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Exploring the interplay of phytohormones and polyamines in drought-stressed Cress (Lepidium sativum) leaves

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Article

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Abstract

The study investigated prolonged drought stress effects on phytohormone and polyamine levels in cress leaves, elucidating plant responses to harsh environments. The study found that various phytohormones, including 12-oxophytodienoic acid (OPDA), Jasmonic acid (JA), Jasmonyl-Isoleucine (JA-Ile), Abscisic acid (ABA), and salicylic acid (SA), increased during drought stress. ABA rose progressively. Similarly, indole-3-acetic acid (IAA) responded similarly to ABA and SA. Cytokinins, zeatin (ZT) and isopentenyl adenine (iP), played diverse roles in cress leaves' drought tolerance. ZT declined, while iP remained stable. Gibberellic acid (GA_3) levels fell, but brassinolide (BL) increased significantly with drought. The study also explored drought stress's impact on polyamines, which decreased, revealing their sensitivity to severe drought. The research highlighted the complex regulatory mechanisms governing plant adaptation to water scarcity. The interplay between phytohormones (OPDA, JA, JA-Ile, ABA, SA, auxin, cytokinins, GA_3, and BL) and polyamines contributes to plants ability to withstand drought stress and maintain physiological balance under adverse conditions. These findings provide insights into cress leaves dynamic response to drought and enhance our understanding of drought tolerance mechanisms in plants.

Introduction

Plants experience both biotic and abiotic factors, and these elements can exert various influences on the growth, development, and overall productivity of plants. The degree of deviation from optimal growth is contingent upon the category, intensity, duration, and amalgamation of exposure to abiotic stressors (Alkhsabah et al. 2018; AlSammarraie et al. 2020). Drought stands as a prominent abiotic stress factor that exerts detrimental effects on agricultural output and yield (Fahad et al. 2017; Dietz et al. 2021). In arid and semiarid regions, drought is one of the most significant and widespread stresses that plants experience (Taleb et al. 2023). The multifaceted stress of drought typically alters plants physiological, biochemical, and molecular characteristics. Furthermore, it may have a detrimental impact on plant growth and yield. Typically, plants cope with drought stress for maintaining cell water homeostasis through physiological, biochemical, and molecular strategies. However, thesis mechanisms depend on the drought intensity and duration of exposure (Wahab et al. 2022). During drought circumstances, plants can acclimate by modulations at different levels including morphological, metabolic, and transcriptional changes (Seleiman et al. 2021). Increased accumulation of phytohormones and polyamines and sophisticated crosstalk among them are one of the most striking metabolic indicators in plants under drought stress (Nandy et al. 2022; Napieraj et al. 2023). In particular, under stress, phytohormone-mediated accumulation of osmolytes and antioxidants. In addition to being key components of carbon and nitrogen metabolism, enzymes also play a significant role in regulating the overall physiological health of plants (Sharma et al. 2019; Wahab et al. 2022). Hence, ABAhas been shown to trigger drought stress responses where ABA is a key signal molecule that promotes root growth, elicits stomatal closure, and regulates gene expression involved in water transport and osmotic adjustment (Muhammad Aslam et al. 2022). Cytokinin synthesis induced by drought expands plants

tolerance to water deficit by improving root-to-shoot signaling and enhancing photosynthetic efficiency under drought stress (Hai et al. 2020; Gujjar et al. 2021). Ethylene and jasmonic acid serve as essential signaling molecules in plants, orchestrating various aspects of growth, development, and defense responses against both biotic and abiotic stresses (Pérez-Llorca et al. 2023). These plant hormones exhibit intricate crosstalk and synergy, enabling plants to finely regulate their responses to changing environmental conditions.

In addition, polyamines, such as putrescine, spermidine, and spermine, are organic compounds of small size that serve crucial functions in the control of plant growth, development, and their ability to withstand abiotic stresses like drought. Polyamines exhibit regulatory functions in terms of stomatal behavior, elevation of photosynthetic efficiency, and safeguarding cellular constituents against oxidative harm when plants experience drought-induced stress conditions (Chen et al. 2020; Shao et al. 2022).

