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ORIGINAL ARTICLE

Strategies induced by methyl jasmonate in soybean seedlings under water restriction and mechanical wounding

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Abstract

Methyl jasmonate (MeJA) is a phytohormone involved in plant defense against stress. However, its application as pretreatment in soybean seeds is limited. Here, we investigated whether seed pretreatment with MeJA mitigated the negative effects of water restriction (WR) and mechanical wounding (MW) in soybean seedlings at the V1 vegetative stage. Seeds of *Glycine max* (Monsoy 6410 variety) were pretreated with water or 12.5 μM MeJA for 14 h. The obtained seedlings were transferred to pots containing substrate (soil and sand) kept in a greenhouse and subjected to different growth conditions: control (no stress), WR (40% water retention), and MW. The experiment was conducted in a 2 × 3 factorial scheme (2 seed pretreatments × 3 growth conditions). The variables analyzed were ethylene levels, hydrogen peroxide, lipid peroxidation, antioxidant system enzymes, sugars, amino acids, proteins, proline, and growth (root and shoot length). WR negatively affected seedling growth, regardless of seed pretreatment, but proline levels increased with MeJA application. In seedlings subjected to MW, MeJA increased ethylene release, which was related to reduced damage. It suggests that pretreatment of soybean seeds with MeJA is a promising tool to mitigate the deleterious effects of biotic and abiotic stresses during seedling establishment, inducing distinct tolerance strategies.

Keywords Ethylene · Seed treatment · *Glycine max* · Proline · MeJA · Mobilization of reserves

Introduction

Plant species can be subjected to hostile environments in which different abiotic stresses trigger defense responses. One of the most substantial approaches to induce tolerance against stresses is biosynthesis and signaling via phytohormones (Dar et al. 2015). Jasmonate (JA) or its methyl ether methyl jasmonate (MeJA) act as signal transduction molecules in plant defense reactions to induce the production of secondary metabolites (Ashry et al. 2018) and are also involved in signaling different stress responses (Mohamed and Latif 2017).

One of the stressors that most affects productivity is water deficit, which reduces photosynthesis and biomass accumulation (Volaire 2018). The water deficit can be even more severe when occuring in the early stages of development, including germination and seedling establishment (Guo et al. 2018). MeJA induces biochemical changes and signal transduction with other phytohormones, promoting greater tolerance to water deficit (Dar et al. 2015). In adult soybean plants, foliar application of MeJA alleviated the deleterious effects of water stress, increased photosynthetic pigments, maintained biomass gain, and led to seeds with the same nutritional quality as non-stressed plants (Mohamed and Latif 2017).

Another stress factor that can reduce plant fitness is injury due to loss of nutrients and entry of pathogens. Plants have defense mechanisms against wounding to prevent pathogen infections (Savatin et al. 2014). Although mechanical wounded stems trigger defense responses similar to those induced by herbivore insects (Rehrig et al. 2014), these stresses also have specific characteristics. Furthermore, mechanical wound is not sufficient to trigger the complete response activated by herbivores (Maffei et al. 2007; Waterman et al. 2019). Herbivory induces MeJA biosynthesis,

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which promotes the synthesis of volatile defense compounds that activate a systemic response (Yu et al. 2018).

MeJA signal transduction pathways involve the activation of antioxidant system enzymes, ROS signaling, and interaction with other phytohormones (e.g., ethylene in abiotic stress events), promoting different tolerance mechanisms (Nazir et al. 2023). Positive effects of the combined action of MeJA and ethylene on cold, freezing, salinity, drought, and heat stresses have been reported in adult plants (Kazan 2015; Nazir et al. 2023). However, it is still unknown whether MeJA can induce ethylene release in seeds and seedlings.

Soybean is one of the most important grain crops worldwide. In 2023, countries such as Brazil and the United States were responsible for exporting approximately 96 and 54 million tons, respectively, of this grain (USDA 2023). The agricultural importance of soybeans reflects the attention given to planting and maintaining the crop to avoid yild losses. Because it is widely cultivated in several countries, soybean is commonly subjected to abiotic stresses, which ultimately reduce productivity. As much of the soybean production worldwide relies on rainwater (Soares et al. 2021; Felisberto et al. 2023), changes in rainfall distribution and mechanical damage lead to reduced photosynthesis and loss of leaf area, impairing crop yield. In addition, plants or their parts are crushed, cut, punctured, rubbed, or hit due to accidental or intentional actions in the field.

