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Effect of ethylene pretreatment on tomato plant responses to salt, drought, and waterlogging stress

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Abstract

Salinity, drought, and waterlogging are common environmental stresses that negatively impact plant growth, development, and productivity. One of the responses to abiotic stresses is the production of the phytohormone ethylene, which induces different coping mechanisms that help plants resist or tolerate stress. In this study, we investigated if an ethylene pretreatment can aid plants in activating stress-coping responses prior to the onset of salt, drought, and waterlogging stress. Therefore, we measured real-time transpiration and CO_2 assimilation rates and the impact on biomass during and after 3 days of abiotic stress. Our results showed that an ethylene pretreatment of 1 ppm for 4 h did not significantly influence the negative effects of waterlogging stress, while plants were more sensitive to salt stress as reflected by enhanced water losses due to a higher transpiration rate. However, when exposed to drought stress, an ethylene pretreatment resulted in reduced transpiration rates, reducing water loss during drought stress. Overall, our findings indicate that pretreating tomato plants with ethylene can potentially regulate their responses during the forthcoming stress period, but optimization of the ethylene pre-treatment duration, timing, and dose is needed. Furthermore, it remains tested if the effect is related to the stress duration and severity and whether an ethylene pretreatment has a net positive or negative effect on plant vigor during stress recovery. Further investigations are needed to elucidate the mode of action of how ethylene priming impacts subsequent stress responses.

KEYWORDS
abiotic stress, drought, ethylene, hypoxia, priming, salt stress, waterlogging

1 | INTRODUCTION

Salinity, drought, and flooding are common environmental stresses that negatively impact plant growth, development, and productivity (Ma et al., 2020). When subjected to abiotic stress, plants initiate a cascade of physiological and biochemical responses that help them tolerate or evade stress. One of these responses is the production of the plant hormone ethylene (Chen et al., 2022), which is involved in adaptation processes toward abiotic stress. For example, ethylene can reduce plant growth through metabolic adaptations and redirect resources to survive during stress periods (Dubois et al., 2018). Ethylene can also induce morphological changes by promoting adventitious

root formation, regulating stem and petiole growth, and controlling stomatal aperture, which leads to a change in physiology to cope with stress (Chen et al., 2022). Furthermore, ethylene signaling can activate genes that help to resist and tolerate stress, such as genes involved in antioxidant production, osmoprotectants, and other stress-related proteins (Husain et al., 2020).

Studies have shown that exposing plants to gentle abiotic stress or a chemical signaling molecule can prime them, enhancing their ability to respond more effectively and rapidly to a severe abiotic stressor (Johnson & Puthur, 2021; Liu, Quan, & Bartels, 2022; Savvides et al., 2016). This process, called priming or sensitization, has shown great promise in enhancing tolerance to different abiotic stresses, such as salinity (Yang et al., 2022). Given that ethylene plays a prominent role in regulating plant adaptation to abiotic stress, it is reasonable to assume that pretreatment with ethylene could prime plants to better cope with future stressors, enhancing their ability to withstand the negative effects of, for example, salinity, drought, and waterlogging.

Many studies have shown that endogenous ethylene can increase salinity stress tolerance in various plants such as *Arabidopsis thaliana* and maize (*Zea mays*; Riyazuddin et al., 2020). Besides the initial osmotic effect, prolonged salt stress leads to the accumulation of sodium (Na^+) and chloride (Cl^-) ions and the production of radical oxygen species (ROS), which can lead to ion toxicity, disruption of normal plant physiology, and cellular damage, resulting in growth inhibition and ultimately death. Ethylene activates antiporters that facilitate the efflux of Na^+ from the cytosol; ROS scavenging enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT); and accumulation of osmolytes such as proline and glycine betaine to maintain water homeostasis and prevent dehydration (Kukreja et al., 2005; Lang et al., 2020; Li et al., 2019; Riyazuddin et al., 2020; Savvides et al., 2016). Furthermore, Lang et al. (2020) demonstrated that the exogenous application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in Arabidopsis activates the expression of the plasma membrane Na^+/H^+ antiporter SOS1 (Salt Overly Sensitive 1) that facilitates the efflux of sodium ions from the cytosol, thereby preventing excessive Na^+ accumulation in the cytoplasm to maintain proper ion homeostasis (Lang et al., 2020; Shi et al., 2000). Moreover, Peng et al. (2014) demonstrated that the overexpression of the *ETHYLENE INSENSITIVE 3* (*EIN3*) resulted in the upregulation of genes involved in stress tolerance (Peng et al., 2014).

During drought stress, ethylene and other hormones (usually abscisic acid [ABA]) can increase the survival of plants (Ullah et al., 2018). A study by Zhang et al. (2009) showed that transgenic tobacco expressing the drought- and ethylene-inducible soybean *GmERF3* gene exhibited higher tolerance to drought stress due to a higher proline and soluble sugar accumulation. Similarly, Scarpeci et al. (2017) showed that overexpressing *AtERF019* in Arabidopsis increased drought tolerance and decreased transpiration rate by reducing stomatal conductance. In a study by Zhang et al. (2021), an ethephon (a liquid formulation that releases ethylene gas after spraying) pre-treatment was administered to Kentucky bluegrass (*Poa pratensis* L.), which subsequently led to an osmotic enhancement when exposed to drought stress by promoting antioxidant enzyme activity and the accumulation of proline. Similarly, priming wheat grains with ethylene has been shown to elicit improved drought tolerance by

regulating auxin and abscisic acid signaling, ROS scavenging, and osmotic regulation (Yang et al., 2021).