This work aimed to unravel phytohormones and polyamines levels that induce the activation of the acclimation response following a drought stress. To understand the dynamic nature of acclimation, the study focused on the involvement of the possible signaling role of phytohormones, polyamines and crosstalk between polyamines and phytohormones during drought stress.

Materials and Methods

Plant specimens and cultivation circumstances

L. sativum seeds (Vilmorin, France) were obtained from local distributer and germinated in Molecular Biology Research Laboratory/ Department of Biological Sciences/ Mutah University, Jordan (AlSammarraie et al. 2020). Cress plants were grown under controlled conditions with 14 hours of light at 150 µmol photon m^{-2}s^{-1}, alternating temperatures (21°C/10h dark, 20°C), and 55–60% humidity. They were potted in a substrate mix of peat moss, perlite, and vermiculite (2:1:1 ratio). After 6 weeks, some plants exposed to varying durations of drought stress, while others remained in controlled conditions. Plant samples were analyzed to study phytohormone and polyamine fluctuations in response to drought duration. The plant collection and use was in accordance with all the relevant guidelines.

Drought application

Drought stress was imposed on plants grown under controlled conditions. Initially, plants were irrigated with tap water three times a week for six weeks. Subsequently, plants experienced varying water deficit periods (3, 6, or 9 days) by withholding water. In contrast, control plants were consistently irrigated three times a week for 3, 6, or 9 days. Following each drought exposure, leaf samples were collected from the plants in a growth chamber and stored at -80°C for further analysis (Alkhsabah et al. 2018).

Phytohormones and polyamines analysis

To analyze phytohormones (OPDA, JA, JA-Ile, ABA, SA, IAA, ZT, GA3, BL, and iP) and polyamines (Putrescine, Cadaverine, Agmatine, Spermine, Spermidine), 50 mg of plant tissue underwent extraction.

The extraction solution was a mixture of methanol (86%) and acetonitrile (10%). After homogenization and vortexing for 3 minutes, the sample was centrifuged at 4000 rpm for 2 minutes. The resulting supernatant was evaporated using N-gas, and the residue was dissolved in 500 µl of HPLC mobile phase.

HPLC separation utilized solvents A (90% acetic acid-water) and B (10% methanol) in a gradient isocratic method. The gradient included 100% B for 20 minutes, followed by 100% B for 15 minutes, and re-equilibration. An Agilent Zorbax Eclipse XDB-C18 column (4.6*125 cm*5µm) at 35°C and an injection volume of 10 µL were used.

The eluent underwent analysis using a Shimadzu LC-MS 8030 with an electrospray ion mass spectrometer (ESI-MS) in positive ion mode. The mass range scanned was 50 to 1200 m/z, with an ESI voltage of 125V and SKIMMER set to 65V. High-purity nitrogen (99.999%) was the drying gas at a flow rate of 200 µL min^{-1}. The fragment nebulizer was at 45 psi, and the capillary temperature was 350°C. Additionally, a 0.1% formic acid solution served as a blank (Cao et al. 2016).

Statistical analysis

The results of the study were presented as the mean ± SD. To determine the statistical significance, a two-way analysis of variance (ANOVA) was performed. If a significant difference was detected, a Tukey post hoc test was conducted to identify which means were significantly different from each other. In the results, different letters were used to denote statistical significance at a significance level of $P < 0.05$.

Results

Leaf phytohormone content

Throughout a prolonged 9-day period of drought stress exposure, cress leaves experienced significant alterations in the content of various phytohormones, highlighting the dynamic response to these conditions. Notably, the content of OPDA in the leaves displayed a remarkable threefold increase after 9-day drought stress compared to the control (Fig. 1A). Similarly, the level of JA exhibited a significant rise during drought stress exposure, with respective fold increases of 1.4, 1.5, and 3.5 after 3, 6, and 9 days of exposure (Fig. 1B). Correspondingly, the levels of JA-Ile in the leaves showed a significant increase during the exposure to drought stress. After 3, 6, and 9 days of exposure, the content of JA-Ile in the leaves increased by approximately 2.3-fold, 1.9-fold, and 1.8-fold, respectively (Fig. 1C). Similarly, the impact of drought stress on the content of ABA in the leaves was observed after 3, 6, and 9 days of exposure. The levels of ABA were measured to be 1.6, 1.6, and 1.8, respectively, compared to the control (Fig. 1D).

