

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

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Abstract

The study investigated prolonged drought stress effects on phytohormone and polyamine levels in cress (*Lepidium sativum* L.) leaves, elucidating plant responses to harsh environments. Phytohormones, 12-oxophytodienoic acid (OPDA), Jasmonic acid (JA), Jasmonyl-Isoleucine (JA-Ile), Abscisic acid (ABA), and salicylic acid (SA) increased, especially ABA that rose consistently. Indole-3-acetic acid (IAA) responded akin to ABA and SA. Cytokinins such as zeatin (ZT) and isopentenyl adenine (iP) diversified drought tolerance, ZT fell sharply, and iP remained stable. Gibberellic acid (GA₃) declined, while brassinolide (BL) surged with prolonged drought. Polyamines reduced, indicating sensitiv-

ity to severe drought. Findings uncovered intricate regulatory mechanisms for plant acclimation to water scarcity. Interaction of OPDA, JA, JA-Ile, ABA, SA, IAA, ZT, iP, GA₃, BL, and polyamines contributes to drought coping and physiological balance. Insights into cress leaf response and drought tolerance mechanisms contribute significantly to plant drought acclimation understanding.

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.^{1,2} Drought stands as a prominent abiotic stress factor that exerts detrimental effects on agricultural output and yield.³ In arid and semiarid regions, drought is one of the most significant and widespread stresses that plants experience.⁴ 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 by maintaining cell water homeostasis through physiological, biochemical, and molecular strategies. However, these mechanisms depend on the drought intensity and duration of exposure.⁵ During drought circumstances, plants can acclimate by modulations at different levels including morphological, metabolic, and transcriptional changes.⁶ Increased accumulation of phytohormones and polyamines and sophisticated crosstalk among them are some of the most striking metabolic indicators in plants under drought stress.^{7,8} In particular, under stress, phytohormone-mediated accumulation of osmolytes and antioxidants increases. This accumulation, in turn, acts as a protective mechanism for the cell's machinery, guarding it against a range of environmental stressors. In addition, the modulation of enzymes related to carbon and nitrogen metabolism plays a crucial role in governing physiological fitness.^{5,9} Hence, abscisic acid (ABA) has 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.¹⁰ Cytokinin synthesis induced by drought expands plants' tolerance to water deficit by improving root-to-shoot signaling and enhancing photosynthetic efficiency under drought stress.^{11,12} 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.¹³ These plant hormones exhibit intricate crosstalk and synergy, enabling plants to finely regulate their responses to changing environmental conditions.

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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.^{14,15}

Lepidium sativum, commonly known as garden cress, belongs to the Brassicaceae family. Garden cress has been utilized for various culinary and medicinal purposes due to its rich nutrient content and potential health benefits. The widespread distribution and adaptability of *L. sativum* make it an interesting subject for botanical and agricultural studies.²

This work aimed to unravel phytohormones and polyamine levels that induce the activation of the acclimation response following 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 of *L. sativum* leaves.

Materials and Methods

Plant specimens and cultivation conditions

Cress seeds that had undergone germination were cultivated in a controlled environment, featuring 55-60% humidity, and 14 h of light exposure with an intensity of 150 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at 20°C. Additionally, they were subjected to darkness at 21°C for 10 h. They were potted in a substrate mix of peat moss, perlite, and vermiculite (2:1:1 ratio). After 6 weeks, plant seedlings were exposed to varying durations of drought stress, while others remained in controlled conditions. The analyses and assays were meticulously performed on plant leaves, ensuring a robust and reliable dataset. Triplicate testing was carried out for each of the three independent biological samples. Plant samples were analyzed to study phytohormone and polyamine fluctuations in response to drought duration.