When plants are submerged or waterlogged, gas exchange is severely restricted leading to a reduction in oxygen, also known as hypoxia (Hartman et al., 2019). Due to the low diffusion of ethylene from the flooded organs to the floodwater, ethylene gets entrapped and accumulates within the plant tissue, triggering a cascade of adaptive morphological and anatomical flood responses such as epinasty or hyponasty, stem and internode elongation, and the development of adventitious roots and aerenchyma (Hartman et al., 2021; Sasidharan et al., 2018). Furthermore, ethylene was shown to enhance the stability of transcription factors ERFVII (Group VII ethylene response factors), which regulate hypoxia responses by controlling hypoxia-responsive gene expression (Hartman et al., 2019). Recently, it was reported that an ethylene pretreatment of only 4 h can effectively increase the survival of Arabidopsis root meristems when subsequently exposed to hypoxic conditions, mediated by NO and ROS scavenging (Hartman et al., 2019; Liu, Hartman, et al., 2022). Moreover, it was revealed that ethylene is involved in the hypoxia acclimation responses in bittersweet and waterlogging-tolerant potato cultivars (Hartman et al., 2020). However, ethylene does not play a similar role in tomato plants and the roots of waterlogging-sensitive potato cultivars (Hartman et al., 2020).

While ethylene is generally known to have a positive effect on regulating stress responses and increasing stress tolerance, in certain plant species, it can have a negative impact by increasing stress sensitivity. For example, transgenic tobacco plants with reduced ethylene biosynthesis showed elevated salinity tolerance (Riyazuddin et al., 2020; Tavladoraki et al., 2012), while an exogenous ethylene treatment in rice led to salinity hypersensitivity (Yang et al., 2015). Similarly, Wang et al. (2016) reported that ethylene-insensitive tobacco mutants exhibited enhanced drought resistance, with a lower leaf water loss due to a smaller stomatal aperture, increased peroxidase and superoxide dismutase activities, and a higher accumulation of proline (Wang et al., 2016).

These studies have indicated that the effects of ethylene pretreatment on plant stress responses can be highly species specific and context dependent, with both positive and negative effects observed. Furthermore, the underlying mechanisms that determine whether ethylene has a positive or negative effect on response to abiotic stress in different plant species are not yet fully understood. Here, we aimed to study the effect of an ethylene pretreatment on tomato (*Solanum lycopersicum*) plant performance during salt, drought, and waterlogging stress. We assessed real-time physiological responses (e.g., epinastic bending, transpiration, and CO_2 assimilation rates) and the effect on plant development (fresh and dry weight).

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

Tomato (*S. lycopersicum*; cultivar Ailsa Craig) seeds were sowed and germinated in the soil. After approximately 2 weeks, or when seedlings developed two true leaves, they were transplanted onto

Rockwool blocks soaked with fertigation solution (Holsteens et al., 2022). The temperature in the growth chamber was 21°C during the day and 18°C during the night with a constant RH of around 40%. Plants were grown under a long day photoperiod (16 h light/8 h dark) with sunlight mimicking LED lamps (N1 spectrum Vegeled™; Colasse SA, Belgium) at a light intensity of approximately 150 μmol s^{-1} m^{-2}. When plants reached the six-leaf stage, they were placed in transparent airtight PMMA boxes (volume 254 L) for further experiments. Temperature and RH in the boxes were controlled and were kept between 25–26°C and 60–70%, respectively.

2.2 | Ethylene treatment and real-time CO_2 consumption

To test the effect of stress with and without ethylene pretreatment on CO_2 consumption rates, 12 boxes were utilized (six for control and six for stress treatment), each containing a single plant. An automated stop-and-flow gassing system was developed using thermal mass-flow controllers (Brooks® SLA5800 series) and valves (SMC 2 port solenoid valves) operated by an Arduino. This system allows controlling gas composition (mixing air as a carrier gas with desired concentrations of CO_2 and ethylene) and gas flow rate (up to 120 L/min) distributed to 12 boxes (each box receiving 10 L/min of the gas mixture) with an accuracy of ±.5%. The CO_2 levels inside each box were kept between 400 and 650 ppm. The operation of the system followed a specific stop-and-flow protocol: when the CO_2 levels (measured and logged with Sensirion SCD30 sensors placed inside the boxes) in at least one of the boxes dropped below the lower threshold of 400 ppm due to plant CO_2 consumption, all the boxes were flushed with a fresh mixture of air, CO_2, and ethylene (for ethylene boxes). The flushing process continued until the CO_2 levels in all the boxes reached the upper threshold of 650 ppm, after which the flushing process stopped and the CO_2 consumption period commenced. The total daily CO_2 consumption for each plant was calculated by determining the difference in CO_2 levels between the start and end of each consumption period throughout the day. To eliminate potential differences in CO_2 consumption rates and total daily CO_2 consumed between each experiment due to variations in plant size, CO_2 consumption rates were normalized to the initial measurement on Day 0 without treatment. The 1 ppm ethylene concentrations were dosed with ±5% accuracy.

All plants were placed inside the box 24 h before the start of the treatment (referred to as Day 0) to allow them to adjust to the box environment while monitoring CO_2 consumption rates. Stress treatments started on the second day of the experiment at 12 a.m. (4 h zeitgeber time). A new set of 12 plants was used to investigate the effect of ethylene pretreatment in combination with one of the three abiotic stresses. All plants were once again placed inside the boxes for 24 h of acclimatization. On the second day of the experiment (referred to as Day 1), six of these plants received a 1 ppm ethylene treatment for 4 h (at 0–4 h zeitgeber time), before undergoing the stress treatment. The stress treatment lasted 3 days.

Throughout experiments with and without ethylene pretreatment, the total CO_2 consumption rate per box was monitored in real-time with Sensirion SCD30 CO_2 sensors. CO_2 consumption rates and daily CO_2 consumption were calculated using a Python script. Plant fresh and dry weight (after drying at 70°C for 14 days) were determined at the end of each experiment.