Exogenous application of MeJA alleviates stresses in adult plants; however, the effects of seed pretreatment in soybean seedlings are poorly known. We hypothethized that soybean seeds which were pretreated with MeJa, attenuate stress effects through ethylene, increasing antioxidant capacity and osmoprotection. Here, we aimed to investigate whether seed pretreatment with MeJA would attenuate the effects of water restriction (WR) and mechanical wounding (MW) in soybean seedlings at the vegetative stage V1.

Materials and methods

Plant material, seed pretreatment, and obtained seedlings

Soybean seeds (*Glycine max* (L.) Merr) of the Monsoy 6410 variety were kept at 4 °C until the experiments were performed. Seeds were pretreated with 12.5 µM MeJA (Sigma Aldrich®) or deionized water (control) for 14 h (in the dark at 25 °C) as this concentration and incubation time did not reduce the percentage or speed of seed germination (Supplementary Material, Fig. S1, Table S1). Afterward, seeds were transferred to gerbox-type boxes and placed in a germination chamber at 30 °C under a 12-h photoperiod (40 µmol m^{-2} s^{-1} of irradiance). Seeds were monitored daily until seedlings with roots ≥ 2 cm were obtained.

Cultivation of soybean seedlings under stressful conditions

The seedlings obtained in the previous experiment were transplanted into 0.5 L polyethylene pots containing a mixture of soil and sand (2:1) and kept in the greenhouse for nine days until they reached vegetative stage V1, (i.e., unifoliate leaves fully expanded in the control samples). Substrate moisture content was maintained by daily monitoring of the pot weights according to the moisture retention curve using the gravimetric method (Souza et al. 2000). WR, retention capacity of 40% based on Supplementary Material, Fig. S2; Table S2) was imposed immediately after seedling transplantation. For the MW experiment, the first pair of unifoliate leaves fully expanded was mechanically wounded 24 h before the plant material was collected by cutting 40% of the leaf area with scissors (Lama et al. 2019).

Sample collection and processing

Seedlings at the V1 stage were removed from the substrate, washed in running water, followed by deionized water, and dried superficially (paper towels). The root and shoot lengths were measured (in cm) with a graduated ruler. For the analyses using fresh or dry matter, seedlings (shoot + root) were frozen in liquid N_2 and stored at -80 °C or dried in an oven of forced air circulation at 75 °C for 72 h.

Quantification of ethylene

Ethylene was quantified as described by López et al. (2022), with modifications., Two fresh cotyledonous leaves (WR condition) or the first pair of unifoliate leaves fully expanded (control and MW conditions) per seedling were incubated in vacutainer tubes for 48 and 72 h, respectively. The timing of incubation was defined regarding the fresh weight of each structure used. The plant material from each seedling was incubated in three separate vials and the gas (3 mL) was removed with a 10 mL plastic syringe. Samples from the same seedling were extracted with the same syringe, and the total volume of 9 mL was subsequently injected into the CI-900 portable ethylene analyzer (Bio-Science) for ethylene quantification. Ethylene evolution rate was expressed as ppm g^{-1} FW h^{-1}.

Hydrogen peroxide (H_2O_2)

The levels of H_2O_2 were quantified by spectrophotometry at 390 nm according to Velikova et al. (2000) method.

Lipid peroxidation

Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) by spectrophotometry at 535 and 600 nm as described by Buege and Aust (1978).

Extraction and quantification of enzymes from the antioxidant system

Aliquots (0.2 g of fresh seedling material) were extracted as described by Biemelt et al. 1998.The activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were assayed in the supernatants (enzymatic extracts) as described by Giannopolitis and Ries (1977), Havir and McHale (1987) and Nakano and Asada (1981), respectively.