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.¹

Phytohormones and polyamines analysis

To analyze phytohormones 12-oxophytodienoic acid (OPDA), Jasmonic acid (JA), Jasmonyl-Isoleucine (JA-Ile), Abscisic acid (ABA), salicylic acid (SA), Indole-3-acetic acid (IAA), zeatin (ZT), Gibberellic acid (GA_3), Brassinolide (BL), and isopentenyl adenine (iP), and polyamines (Putrescine, Cadaverine, Agmatine, Spermine, Spermidine), 50 mg of leaves 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 x 125 mm with a particle size of 5 μm was employed at a temperature of 35°C, and the sample injection volume was set at 10 μL .

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 $\mu\text{L min}^{-1}$. 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.¹⁶

The identification and quantification of phytohormones and polyamines were determined by comparing the retention time of the prepared standard phytohormones and polyamine solutions with the retention time from the collected data for the samples after each run.

Statistical analysis

The study results were expressed as the mean \pm standard deviation (SD) (n=3) of three independent biological samples. Subsequently, a two-way analysis of variance (ANOVA) was executed, followed by a Tukey post hoc test to pinpoint significant differences among means. This analysis compared the means of various durations of drought stress with their respective controls. Notably, distinct letters were employed in the results to indicate statistical significance, adhering to a significance level of $P \leq 0.05$.

Results

Leaf phytohormones 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 (Figure 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 (Figure 1B). Correspondingly, the levels of JA-Ile in the leaves showed a significant increase during 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 (Figure 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 (Figure 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 (Figure 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 (Figure 1F).