2.3 | Waterlogging treatment

To test the effect of root hypoxia on plant CO_2 consumption rates and epinastic bending of leaves with and without an ethylene pretreatment, plants were placed in transparent, airtight PMMA boxes, at 8 a.m. (0 h zeitgeber time). Following a 24-h period for acclimatization, the hypoxia treatment started on the second day of the experiment at 12 a.m. (4 h zeitgeber time). Six plants received waterlogging stress by filling individual trays with distilled water up to 4 cm above the Rockwool surface to induce root hypoxia through natural oxygen consumption. Another six plants received normal nutrient solution (no waterlogging). Stress conditions were given for 3 days, followed by a reoxygenation phase. To assess the waterlogging treatment, we measured the angle between the adaxial side of the leaf petiole and the stem using a protractor, at the first 2 cm of the petiole base as described in Geldhof et al. (2021). The measurements were taken on the first day of the experiment during the acclimatization period (Day 0) at 8 h zeitgeber time, on the second day of the experiment (Day 1) after ethylene pretreatment (if given) and before the hypoxia treatment at 4 h zeitgeber time, and at the end of the experiment (Day 4), after three full days of hypoxia, at 8 h zeitgeber time. For these protractor angle measurements, plants were taken out of the box and put back in afterward, without interfering with the waterlogging treatment itself. To investigate the impact of ethylene pretreatment, the experiment was replicated with a pretreatment of 1 ppm ethylene administered to the plants for 4 h before the onset of stress, as described in the previous section.

2.4 | Drought stress

To investigate the effect of ethylene pretreatment on plants experiencing water deficit, real-time transpiration was measured using digital lysimeters (Holsteens et al., 2022). The water content of the Rockwool block was measured with a TEROS 12 soil moisture sensor throughout the experiments. Prior to initiating the drought experiments, plants were not irrigated until a uniform water content level, ranging from 150 to 200 m^3/m^3 volumetric water content (VWC), was achieved across all Rockwool blocks. Afterward, plants were placed onto digital lysimeters in PMMA boxes for 24 h of acclimatization while measuring CO_2 consumption rates, as previously described. On the second day of the experiment (Day 1), control plants were given nutrient solution to recover from drought and then returned to their respective boxes for additional measurements. The treated plants were continued to be subjected to drought stress conditions

for three full days by withholding nutrient solution. To test the effect of ethylene pretreatment on these parameters, a new set of plants that were treated with drought stress were treated with 1 ppm ethylene for 4 h prior to the experiment (Day 1, 0–4 h zeitgeber time) as previously described. Transpiration and CO_2 consumption rates were measured in real-time through the experiments. Plant fresh and dry weights were determined at the end of each experiment, as previously described.

2.5 | Salt stress

A control nutrient solution (EC 2.5 dS/m) was supplemented with a concentrated NaCl stock solution (6.16 M) to reach severe salinity (10 dS/m; [NaCl] = 94 mM) according to Holsteens et al. (2022). The salt treatment started on the second day of the experiment at 12 a.m. (Day 1; 4 h zeitgeber time) by irrigating the Rockwool blocks of six plants continuously for 5 min to ensure complete drainage and saturation of the blocks with the salt solution. Control plants received the normal nutrient solution. Electrical conductivity (EC) values were measured using TEROS 12 sensors. In order to assess the impact of ethylene pretreatment, the experiment was repeated with a new set of plants, as described in previous sections. CO_2 consumption rates, transpiration, and fresh and dry weight were measured at the end of each experiment.

2.6 | Data and statistical analysis

Data from two separate experiments (with and without ethylene pretreatment) were consolidated into a single graph. The control samples (no abiotic stress) of the two separate experiments were merged. All data were analyzed and visualized using GraphPad Prism 8. Treatments were compared using one-way ANOVA with post hoc Tukey test with the significance threshold $p = .05$ and 95% confidence interval.

3 | RESULTS

3.1 | Ethylene pretreatment did not alleviate the detrimental impact of salt stress

To investigate the effect of salt stress, with and without an ethylene pretreatment, we studied whole plant CO_2 consumption and transpiration rates. To induce salt stress, the EC of Rockwool blocks was increased from 2.3 to 10 mS/cm (equals severe salinity) by administrating a saline solution, immediately after the ethylene pretreatment. Salt-stressed plants with and without ethylene pretreatment showed significantly lower CO_2 consumption rates compared to the unstressed control group (Figure 1a). However, there was no effect of the ethylene pretreatment during salt stress, except on the first day, right after the ethylene treatment, and prior to the salt stress

(Figure 1a). Our data further showed that, similarly to CO_2 consumption rates, whole plant transpiration was negatively affected by salt stress and that an ethylene pretreatment did not alleviate or worsen the transpiration rate during stress (Figure 1b). Salt stress also resulted in a reduction in both fresh and dry weights independent of the ethylene pretreatment (Figure 1c,d).

3.2 | Ethylene pretreatment has a negative effect on the transpiration of plants subjected to drought stress

To assess the drought stress treatment, the water content of Rockwool blocks was monitored throughout the experiments, clearly indicating that desiccation occurred (Figure S1). Our results indicated that drought-stressed plants, with and without ethylene pretreatment, exhibited a notable reduction in CO_2 and water consumption rates when compared to the control group on day 2 of the experiment (Figure 2a,b). Furthermore, it seems that an ethylene pretreatment in combination with drought stress led to a stronger reduction in CO_2 consumption compared to drought stress alone (Figure 2a). This negative effect of the ethylene pretreatment was also observed on whole plant transpiration rates, which were significantly lower throughout the experiment (Figure 2b), except during the last 2 days when the transpiration rate of drought-stressed plants further decreased. Drought stress exhibited a significant reduction in both fresh and dry weights independent of the ethylene pretreatment (Figure 2c,d).