Extraction and quantification of sugars, amino acids, proline, and proteins

Aliquots (0.1 g of seedling dry matter) were extracted with different solvents for the quantification of metabolites and proteins by spectrophotometry. Total soluble sugars (TSS) and reducing sugars (RS) were extracted according to Zanandrea et al. 2010 and quantified following the methodology of Yemm and Willis (1954) and Miller (1959), respectively. Non-reducing sugars (NRS) were obtained by the difference between TSS and RS. Total free amino acids (AA) were determined by the ninhydrin method of Yemm et al. (1955). Proline levels and total soluble proteins were measured according to Bates et al. (1973) and Bradford (1976), respectively.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design in a 2 × 3 factorial scheme (2 seed pretreatments—water and MeJA vs. 3 growth treatments—WR, MW, and control) with five replicates consisting of ten seedlings each. The number of seedlings per replicate differed in some analyses five seedlings for the analyses of hydrogen peroxide, lipid peroxidation, and enzymes of the antioxidant system; three seedlings for quantification of ethylene (data transformed by the square root), and two seedlings for the analyses of growth, sugars, amino acids, proteins, and proline. Biochemical analysis using spectrophotometer were carried out with in triplicates in each biologic replicate. Significant differences among treatments were assessed by analysis of variance (ANOVA) followed by post hoc Tukey's test at 5% probability using the ExpDes.pt package (Ferreira et al. 2021) of the statistical software R version 4.0.5 (R Core Team 2021).

Results

Seedlings from seeds pretreated with MeJA showed a significant reduction of 21.41 and 20.30% in the root and shoot length, respectively, in the WR condition compared to the control. However, under the WR treatment, MeJAled to an increase of 40.79% in the shoot length and a decrease of 47.96% in root length compared to water (Fig. 1a, b).

Seeds exposed to the pretreatment with MeJA had higher levels of MDA compared to water in all growth conditions (WR, MW, and control). Comparatively, this increase was more prominent in the WR treatment than in the control and MW. Figure 2a, b show that pretreatment of seeds with MeJA induced higher concentrations of H_2O_2 and MDA in seedlings subjected to WR treatment compared to water.

Regarding the enzyme activity of the antioxidant system, the pretreatment of seeds with MeJA increased the activity of CAT by 46.26% when compared to seedlings treated with water in the control. Under stressful growth conditions (WR and MW), the activity of this enzyme was strongly reduced, with no significant differences between them independent from the seed pretreatment (Fig. 3). The activities of the SOD and APX enzymes were not significantly affected (data not shown).

According to Fig. 4a–d, seedlings from seeds pretreated with MeJA and subjected to WR had a reduction of 71.04, 69.44, 64.02 and 93.31% in TSS, proteins, NRS, and AA, respectively, than seedlings from seeds pretreated with water. For the proline content, an increase of 61.92% was observed in the soybean seedlings when the seeds were pretreated with MeJA compared to water (Fig. 5) under WR. Pretreatment with MeJA increased the release of ethylene by the seedlings only in the MW condition (by eightfold after 72 h when compared to water) (Fig. 6).

Discussion

It has been described that some substances application are capable to modulate antioxidant and homonal resposes alleviating biotic and abiotic stresses (Nazir et al. 2023). This study demonstrated that the pretreatment of soybean seeds with MeJA can induce different effects and tolerance mechanisms depending on the stress conditions experienced

