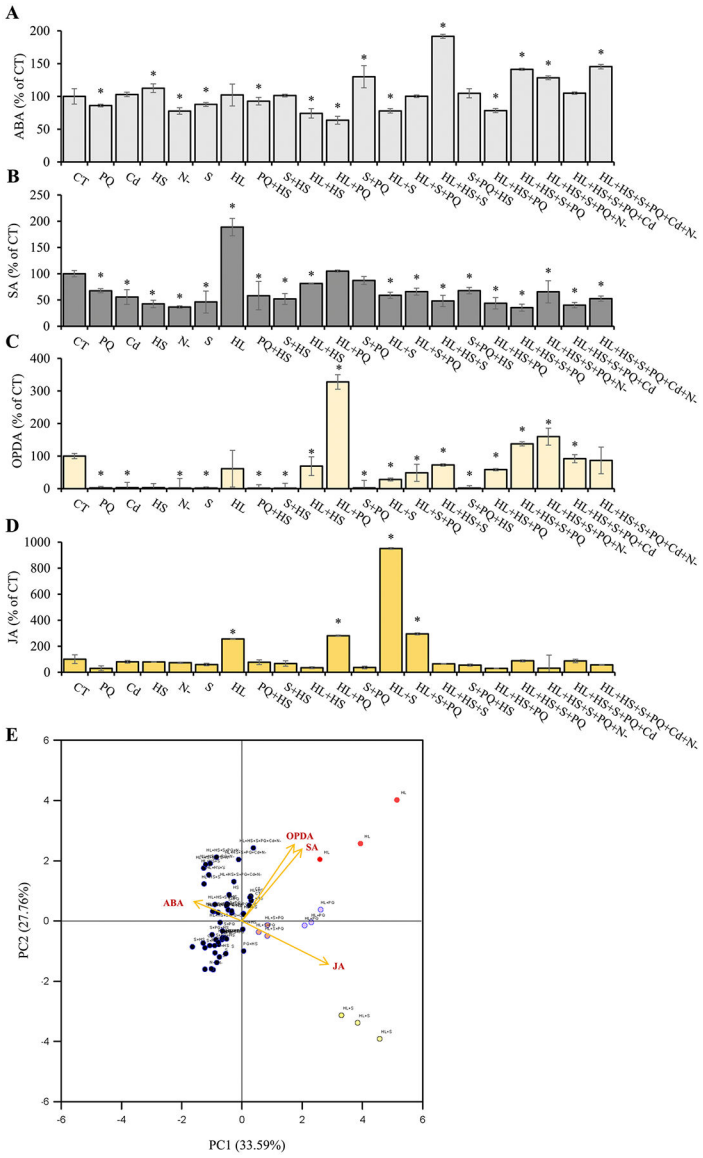

Fig. 5.

The impact of multifactorial stress combination on hormone levels in tomato plants. (A-D) The effect of multifactorial stress conditions (nitrogen deficiency, heat stress, salinity, high light, heavy metal as cadmium treatment, and the herbicide paraquat) applied up to a combination of all six factors on ABA (A), SA (B), OPDA (C), and JA (D) accumulation. (E) PCA plot showing differences on ABA, SA, OPDA, and JA accumulation between the different MFSC. Colored dots depict combinations of HL with S and/or PQ. Loading plot is shown as arrows representing the influence of MFSC on ABA, SA, OPDA, and JA accumulation. Statistical analysis was performed by Student's t-test (asterisks denote statistical significance at $P < 0.05$ compared to CT). Abbreviations: ABA, abscisic acid; CT, control; HL, high light; HS, heat stress; JA, jasmonic acid; N−, nitrogen deficiency; OPDA, 12-oxo-phytodienoic acid; PC, principal component; PCA, principal component analysis; PQ, paraquat; S, salinity; SA, salicylic acid.

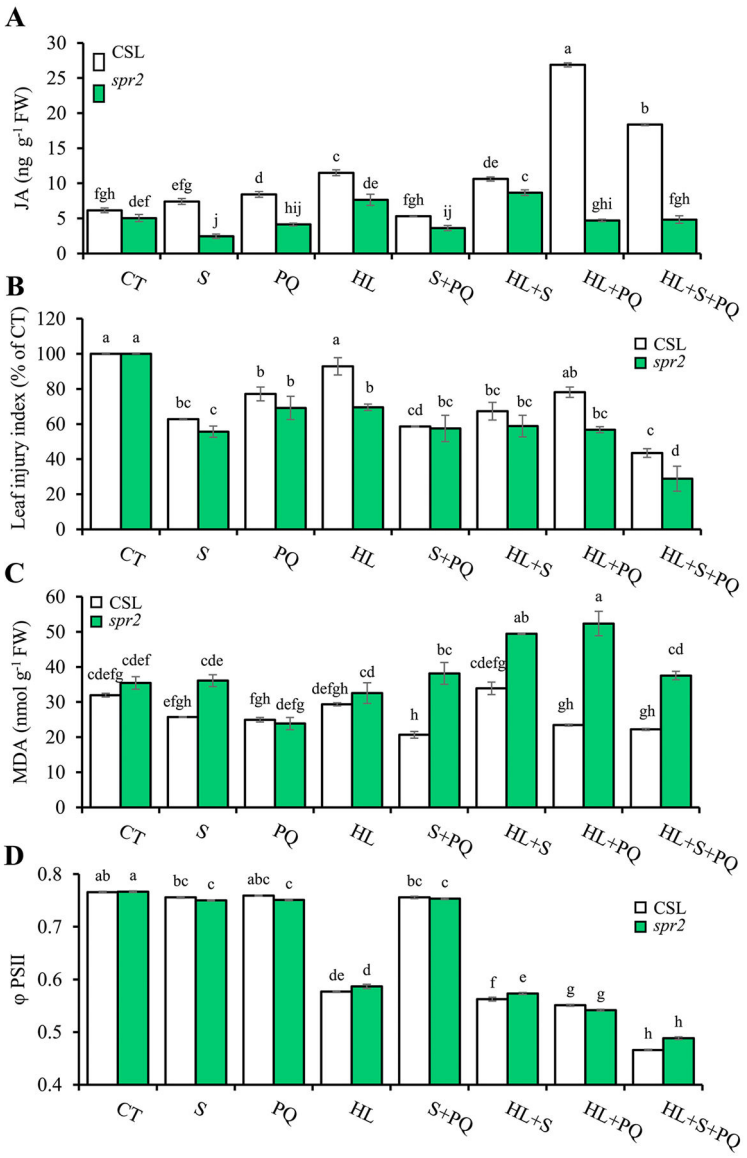

Fig. 6.

The impact of high light stress and its combination with paraquat and/or salinity on tomato leaf injury, MDA accumulation and PSII efficiency of wild type (CSL) and JA-deficient mutant (*spr2*) plants. (A) The effect of HL and its combination with salinity and/or paraquat on JA accumulation of wild type and *spr2* plants. (B-D) The effects of salinity, paraquat, and high light applied in all possible combinations on leaf injury index (B), MDA accumulation (C) and PSII efficiency (D) of wild type and *spr2* plants. Statistical analysis was performed by two-way ANOVA followed by a Tukey post hoc test (different letters denote statistical significance at $P < 0.05$). Abbreviations: CT, control; CSL, Castlemar; HL, high light; JA, jasmonic acid; MDA, malondialdehyde; PQ, paraquat; PSII, photosystem II; S, salinity.