3.3 | Ethylene pretreatment did not reduce root hypoxia-induced epinastic bending and CO_2 consumption rates

To investigate the effect of an ethylene pretreatment on waterlogging tolerance, we measured the epinastic bending of tomato leaves of different ages (leaf 1–7/8), at three different time points: before the stress on Day 0 (Figure 3a), just before the start of the root hypoxia stress without (green) and with (light blue) 4 h ethylene pretreatment on Day 1 (Figure 3b), and after 3 days of hypoxia stress (Figure 3c). Furthermore, we monitored real-time CO_2 consumption rates throughout the experiment. Our results showed that epinastic bending was evident in both the waterlogging-stressed and ethylene pretreatment groups, in contrast to the absence of epinasty observed on Day 0 (Figure 3a). The ethylene pretreatment (1 ppm for 4 h) prior to the onset of waterlogging resulted in an epinastic bending of all leaves except the youngest ones (Leaves 5 and 6; Figure 3b), consistent with previous reports on ethylene-induced epinastic bending of tomato. After 3 days of root hypoxia stress, all leaves with or without the ethylene pretreatment showed significant epinastic bending compared to the unstressed control group (Figure 3c). Data obtained over 4 days and normalized to the initial CO_2 consumption rate before the treatment (Day 0) showed that waterlogging reduced CO_2 consumption rates, however only
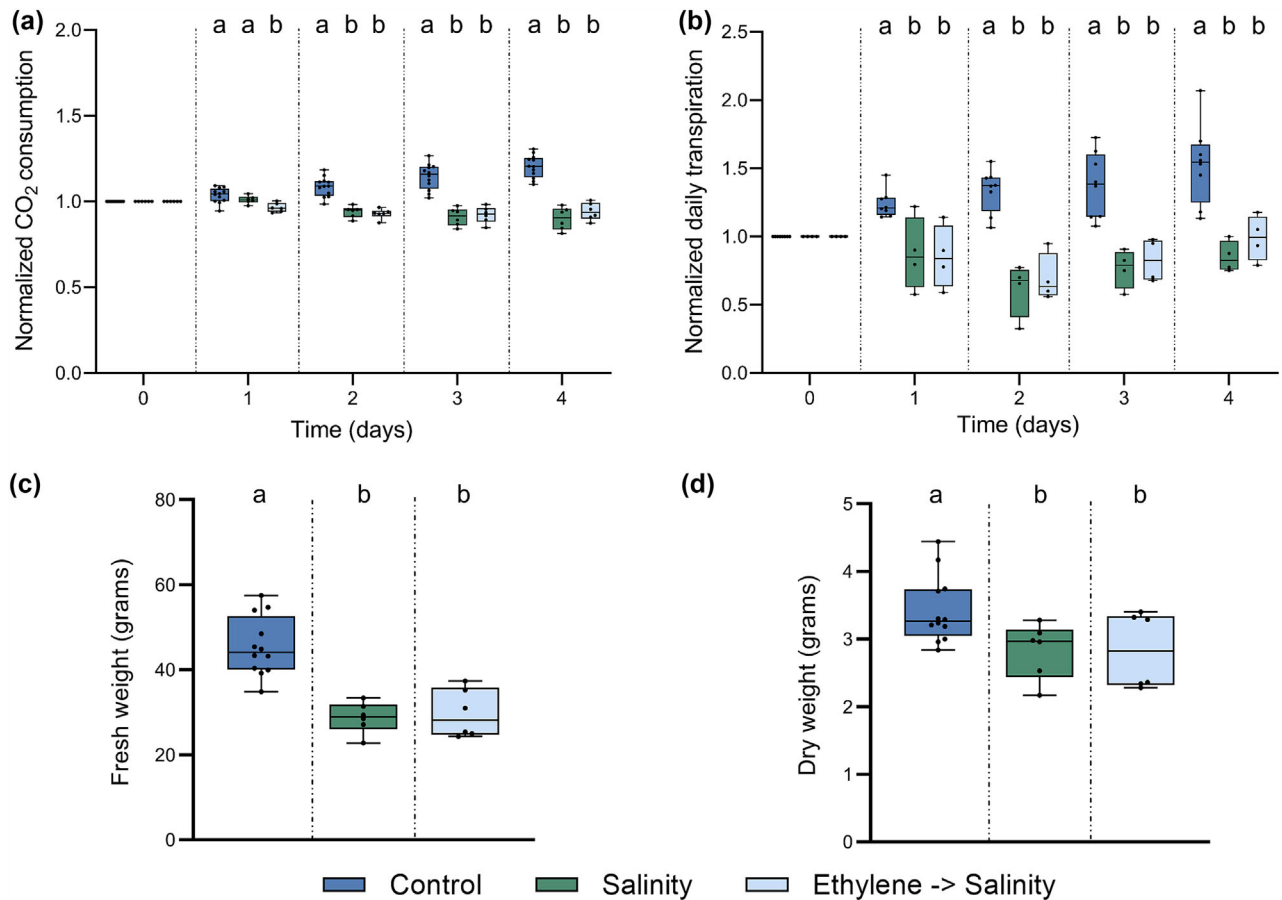
FIGURE 1 Effect of salt stress (10 dS/m) in combination with ethylene pretreatment (light blue; 1 ppm for 4 h) or without (green). (a) Average daily CO2 consumption of control ($n = 12$) and salt-stressed tomato plants ($n = 6$) normalized to the CO2 consumption rate of the first day (Day 0). (b) Average daily transpiration of control ($n = 12$) and salt-stressed tomato plants ($n = 6$) normalized to the transpiration of the first day (Day 0). (c) Plant fresh and (d) dry weight, determined at the end of experiment for control ($n = 12$) and salt-stressed tomato plants ($n = 6$). Control is shown in dark blue ($n = 12$). Error bars represent standard deviation. Significantly different treatments are indicated by different letters, with $\alpha = .05$ as the threshold for significance.

significantly after 4 days of stress. Furthermore, an ethylene pretreatment did not alleviate the negative effect of waterlogging on CO_2 consumption rates. On the contrary, it seems that ethylene in combination with root hypoxia caused an even stronger reduction of the CO_2 consumption rate, already significant on the second day of stress (Figure 3d), despite having a similar degree of epinastic bending. This trend is also visible in the dry and fresh weight measured at the end of the experiment, for which the ethylene pretreated group showed the lowest dry and fresh weight compared to the unstressed control group but not significantly different compared to group receiving only waterlogging stress (Figure 3e,f).