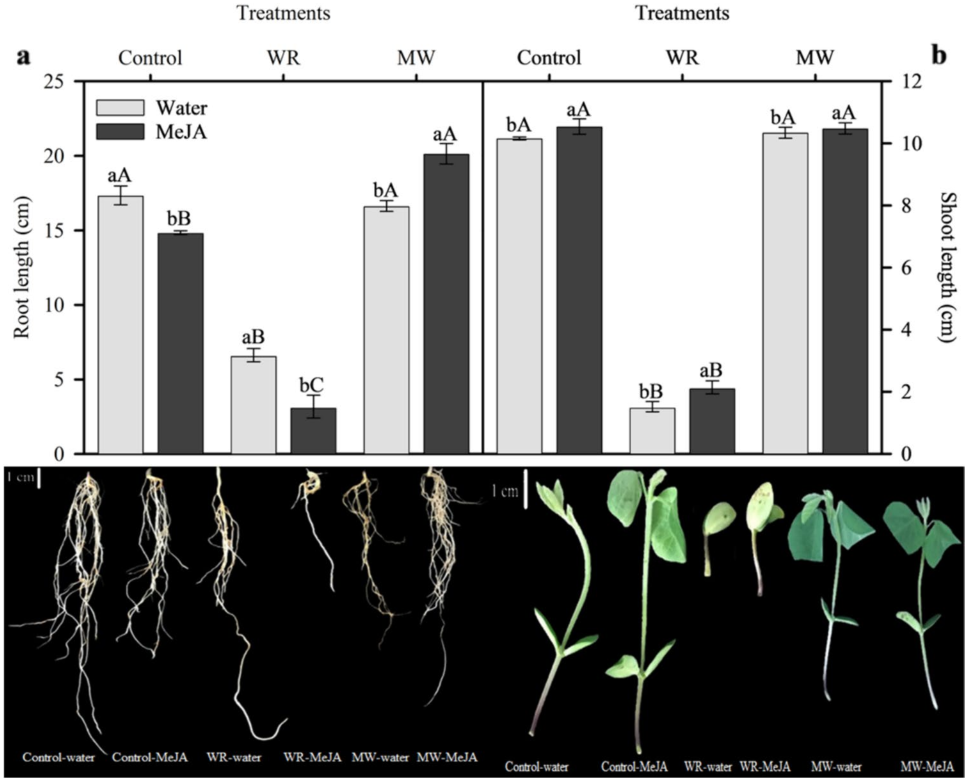


Fig. 1 Root and shoot length of soybean seedlings from seeds pretreated with MeJA or water subjected to no stress (control) water restriction (WR), and mechanical wounding (MW) . Data are means ± standard error ($n = 5$). Lowercase and upper-caseletters represent significant differences (ANOVA, Tukey's test ($P < 0.05$)) between seed pretreatments and among growth conditions, respectively

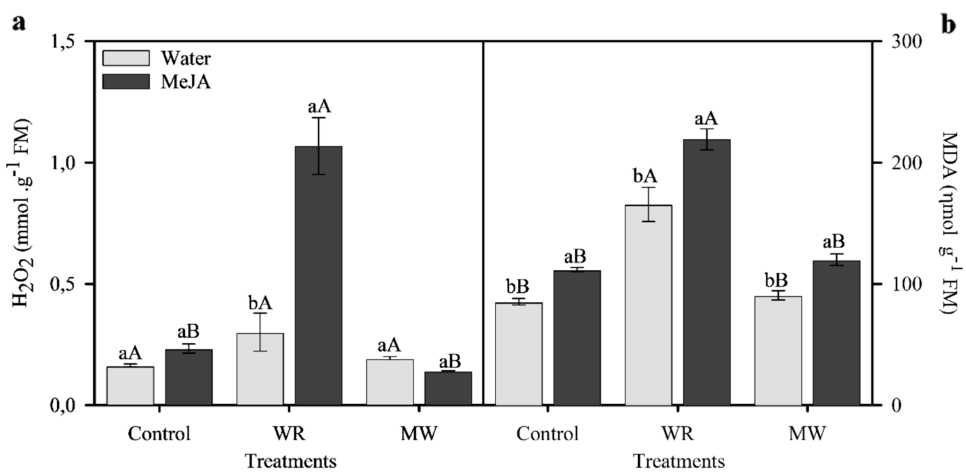


Fig. 2 H_2O_2 and MDA levels of soybean seedlings from seeds pretreated with MeJA or water subjected to no stress (control) water restriction (WR), and mechanical wounding (MW). Data are means ± standard error ($n=5$). Lowercase and uppercase letters represent significant differences [ANOVA, Tukey's test ($P<0.05$)] between seed pretreatments and among growth conditions, respectively

by the seedlings. The activated mechanisms were different when seedlings were subjected to WR and MW. Seed pretreatment with MeJA was not effective in mitigating the effects of WR in soybean seedlings at stage V1, but induced greater ethylene release and increased stress tolerance in seedlings under MW conditions. Therefore, we investigated the mechanisms by which we found these results.

WR applied in the early stages of soybean development reduced seedling growth, especially in roots. These results are similar to those of Li et al. (2018), who concluded that foliar application of increasing concentrations of MeJA after plant emergence significantly decreased the growth of soybean, tomato, and sunflower plants. On the other hand, the results presented here indicate that when soybean seeds were pretreated with MeJA, seedlings at the V1 vegetative stage invested in the aerial part to mitigate the effects WR. Sheteiwy et al. (2018) observed that 2.5 mM MeJA alleviated the water stress of rice seedlings and positively influenced both the length of the roots and the aerial part. In the same way, Sirhindi et al. (2016) found positive effects on the root and shoot growth of soybean seedlings treated with 2 mM MeJA. This positive effect on root growth was related to increased cell division, which may have the participation of cytokinins (Avalbaev et al. 2016).