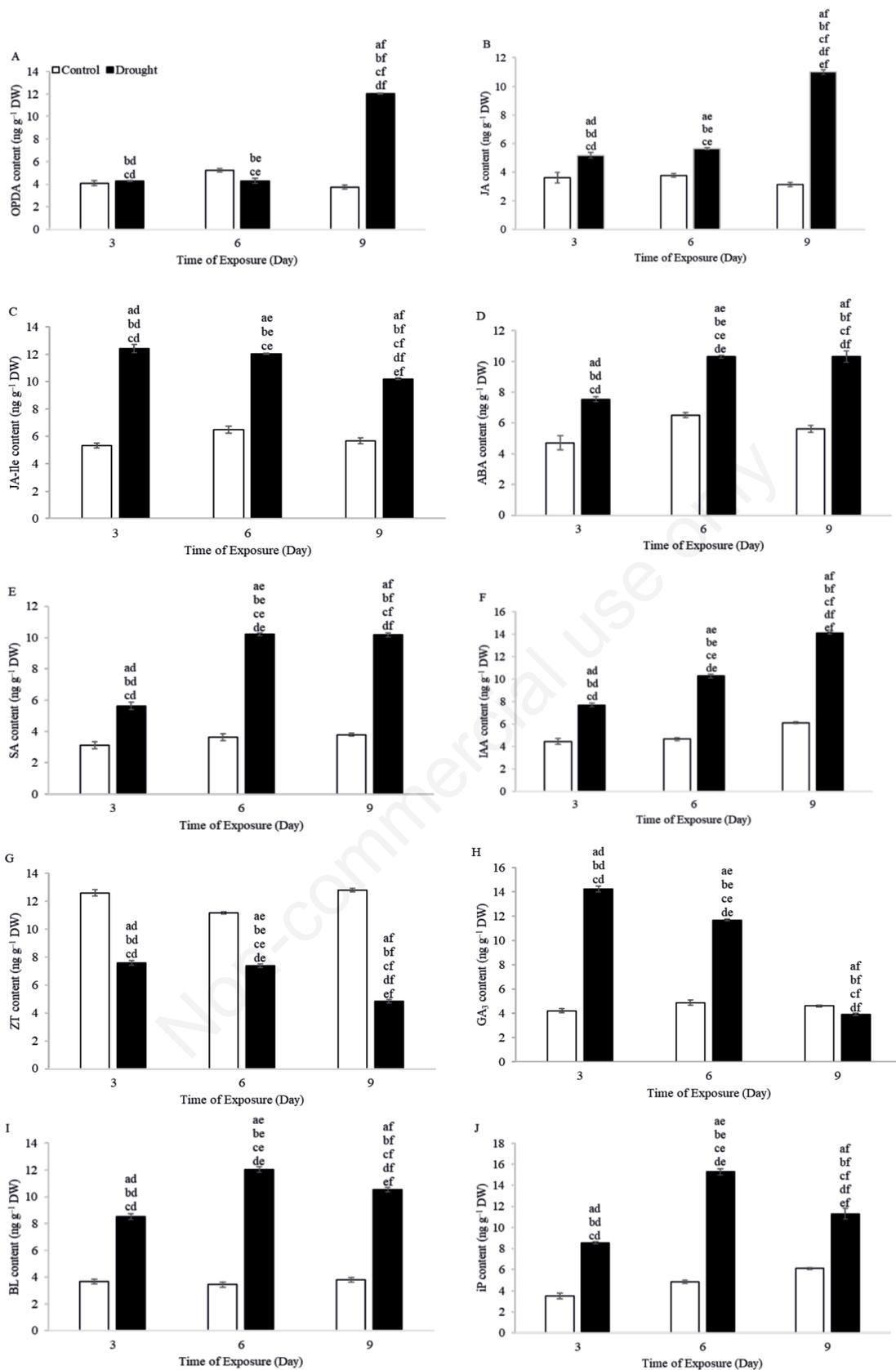


Figure 1. Levels of phytohormone contents in *L. sativum* under drought stress, as measured by LC-MS/MS. A- OPDA, B- JA, C- JA-Ile, D- ABA, E- SA, F- IAA, G- ZT, H- GA₃, I- BL, and J- iP were estimated in various drought stress time points (black histograms 3, 6 and 9 days) in comparison to the control phytohormones contents (white histograms). Data showing replicate means±SD. Significant differences are indicated by different letters (Tukey's test; $P \leq 0.05$).

In contrast, during drought stress, the levels of zeatin demonstrated a significant reduction in comparison to control conditions. This reduction was particularly pronounced, with the zeatin 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 (Figure 1G).

The study also aimed to examine the impact of drought overtime on the leaf content of GA₃, a plant hormone. It was observed that the GA₃ leaf content underwent significant changes during drought stress. Specifically, compared to the control (Figure 1H), over 3 days, the GA₃ 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 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 (Figure 1I). Additionally, the IP cross 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 (Figure 1J).

Leaf polyamines content

Drought stress significantly reduced the levels of all polyamines in leaves in comparison with those of controls (Figure 2). The adverse effect on leaves' polyamine content 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 (Figure 2A). Cadaverine content in leaves decreased to 0.9-fold after 3 days of drought exposure, remaining constant after that (Figure 2B).