4 | DISCUSSION

The capacity of ethylene to prime plants to alleviate subsequent abiotic stress responses has a lot of potential for future agricultural applications. Recently, it was shown that an ethylene treatment during dark-grown seedling development can stimulate subsequent growth

during the light phase and alleviate abiotic stressors in Arabidopsis (Brenya et al., 2023). Our work tested an ethylene priming treatment on young tomato plants subsequently challenged by salt, drought, and waterlogging stress. Overall, the ethylene priming effects were minimal, with some beneficial effects during drought, some negative effects during salinity, and no observable effects during waterlogging stress. However, it is worth mentioning that the timing, dose, and duration of the ethylene priming treatment are crucial. Ethylene signaling requires some time to initiate stress response pathways. Perhaps the 4 h ethylene pretreatment was not long enough, or the time between the priming treatment and the actual stress response was too short, or the dose used was sub-optimal. It is possible that certain pathways involved in stress resilience are induced at a later moment or require more time. These experimental conditions could explain why we did not observe very strong beneficial effects. To fully realize ethylene priming benefits, it would be advised to have additional time between the priming and stress treatment, to allow for a more robust and effective stress resilience response. Furthermore, the ideal ethylene pre-treatment will also depend on the actual stress duration and
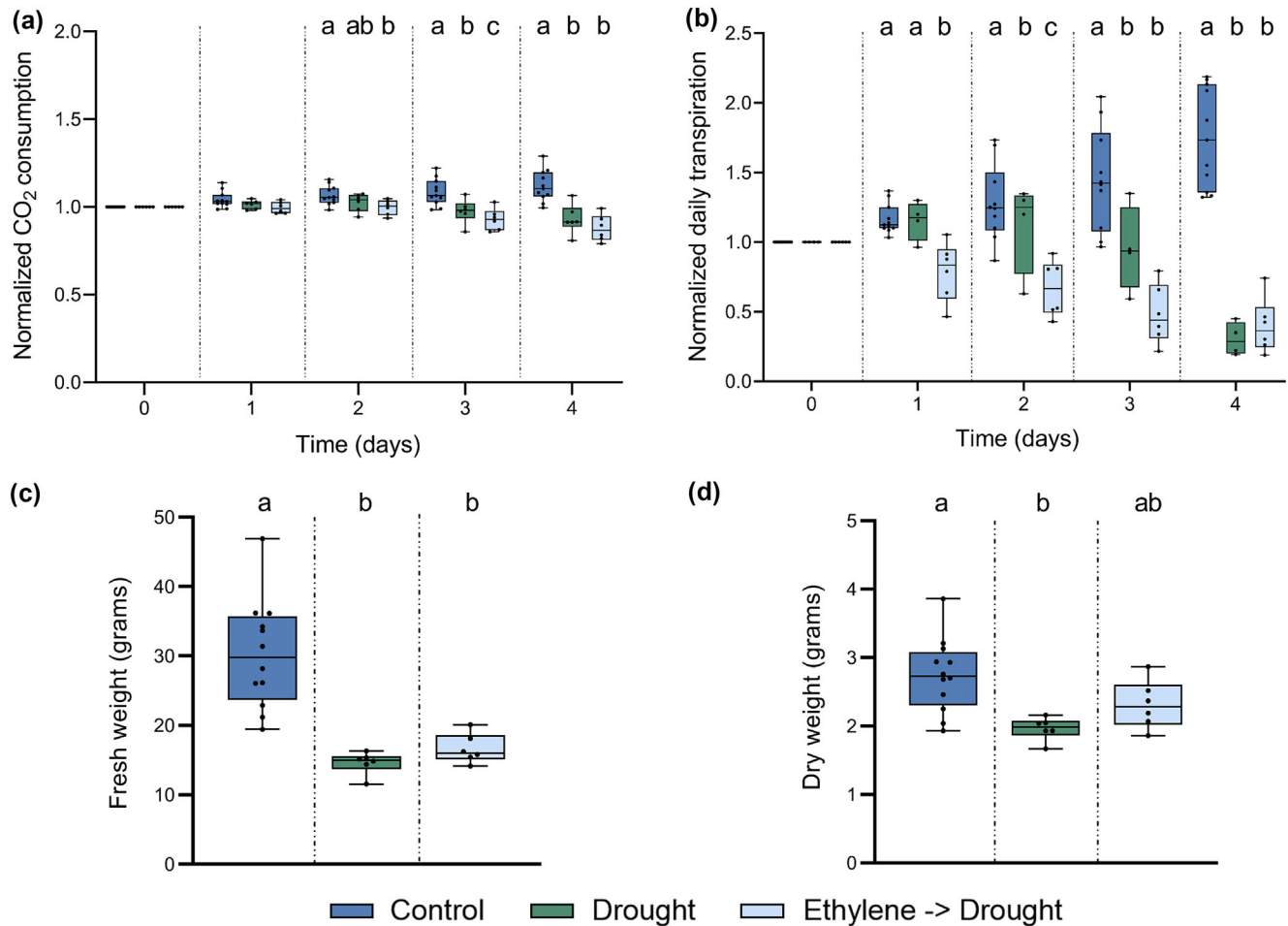
FIGURE 2 Effect of drought stress in combination with ethylene pretreatment (light blue; 1 ppm for 4 h) or without (green). (a) Average daily CO2 consumption of control ($n = 12$) and drought-stressed tomato plants ($n = 6$) normalized to the CO2 consumption rate of the first day (Day 0). (b) Average daily transpiration of control ($n = 12$) and drought-stressed tomato plants ($n = 6$) normalized to the transpiration of the first day (Day 0). (c) Plant fresh and (d) dry weight, determined at the end of experiment for control ($n = 12$) and drought-stressed tomato plants ($n = 6$). Control is shown in dark blue ($n = 12$). Error bars represent standard deviation. Significantly different treatments are indicated by different letters, with $\alpha = .05$ as the threshold for significance.

severity, its subsequent recovery period, or the actual plant, and tissue age at the moment of stress (Rankenberg et al., 2021). Overall, finding the optimal ethylene priming treatment to alleviate abiotic stress certainly requires more work.