During periods of drought stress, the leaves exhibited a substantial increase in the presence of SA. Notably, this augmentation in SA content was pronounced, with fold enhancements of 1.8, 2.8, and 2.7 after 3, 6, and 9 days of exposure to the drought conditions, respectively(Fig. 1E). The fluctuation of IAA content in the leaves underwent dynamic changes during the period of drought exposure, exhibiting fold

increments of 1.7-fold after 3 days, 2.2-fold after 6 days, and 2.3-fold after 9 days of exposure, respectively, when compared to the control conditions (Fig. 1F). In contrast, during drought stress, the levels of ZT, demonstrated a significant reduction in comparison to control conditions. This reduction was particularly pronounced, with the ZT content reaching 0.6, 0.7, and 0.4-fold of the original levels of 3 days, 6 days, or 9 days of exposure to the stress, respectively (Fig. 1G).

The study also aimed to examine the impact of drought over time on the leaf content of GA_3, a plant hormone. It was observed that the GA_3 leaf content underwent significant changes during drought stress. Specifically, compared to the control (Fig. 1H), over the course of 3 days, the GA_3 content increased by 3.4-fold, which further increased to 2.4-fold after 6 days and 0.9-fold after 9 days.

Moreover, the BL phytohormone, known for its ability to enhance drought tolerance, showed a notable increase in leaf content during drought stress. Following 3, 6, and 9 days of exposure, the BL content increased by 2.3-, 3.5-, and 2.8-fold, respectively (Fig. 1I). Additionally, the iP cress leaf content demonstrated fold increases of 2.4, 3.2, and 1.9 after 3, 6, and 9 days of exposure, respectively, compared to the control (Fig. 1J).

These findings emphasize the significant alterations in the content of various phytohormones in cress leaves during prolonged drought stress. The changes in hormone levels indicate the plants dynamic response and adaptation to unfavorable environmental circumstances.

Leaf polyamines content

Drought stress significantly reduced the leaves levels of all polyamines relative to those of controls (Fig. 2). The adverse effect on polyamines leaves content was varied with drought duration exposure. Specifically, the content of putrescine in leaves showed a slight reduction, reaching 0.9-fold of the control levels across all durations of drought exposure (Fig. 2A). Cadaverine content in leaves decreased to 0.9-fold after 3 days of drought exposure, remaining constant thereafter (Fig. 2B). Agmatine content decreased progressively: 0.8-fold at 3 days, 0.9-fold at 6 days, and 0.3-fold at 9 days (Fig. 2C). Spermidine content decreased: 0.6-fold (3 days), 0.6-fold (6 days), and 0.5-fold (9 days) (Fig. 2D). Spermine content reduced: 0.6-fold (3 days) and 0.4-fold (6 days) compared to controls (Fig. 2E).

Discussion

The results obtained from the study on the alterations in phytohormones and polyamines levels in cress leaves during prolonged drought stress have significant implications for understanding the plants response to adverse environmental conditions. Firstly, the changes in phytohormones levels during drought stress highlight the dynamic response of cress leaves. The content of OPDA displayed a remarkable increase after 9 days of drought stress. This finding aligns with earlier research demonstrating the role of OPDA in plant responses to drought stress (Savchenko et al. 2014). Furthermore, OPDA translocates from the chloroplast to the peroxisome where it undergoes reduction to form JA. This translocation and reduction process has been established as a key step in JA biosynthesis

(Kienow et al. 2008). Additionally, OPDA functions as a signaling molecule, influencing gene expression independently or in conjunction with the canonical JA signaling framework (Ribot et al. 2008). The results of the study propose that under drought conditions, there is a potential augmentation in the movement of OPDA and/or an induction of subsequent peroxisomal stages in the biosynthesis of JA. The support of this suggests that the leaves content of both OPDA and JA are raised in a time-dependent manner to drought stress (Fig. 1A and B). This indicates that OPDA is responsive to drought stress and its concentration increases significantly over time of drought exposure and that JA contributes to the cress leaves response to water scarcity and its concentration increases as the stress duration extends. OPDA and JA late rising of drought exposure can stimulate stomatal closure to improve drought resistance (Savchenko et al. 2014). Moreover, JA plays a pivotal role in plant responses to drought stress that when plants experience water scarcity, activate several defense mechanisms to safeguard themselves. These processes include the expression of stress-related genes, accumulating specific metabolites, fostering the synthesis of osmoprotectants and antioxidants (Ali and Baek 2020; Iqbal et al. 2022), orchestration of plant water balance through coordinating processes such as root water uptake, transpiration regulation, and water loss mitigation (Per et al. 2018; Iqbal et al. 2022).