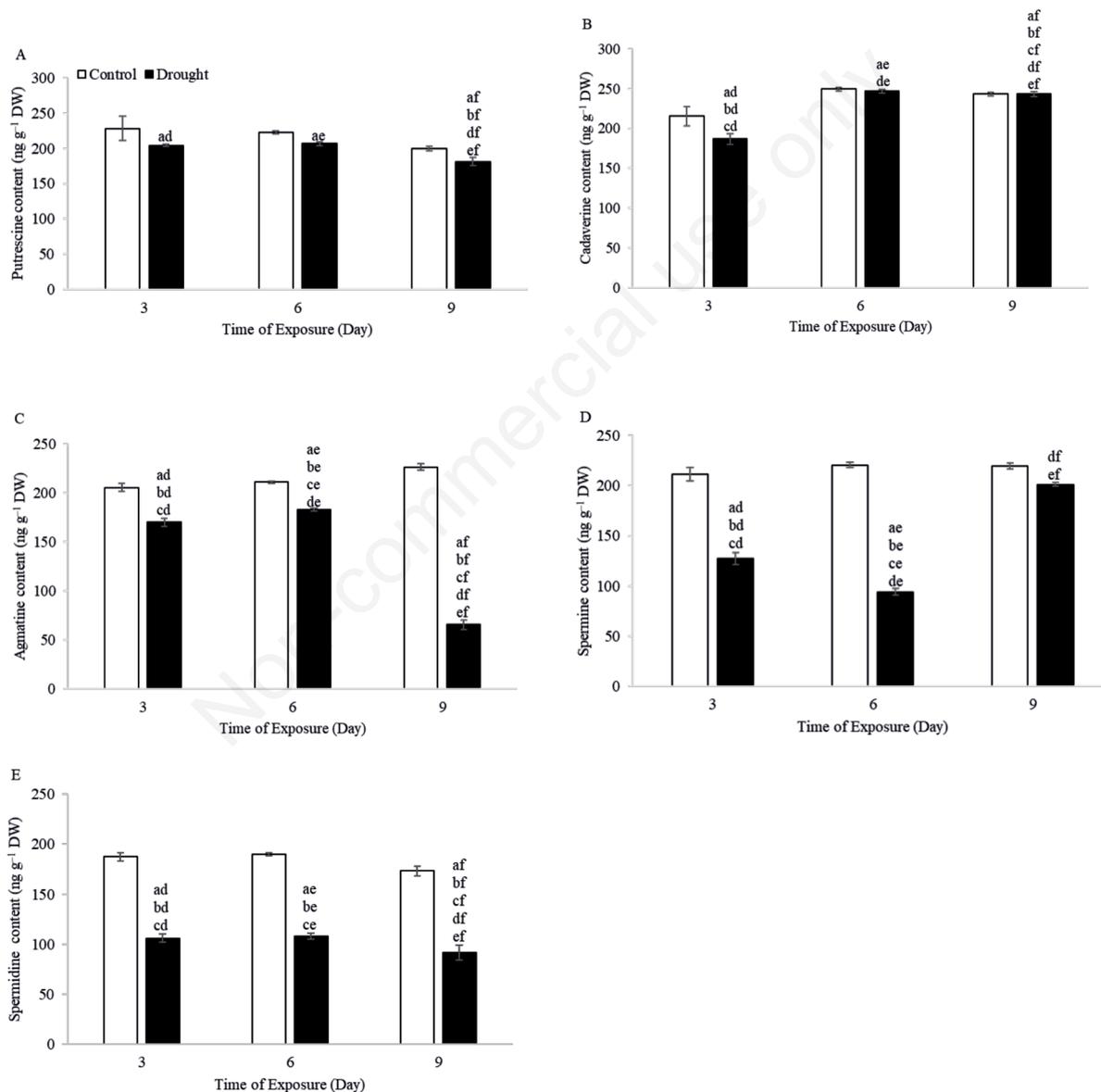


Figure 2. Levels of polyamine contents in *L. sativum* leaves under drought stress as quantified by LC-MS/MS. A- Putrescine. B- Cadaverine. C- Agmatine. D- Spermine. E- Spermidine were estimated in various drought stress time points (black histograms 3, 6, and 9 days) in comparison to the control polyamine contents (white histograms). Data showing replicate means±SD. Significant differences are indicated by different letters (Tukey's test; $P \leq 0.05$).

Agmatine content decreased progressively: 0.8-fold at 3 days, 0.9-fold at 6 days, and 0.3-fold at 9 days (Figure 2C). Spermidine content decreased: 0.6-fold (3 days), 0.6-fold (6 days), and 0.5-fold (9 days) (Figure 2D). Spermine content reduced: 0.6-fold (3 days) and 0.4-fold (6 days) compared to controls (Figure 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 plant's response to adverse environmental conditions. Firstly, the changes in phytohormone 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.¹⁷ 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.¹⁸ Additionally, OPDA functions as a signaling molecule, influencing gene expression independently or in conjunction with the canonical JA signaling framework.¹⁹ 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 leaf content of both OPDA and JA is raised in a time-dependent manner to drought stress (Figure 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 following drought exposure can stimulate stomatal closure to improve drought resistance.¹⁷ Moreover, JA plays a pivotal role in plant responses to drought stress; when plants experience water scarcity, JA activates 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,^{20,21} orchestration of plant water balance through coordinating processes such as root water uptake, transpiration regulation, and water loss mitigation.^{21,22}