4.1 | Ethylene pretreatment increases sensitivity to water loss during salt stress

Salt stress limits water uptake, leading to cell dehydration and changes in cell turgor, generating osmotic stress. During severe salinity, the increase in endogenous ABA levels induces stomatal closure to regulate water balance and osmotic homeostasis (Verma et al., 2016). By analyzing the real-time transpiration rates during salt stress, we observed no significant effect of the ethylene pretreatment (Figure 1b). On the other hand, the stomatal aperture must be carefully regulated to ensure optimal CO_2 uptake for photosynthesis while

protecting plants against excess water loss (Nilson & Assmann, 2007; Postiglione & Muday, 2020); otherwise, it could hinder plant growth (Liu, Song, et al., 2022). Our data showed that CO_2 consumption rates declined gradually during salt stress, indicating a reduction in photosynthetic activity and matching the reduction in transpiration (Figure 1b). However, the ethylene pretreatment had no impact on plant photosynthesis, also evidenced by the unaffected fresh and dry weights (Figure 1c,d).

It is plausible that an ethylene pretreatment may not exhibit any noticeable effects during the first osmotic phase of salt stress (Yang & Guo, 2018). Perhaps ethylene is more effective in mitigating ion toxicity associated with salt stress at a later stage, by regulating other mechanisms such as ion transport or ROS scavenging. Furthermore, Borbély et al. (2020) showed that an ACC treatment of tomato plants lowered the cyclic electron flow around photosystem I and enhanced PSII photochemistry after 6 h of lethal salt stress, which, contrary to our results, sustained positive carbon assimilation. In their study,
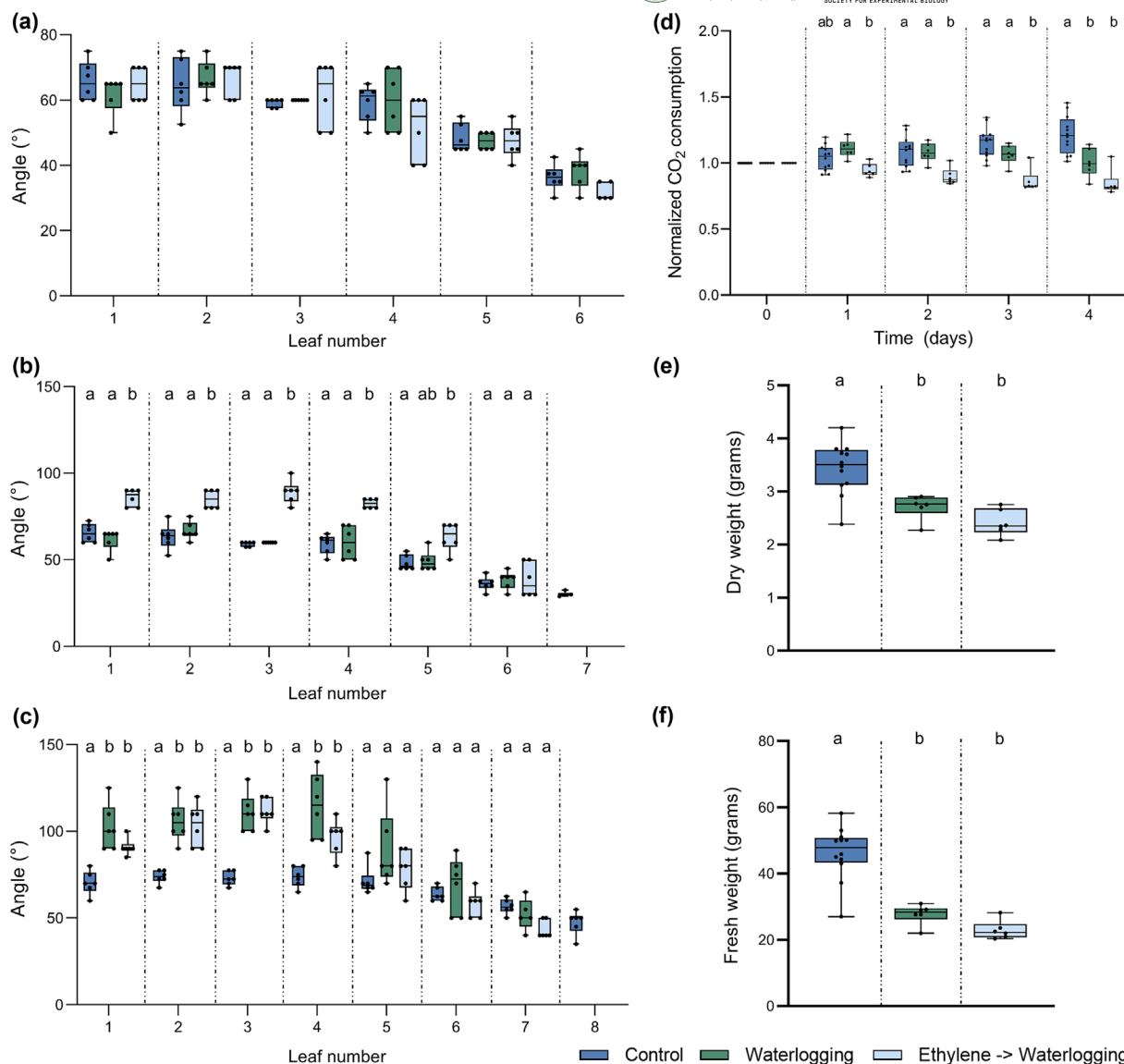
FIGURE 3 Effect of waterlogging stress in combination with ethylene pretreatment (light blue; 1 ppm for 4 h) or without (green) on the epinastic angles of leaves of different ages for control ($n = 12$) and waterlogging-treated tomato plants ($n = 6$) at three time-points: (a) prior to the ethylene pretreatment (Day 0); (b) after the ethylene pretreatment (1 ppm for 4 h; day 1); and (c) after 3 days of waterlogging stress. (d) Average daily CO_2 consumption of control ($n = 12$) and waterlogging-stressed tomato plants ($n = 6$) normalized to the CO_2 consumption rate of the first day (Day 0). (e) Plant fresh and (f) dry weight, determined at the end of experiment for control ($n = 12$) and waterlogging-stressed tomato plants ($n = 6$). Control is shown in dark blue ($n = 12$). Error bars represent standard deviation. Significantly different treatments are indicated by different letters, with $\alpha = .05$ as the threshold for significance.

however, they employed a different pretreatment approach where they exposed plants to ACC-containing nutrient solution for 1 h prior to adding NaCl. In contrast to our study, where the treatment lasted for 4 h and did not involve a continuous ethylene application, ethylene production persisted for at least 6 h during salt stress, which may have triggered distinct responses and mechanisms with greater intensity, ultimately leading to positive CO_2 assimilation (Borbély et al., 2020). This points toward the importance of an optimal mode of application of ethylene (or its biosynthesis precursor) to alleviate salt stress, and that the timing and length of the pretreatment could be crucial in stress tolerance.