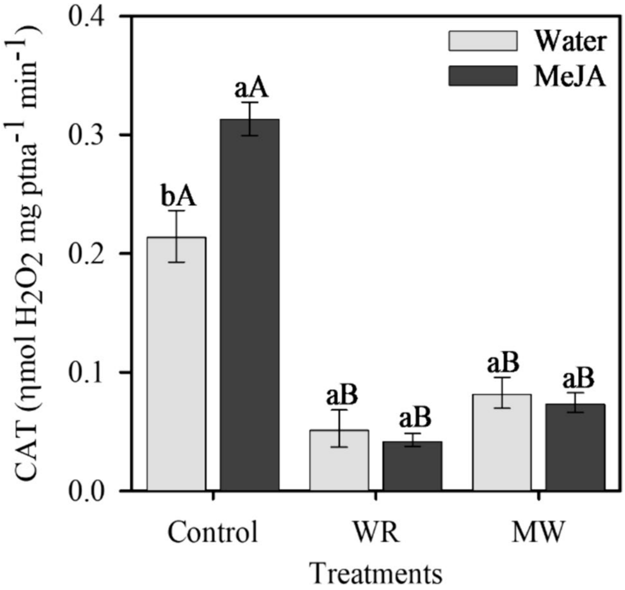
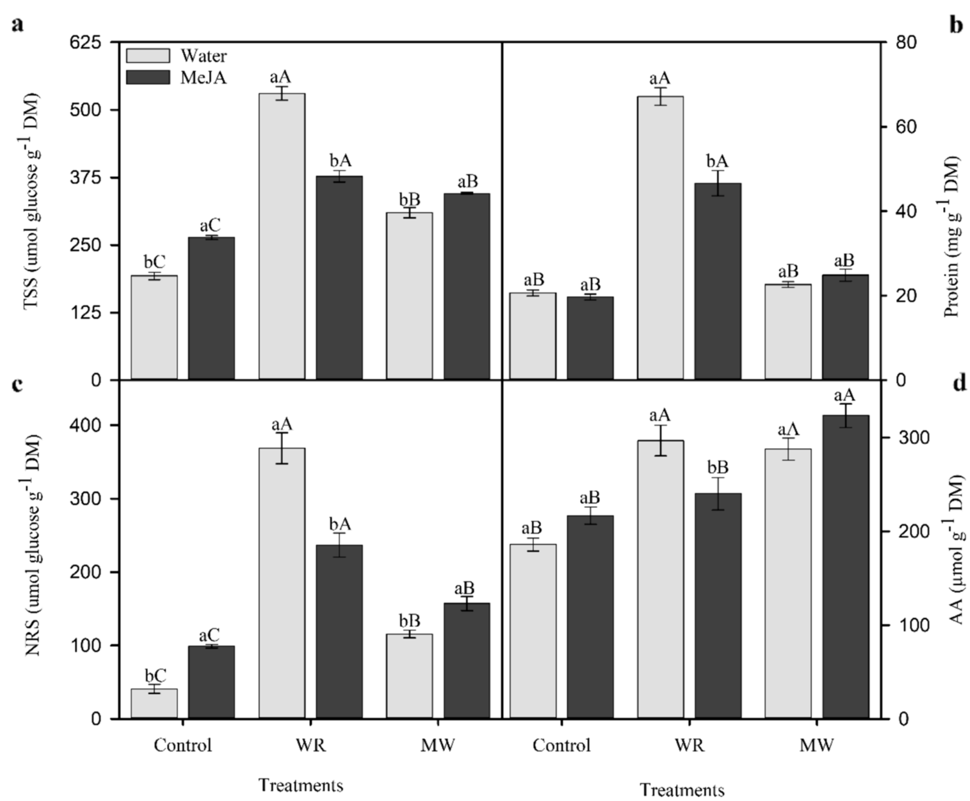


Fig. 3 CAT activity in soybean seedlings from seeds pretreated with MeJA or water subjected to no stress (control), water restriction (WR), and mechanical wounding (MW). Data are means ± standard error ($n = 5$). Lowercase and uppercase letters represent significant differences [ANOVA, Tukey's test ($P < 0.05$)] between seed pretreatments and among growth conditions, respectively. (ptna = protein)

Thus, we can infer that WR negatively influenced the growth variables, causing oxidative stress, regardless of the application of MeJA. This was probably due to the initial seedling stage when the WR was imposed. This finding is corroborated by the H_2O_2 and MDA quantification data. Pretreatment with MeJA promoted lipid peroxidation in relation to water in all treatments, especially in WR. However, MeJA applied exogenously to the seeds may have been the target of lipid peroxidation, as this phytohormone is derived from the oxidation of polyunsaturated fatty acids (Muñoz and Munné-Bosch 2020), which may have contributed to the increase in MDA levels. To better understand the oxidative damage regarding the MeJa application, we investigated the activity of antioxidant enzymes.