The interplay between JA and other phytohormones, particularly ABA(Fig. 1D), is essential for coordinating the complex plant responses to drought stress (Salvi et al. 2021; Wang et al. 2021). The JA signaling pathways play a crucial role in mitigating the impact of drought stress and enhancing plants ability to tolerate drought conditions. These pathways achieve this by promoting the production of organic osmoprotectants, which help maintain cellular water balance, and by increasing the activity of antioxidative enzymes that counteract oxidative stress caused by drought (Ali and Baek 2020; Iqbal et al. 2022). The modulation of these pathways provides insights into potential strategies for improving crop resilience against drought-induced challenges.

OPDA, JA, and JA-Ile act as signaling molecules to stress (Alsharafa et al. 2014). JA-Ile the active form of JA, is a jasmonate conjugate. It acts as a critical regulator in the jasmonate signaling pathway (Yi et al. 2023). JA-Ile plays a pivotal role in regulating the expression of genes specifically associated with JA-Ile signaling (Arnold et al. 2016). It serves as a mobile signal and can be transported from leaves to the roots to coordinate plant growth (Schulze et al. 2019). This work results showed that under drought stress conditions, in cress leaf tissues, during the progressive drought stress, there is an early increase in JA-Ile levels (Fig. 1C). Notably, this increase in JA-Ile levels occurs concurrently with low levels of ABA presence (Fig. 1D). The basal level and magnitude change under drought stress of OPDA, JA, and JA-Ile indicate that the production of JA from OPDA is not the limiting factor for JA-Ile synthesis (Wasternack and Song 2017). Drought stress influences the levels of OPDA, JA, and JA-Ile, suggesting their involvement in plant responses to abiotic stresses (Yang et al. 2019). Early JA-Ile accumulation of drought-stressed plants may affect both the aperture and density of stomata, which together can affect transpirational water loss and modulate plant scavenging of elevating H_2O_2 content as mentioned previously (Mahmud et al. 2021).

During periods of drought stress, plants detect the water shortage and respond by synthesizing ABA (Muhammad Aslam et al. 2022). Our study indicates that the levels of endogenous ABA increase early on and continue to rise as the drought stress progresses (Fig. 1D). ABA acts as a signaling molecule that helps plants perceive the drought stress and initiate appropriate responses to cope with limited water availability. Consequently, ABA regulates various adaptive strategies in plants, including the closure of stomata, modulation of the root system, activation of gene expression at the transcriptional and post-transcriptional levels, and modulation of signal transduction pathways (Chen et al. 2020; Korek and Marzec 2023).

SA plays a vital role in plant defense mechanisms and their ability to cope with various stresses, such as drought. It functions as a signaling molecule and is involved in regulating plant growth, development, and stress responses (Pandey and Chakraborty 2015). When plants are exposed to drought stress, SA levels in their leaves increase. This response is characterized by an early rise in endogenous salicylic acid levels, which continue to increase as the drought stress progresses (Fig. 1E). SA may contribute to plant adaptation to drought stress through several processes. Firstly, it can induce stomatal closure, reducing water loss through transpiration. Additionally, SA enhances plants antioxidant defense system, which helps mitigate oxidative damage caused by drought stress (Seleiman et al. 2021). It also influences the expression of stress-responsive genes, including those involved in drought tolerance, and stimulates the accumulation of compatible solutes (Sidhu and Bali 2022). Furthermore, SA interacts with other plant hormones to facilitate plant drought tolerance (Pandey and Chakraborty 2015; Singh et al. 2019).

The IAA exhibited similar changes in response to drought stress, similar to the hormones ABA and SA (Fig. 1F). This finding suggests that IAA, like ABA and SA, is responsive to drought stress (Salehin et al. 2019; Zhang et al. 2020).