4.2 | Ethylene pretreatment reduces water losses during drought stress

Similarly to salt stress, the immediate response of plants toward drought stress is to prevent water loss by stomatal closure (Basu et al., 2016), which is predominantly mediated by ABA. It has been shown that bulk leaf ABA levels increase with increasing drought stress (Beardsell & Cohen, 1975; Franks & Farquhar, 2001; Pierce & Raschke, 1980).

Our data (Figure 2b) show a decrease in transpiration rates throughout the drought stress period, likely coordinated by stomatal

closure. With prolonged drought stress, the overall daily plant transpiration decreases significantly in comparison to the well-watered control group. Plants subjected to an ethylene pretreatment prior to drought stress exhibited a slightly more rapid decrease in transpiration rates compared to those exposed to drought alone. Previous research has indicated that the application of exogenous ethylene to wild-type Arabidopsis plants can induce stomatal closure and decrease stomatal conductance, thereby restricting transpiration rates (Desikan et al., 2006; Tholen et al., 2004). Perhaps the ethylene pretreatment in our experiment already induced stomatal closure. Once drought stress started, perhaps stomata closed even more, due to the well-known ABA-mediated stomatal closure response, leading to further reductions in transpiration (Figure 2b). This adaptive response due to the ethylene pretreatment might help plants to conserve more water and reduce dehydration during the drought stress period. However, we only observed a moderate effect on photosynthesis, which was only significantly lower for the ethylene-pretreated plants on Day 3 of the drought experiments (Figure 2a) and which did not lead to a less significant reduction in plant fresh or dry weight at the end of the experiment (Figure 2c,d).

Interestingly, other studies examining the interaction between ethylene and ABA in regulating stomatal closure involved applying ABA during or after the ethylene treatment (Desikan et al., 2006; Watkins et al., 2014). In such cases, ethylene has been found to inhibit ABA-mediated stomatal closure. However, we observed that the ethylene pretreatment further reduced plant transpiration, so likely, this ethylene-mediated inhibitory effect on drought-induced ABA-mediated stomatal closure did not occur.

The results of our experiments indicate that an ethylene pretreatment may be beneficial for plants facing drought stress; however, the timing of the pretreatment might be further optimized. Moreover, it should be noted that plants subjected to extreme drought stress, resulting in complete depletion of water content, are unlikely to benefit largely from any priming treatment. To gain a more comprehensive understanding of the possible long-term effects of an ethylene pretreatment, it would be beneficial to maintain a constant state of low water availability (mild drought stress), potentially improving overall plant survival rates.

4.3 | Ethylene- and hypoxia-induced epinasty lower CO_2 assimilation rates during waterlogging stress

In response to waterlogging, stomata also close, which restricts transpiration enabling the maintenance of turgor pressure within the plant (Bradford & Yang, 1981). During waterlogging, ACC accumulates in hypoxic roots and is then transported to the normoxic shoot through the xylem (Bradford & Yang, 1980), where it is converted into ethylene ultimately leading to the epinastic response (English et al., 1995). Geldhof et al. (2022) showed that the stomatal conductance of all leaves of tomato plants decreased drastically during waterlogging, which could limit CO_2 availability. Furthermore, ethylene and/or ABA directly or indirectly enhance H_2O_2 production under waterlogging stress conditions contributing to stomatal closure (Bashar et al., 2019). Epinasty and partial stomatal closure may act together to restrict transpiration losses soon after waterlogging.