Besides CAT activity in control seedlings from seeds treated with MeJA, antioxidant system enzymes were not induced by MeJA in soybean seedlings, which could attenuate the effects of oxidative stress under the stress conditions tested. These results disagree with the increased activity of antioxidant system enzymes in adult plants of maize (Tayyab et al. 2020) and soybean (Mir et al. 2018) subjected to water stress. It was discussed that MeJA also modulates antioxidant system to improve tolerante to biotic stress in plants (Demiwal et al. 2024) and seeds (Kaushik et al. 2024). In this work it was not the strategy induced by MeJA in the soybean seeadlings. However, the increasing activity of CAT mediated by MeJA treatment in control seems to be a promissor mechanism induced by methyl

Fig. 4 Content of TSS, proteins, NRS, and AA in soybean seedlings from seeds pretreated with MeJA or water subjected to no stress (control), water restriction (WR), and mechanical wounding (MW). Data are means ± standard error ($n = 5$). Means followed by the same Lowercase and uppercase letters represent significant differences [ANOVA, Tukey's test ($P < 0.05$)] between seed pretreatments among growth conditions, respectively



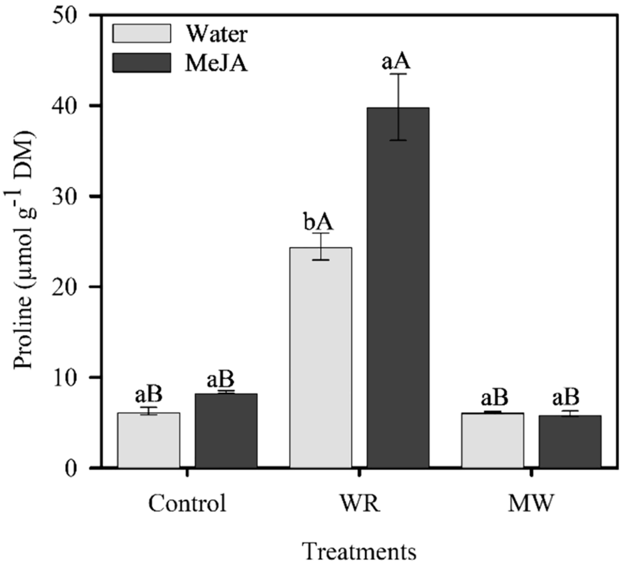


Fig. 5 Proline content in soybean seedlings from seeds pretreated with MeJA or water subjected to no stress (control), water restriction (WR), and mechanical wounding (MW). Data are means ± standard error ($n=5$). Means followed by the same Lowercase and uppercase letters represent significant differences [ANOVA, Tukey's test ($P<0.05$)] between seed pretreatments and among growth conditions, respectively

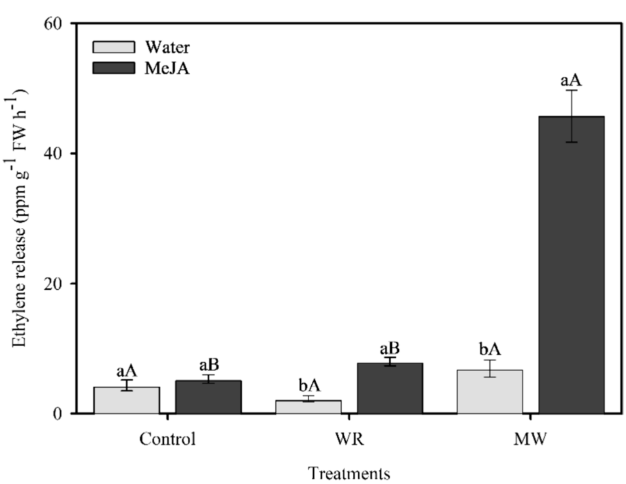


Fig. 6 Ethylene release in soybean seedlings from seeds pretreated with MeJA or water subjected to the no stress (control), water restriction (WR), and mechanical wounding (MW). Data are means ± standard error ($n=5$). Means followed by the same Lowercase and uppercase letters represent significant differences [ANOVA, Tukey's test ($P<0.05$)] between seed pretreatments and among growth conditions, respectively

jasmonate, that can be deeper studied. Therefore, the effects of the stress condition associated with the stage of soybean seedlings could not be attenuated by MeJA application on improving antioxidant enzymes system. It suggests that other physiologic mechanisms could be induced by MeJA.