Cytokinins have a diverse and multifaceted role in enhancing drought tolerance in plant leaves by regulating various physiological and molecular processes (Hai et al. 2020). They contribute to maintaining cell turgor, improving water-use efficiency, and facilitating osmotic adjustments. Additionally, cytokinins influence the expression of genes involved in drought responses. Moreover, cytokinins also exhibit crosstalk with other phytohormones, forming a coordinated network that aids in plant stress tolerance (Verma et al. 2016). ZT and iP are recognized as the primary active isoprenoid cytokinins (Kudo et al. 2010). When cress leaves are subjected to drought stress, the content of ZT declines rapidly, especially with prolonged treatment. Conversely, the content of iP increases early and remains relatively stable with long-term exposure. These findings emphasize the distinct roles of ZT and iP in drought tolerance, which have been observed previously (Kudo et al. 2010; Hallmark and Rashotte 2020). This can be explained by the significant role of iP in delaying leaf senescence. iP upregulates photosynthetic and translation machinery while downregulating catabolic processes commonly associated with senescence and cell death. However, a sudden decrease in ZT at the onset of stress might contribute to stomata closure (Hallmark and Rashotte 2020).

Contrarily, GA_3 displayed similar responses to drought stress as JA-Ile (Fig. 1H), but with a more pronounced effect as the duration of drought stress exposure increased. The findings of this study suggested that drought stress reduced the levels of GA_3 in cress leaves and this accelerated stomatal closure which was also detected previously in wheat and tomato leaves by promoting ABA-induced stomatal closure at the early stages of sensed drought severity (Bano and Yasmeen 2010; Shohat et al. 2021).

Under early drought stress exposure, the levels of biologically active BL, the active form of brassinosteroids, demonstrated a significant increase and continued to rise with prolonged stress duration. This response is mediated through a network involving the production of SA and ABA (Fig. 1D, E, and I), indicating that BL may regulate physiological reactions to drought stress conditions. Research suggests that BL plays a critical role in alleviating drought stress by modulating the content of ABA, which confers protection through regulating stomatal conductance in synergy with SA, thereby minimizing water loss. These mechanisms contribute to maintaining plant water balance and enhancing drought tolerance (Chen et al. 2020; Muhammad Aslam et al. 2022; Boero et al. 2023).

The research focused on investigating the impact of drought stress on the levels of endogenous polyamines in cress leaves. It was found that drought stress has a negative effect on the levels of polyamines in cress leaves. The extent of reduction varied depending on the specific polyamine and the duration of exposure to drought conditions. The study suggests that the decline in polyamines indicates the sensitivity of the polyamine biosynthetic pathway to prolonged and severe drought stress (Zhang and Huang 2013). This finding highlights the importance of endogenous polyamine accumulation as a mechanism for plants to cope with water scarcity and enhance their tolerance to drought conditions (Napieraj et al. 2023) Furthermore, there is a connection between polyamine metabolism and ABA. ABA acts as an upstream signal for the induction of polyamine catabolism and regulates genes expression involved in polyamine production and catabolism in response to drought stress (Chen et al. 2020; Skowron and Trojak 2021). This relationship aids in the regulation of stomatal closure and promotes the biosynthesis of osmoprotectants as a response to drought stress.

Conclusion

The study demonstrates that alterations in phytohormones and polyamines levels occur in cress leaves during prolonged drought stress. These changes reflect the dynamic response of the plant and have significant implications for understanding the plant's adaptation to adverse environmental conditions. The interplay between different phytohormones, such as JA, ABA, SA, IAA, and cytokinins, plays a crucial role in coordinating the plant's response to drought stress and enhancing its drought tolerance. The findings of this study contribute to our understanding of the complex regulatory mechanisms involved in plant adaptation to water scarcity.

Declarations

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Author contribution

The project was designed by KA, who also conducted the research, supervised the results, and wrote the article.

Competing interests

The author(s) declare no competing interests.

Data availability

Correspondence and requests for materials should be addressed to K. A

Ethical approval Not applicable.