It is known that exogenous ethylene causes leaf epinasty in tomatoes (Doubt, 1917). Our data show that an ethylene pretreatment caused epinasty immediately after 4 h of the pretreatment (Figure 3b). At the end of the waterlogging treatment, both ethylene-pretreated and untreated waterlogged plants showed strong epinastic bending, which was maintained throughout the experiment (Figure 3a–c). On the other hand, CO_2 assimilation was significantly reduced by ethylene pretreatment (Figure 3d). One of the reasons could be due to a faster epinastic bending response that limits light perception and thus reduces photosynthesis. It is worth mentioning that the ethylene-induced epinasty was also observed in salt and drought stress experiments; however, plants recovered soon after the ethylene pre-treatment stopped, thus restoring normal light perception. Overall, it seems that the ethylene pretreatment lowered CO_2 assimilation compared to both the stressed and control groups, which is also reflected by lower fresh and dry weights (Figure 3b,c). Furthermore, it was shown that ethylene could induce hypoxia tolerance at a molecular level. In Arabidopsis, it was demonstrated that ethylene enhances the stability and production of the group VII Ethylene Response Factor (ERFVII) transcription factors (Hartman et al., 2019), which regulate a conserved set of genes that respond to hypoxia, giving them the ability to tolerate hypoxic conditions. Ethylene pretreatment can activate ERFVII even before the onset of hypoxia, which, in turn, increases the transcription of downstream target genes, leading to improved energy homeostasis and oxidative stress tolerance during both hypoxia and re-oxygenation events (Hartman et al., 2020, 2019). This positive effect of ethylene priming on waterlogging resilience seems species-specific, as its beneficial effect was shown for bittersweet (*Solanum dulcamara*) and a waterlogging-tolerant potato cultivar (*Solanum tuberosum*) (Hartman et al., 2020), as well as for maize (*Zea mais*) and okra (*Abelmoschus esculentus*) (Vwioko et al., 2017). However, ethylene priming does not have the same positive effect in tomato, indicating that the ethylene priming effect on waterlogging tolerance is species-specific (Hartman et al., 2020). Our work corroborates these findings (Hartman et al., 2020), where there was no positive effect of ethylene priming on tomato tolerance toward waterlogging. This might suggest that ethylene-controlled ERFVII levels may not always be directly correlated with enhanced hypoxia tolerance (Hartman et al., 2020). Furthermore, ethylene enhances only a subset of ERFVII proteins, and only specific ERFVIIs can mediate hypoxia tolerance (Hartman et al., 2020). Perhaps our ethylene pretreatment was not strong or long enough to fully activate the specific ERFVIIs in tomato. In addition, the negative effects of the ethylene-induced epinasty could not be compensated by the putative positive effects of ethylene priming against hypoxia stress.
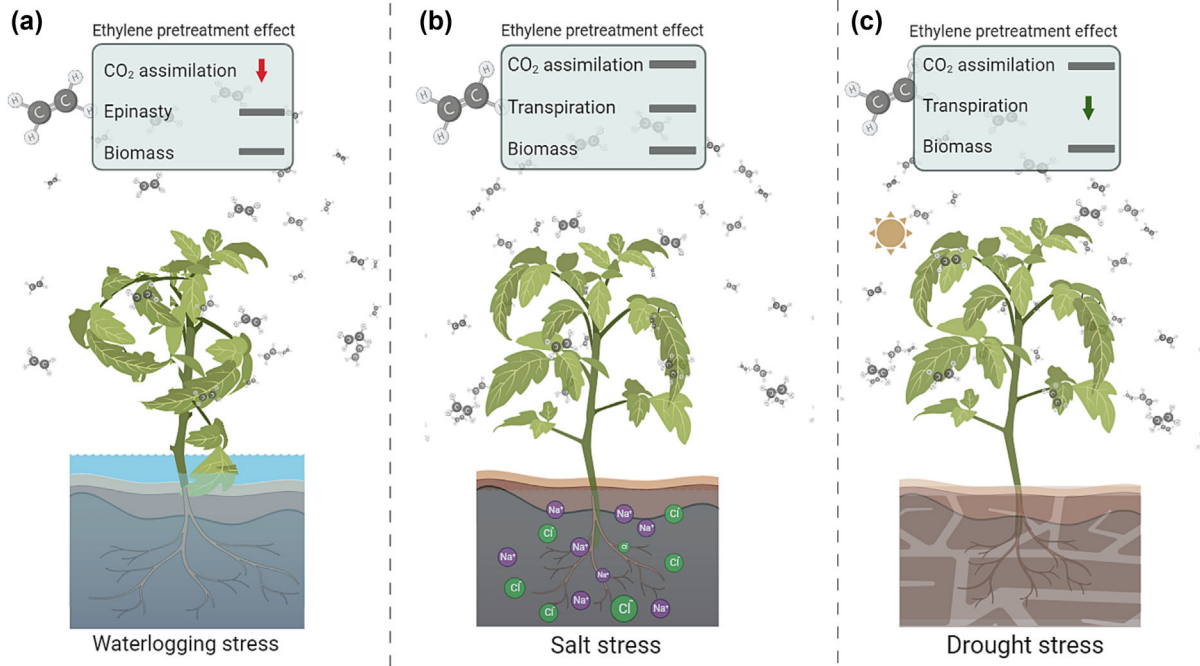
FIGURE 4 Summary of ethylene pretreatment effect on different parameters after 3 days of waterlogging, salt, and drought stress compared to stressed-only plants. (a) After 3 days of waterlogging stress, plants of both stressed groups showed hypoxia-induced epinasty, with no difference in epinastic bending in plants subjected to ethylene pretreatment (indicated with gray, horizontal bar). Plants subjected to ethylene pretreatment displayed lower CO_2 assimilation (indicated with red, downward arrow), but no effect on overall fresh and dry weight at the end of the experiment. (b) Ethylene pretreatment did not alleviate the detrimental impact of salt stress on any of the tested parameters. (c) Ethylene pretreatment reduced transpiration rates of drought-stressed plants and prevented water loss without affecting biomass production.

5 | CONCLUSION

The regulation of plant growth and development under abiotic stress is a complex process. Variations are observed between different crops, as well as between different cultivars and growth stages within the same crop (Rankenberg et al., 2021). Moreover, different plant species have evolved unique adaptation strategies to cope with stress (Godoy et al., 2021). Our study provided insights into the effect of an ethylene pretreatment on real-time transpiration and CO_2 consumption during salinity, drought, and waterlogging. While ethylene pretreatment did not impact the response during salt stress, it amplified the drop in transpiration during drought stress, and it increased the reduction in photosynthesis during waterlogging stress. We did not observe changes in the biomass of plants with or without the pretreatment, eliminating a possible yield penalty during abiotic stress. The effects of ethylene pretreatment are summarized in Figure 4, and our study has shown that an ethylene pretreatment can indeed modulate plant physiology and morphology and that these changes can be either beneficial or harmful during stress conditions. Our findings open the door for further research, particularly regarding the optimal timing and duration of the ethylene pretreatment. Furthermore, our work only focused on 3 days of stress, which might not be enough to spot strong effects. Whether the observed physiological adjustments also benefit plants during stress recovery remains to be studied.

AUTHOR CONTRIBUTIONS

Petar Mohorović and Bram Van de Poel designed the experiments; Petar Mohorović performed the research; Batist Geldhof assisted with the design, development, and programming of the ethylene gassing system, epinasty curvature measurements, and data analysis; Kristof Holsteens assisted with setting-up lysimeters and data analysis; Marilien Rinia assisted with ethylene priming experiments and measurements; Johan Ceusters and Bram Van de Poel supervised experiments and reviewed data and the manuscript; and Petar Mohorović and Bram Van de Poel wrote the manuscript with the input of all authors. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available in the supporting information for this article.

DATA AVAILABILITY STATEMENT

Data will be made available upon a reasonable request.

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