Regarding the reserves compounds, the application of MeJA was efficient in increasing carbohydrate levels in soybean seedlings under the control and MW conditions. However, under WR, the opposite effect was observed. Other studies have shown that the foliar application of MeJA in adult soybean plants increased carbohydrates to maintain cellular osmotic adjustment under water stress (Mohamed and Latif 2017). The Me-JA-induced carbohydrates homeostasis was also positively affected in rice plants under arsenic stress (Nazir et al. 2023). Seedlings subjected to WR had a significant developmental delay (i.e., cotyledons still present during sampling) (Fig. 1). One possible explanation is that treatment with MeJA may have reduced the mobilization of reserves during seedling establishment, as also observed by Yang et al. (2018) for *Astragalus membranaceus* seedlings, making it not possible to maintain osmotic adjustment when subjected to WR. This would delay the mobilization of reserves.

Besides carbohydrates, proteins levels can better explain the mechanisms mediated by MeJA in soybean seedlings. Proteins are hydrolyzed by proteases to provide amino acids for cell storage, transfer, and osmotic adjustment. Thus, during WR and with the use of MeJA, protein contents probably decreased to protect macromolecules, regulate osmosis, maintain the pH, detoxify, and control free radicals in the cell (Parida et al. 2004). MeJA increased proline in seedlings under WR, corroborating the results observed by Sheteiwy et al. (2018) for rice seedlings. Furthermore, this result is in agreement with other studies and supports the idea that proline accumulation occurs in plants exposed to water stress (Sohag et al. 2020; Javadipour et al. 2021) due to its property of stabilizing subcellular structures, eliminating free radicals, and mitigating cellular redox potential (Zulfiqar and Ashraf 2023).

However, the increase in proline by treatment with MeJA was not sufficient to completely mitigate the effects of water stress in soybean seedlings. The results obtained here are nevertheless promising for future studies and corroborate the assumption that MeJA induces the synthesis of compatible osmolytes, especially proline, as a tolerance mechanism to various stresses (Sheteiwy et al. 2018). Our results suggest that this mechanism can be induced from the early stages of plant development. Therefore, together with carbohydrates, proline can be an important mechanism mediated by MeJA application.

Interestingly, the increase in ethylene release was only observed in seedlings from seeds pretreated with MeJA and subjected to MW, suggesting that this mechanical stimulus induces a synergistic action between MeJA and ethylene to attenuate the stress effects. A molecular crosstalk between jasmonic acid and ethylene to attenuate the wonding effect through reactive oxygen species was recently described in broccoli (Torres-Contreras et al. 2023). Mechanical stimulation is widely used in the scientific community to simulate the attack of herbivorous insects (Waterman et al. 2019; Cunha et al. 2023). According to Waterman et al. (2019), simulated herbivory can be used to complement true herbivory to decipher the mechanisms of plant defense responses. After an attack by herbivores, some secondary metabolites(e.g., phenolic compounds, isoflavonoids, and flavonoids) are produced as a plant defense response (Zaynab et al. 2018; Dillon et al. 2017). However, according to Dillon et al. (2018), isoflavonoids are induced exclusively by ethylene in soybean plants, which indicates that seed pretreatment with 12.5 µM MeJA is promising to attenuate the damage induced by herbivory.

Conclusions

The pretreatment of soybean seeds with MeJA is a promising technology to mitigate the deleterious effects of abiotic stresses. MeJA increased proline content under WR conditions, pointing to an important tolerance mechanism induced by compatible osmolytes. However, a different mechanism may be related to the tolerance to MW, in which MeJA increased ethylene release. This finding corroborates that the biosynthesis of ethylene under biotic stress can be induced by jasmonates in soybean. These different strategies and mechanisms may help in future breeding projects aiming to obtain seedlings more tolerant to biotic and abiotic stress.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-024-03692-2.

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Author contribution statement AOF, EMB and VCR conceptualized the focus article. AOF and VCR prepared the first draft of the manuscript. LVV and GSD performed antioxidant analysis. MEL performed the ethylene assay. All authors contributed with the article corrections. EMB corrected and finalized the manuscript.

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Data availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Conflict of 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.

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