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Figures

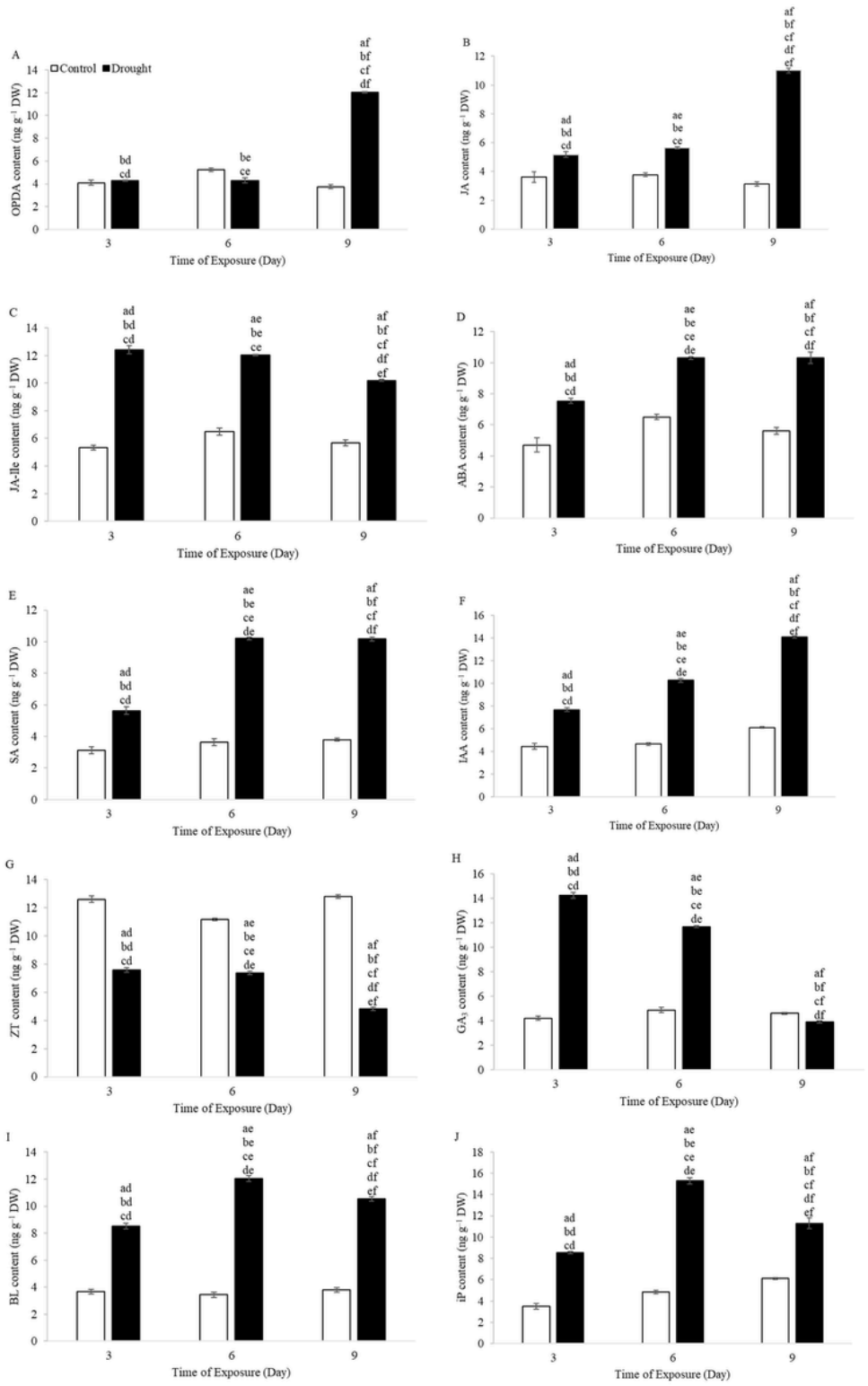


Figure 1

Levels of phytohormones contents in *L. sativum* under drought stress, as measured by LC-MS/MS. A- OPDA, B- JA, C- JA-Ile, D- ABA, E- SA, F- IAA, G- ZT, H- GA_3, I- BL, and J- iP were estimated in various drought stress time points in comparison to the control phytohormones contents. Data showing replicate means ± SD. Significant differences are indicated by different letters (Tukeys test; $P \leq 0.05$).

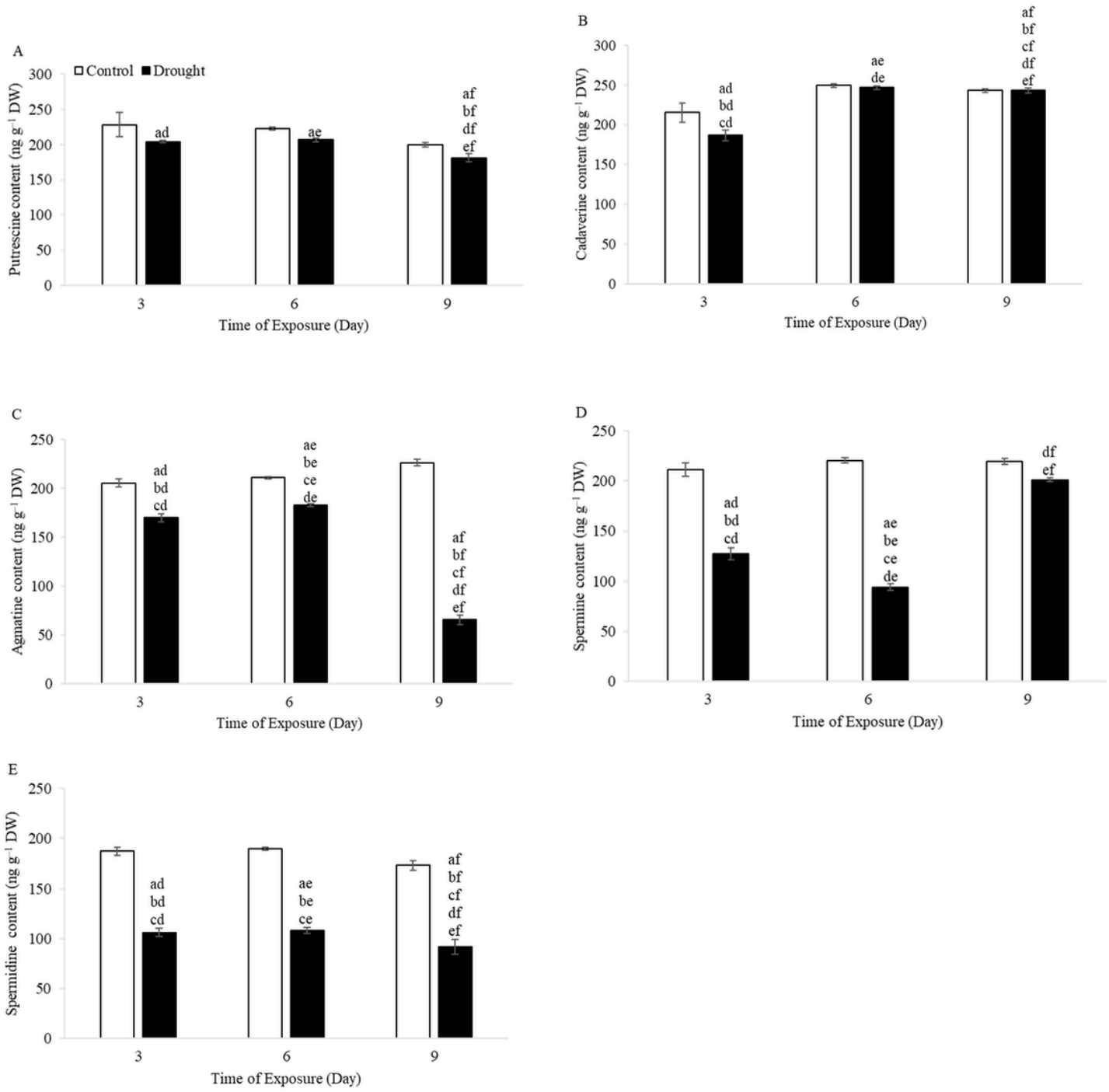


Figure 2

Levels of polyamine contents in *L. sativum* leaves under drought stress as quantified by LC-MS/MS. A- Putrescine. B- Cadaverine. C- Agmatine. D- Spermine. E- Spermidine were estimated in various drought stress time points in comparison to the control polyamine contents. Data showing replicate means ± SD. Significant differences are indicated by different letters (Tukeys test; $P \leq 0.05$).