The interplay between JA and other phytohormones, particularly ABA (Figure 1D), is essential for coordinating the complex plant responses to drought stress.^{23,24} 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 of cellular water balance, and by increasing the activity of antioxidant enzymes that counteract oxidative stress caused by drought.^{20,21} 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.²⁵ JA-Ile the active form of JA, is a jasmonate conjugate. It acts as a critical regulator in the jasmonate signaling pathway.²⁶ JA-Ile plays a pivotal role in regulating the expression of genes specifically associated with JA-Ile signaling.²⁷ It serves as a mobile signal and can be transported from leaves to the roots to coordinate plant growth.²⁸ This work results showed that under drought stress conditions, in cress leaf tissues, during progressive drought stress, there is an early increase in JA-Ile levels (Figure 1C). Notably, this increase in JA-Ile levels occurs concurrently with low levels of

ABA presence (Figure 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.²⁹ Drought stress influences the levels of OPDA, JA, and JA-Ile, suggesting their involvement in plant responses to abiotic stresses.³⁰ 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 H₂O₂ content as previously mentioned.³¹

During periods of drought stress, plants detect the water shortage and respond by synthesizing ABA.¹⁰ Our study indicates that the levels of endogenous ABA increase early on and continue to rise as the drought stress progresses (Figure 1D). ABA acts as a signaling molecule that helps plants perceive 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.¹⁴

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.³² When plants are exposed to drought stress, SA levels in their leaves increase. This response is characterized by an early rise in endogenous SA levels, which continue to increase as the drought stress progresses (Figure 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.⁶ It also influences the expression of stress-responsive genes, including those involved in drought tolerance, and stimulates the accumulation of compatible solutes.³³ Furthermore, SA interacts with other plant hormones to facilitate plant drought tolerance.^{32,34}

IAA exhibited similar changes in response to drought stress, similar to the hormones ABA and SA (Figure 1F). This finding suggests that IAA, like ABA and SA, is responsive to drought stress.³⁵

Cytokinins have a diverse and multifaceted role in enhancing drought tolerance in plant leaves by regulating various physiological and molecular processes.¹¹ 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.³⁶ ZT and iP are recognized as the primary active isoprenoid cytokinins. 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.³⁷ 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.³⁷

Contrarily, GA₃ displayed similar responses to drought stress as JA-Ile (Figure 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 GA₃ 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.³⁸

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 (Figure 1 D, 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.^{10,14}

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.³⁹ 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.⁸ Furthermore, there is a connection between polyamine metabolism and ABA. ABA acts as an upstream signal for the induction of polyamine catabolism and regulates gene expression involved in polyamine production and catabolism in response to drought stress.^{14,40} This relationship aids in the regulation of stomatal closure and promotes the biosynthesis of osmoprotectants as a response to drought stress.

Conclusions

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 hold significant implications for understanding the plant's acclimation 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 comprehension of the complex regulatory mechanisms involved in plant acclimation to water scarcity.

References

- Alkhsabah IA, Alsharafa KY, Kalaji HM. Effects of abiotic factors on internal homeostasis of *Mentha spicata* leaves. *Appl Ecol Env Res* 2018;16:2537-64.
- Al-Sammarraie ON, Alsharafa KY, Al-Limoun MO, et al. Effect of various abiotic stressors on some biochemical indices of *Lepidium sativum* plants. *Sci Rep* 2020;10:21131.
- Dietz KJ, Zörb C, Geilfus CM. Drought and crop yield. *Plant Biol (Stuttg)* 2021;23:881-93.
- Taleb MH, Majidi MM, Pirnajmedin F, Maibody SAMM. Plant functional trait responses to cope with drought in seven cool-season grasses. *Sci Rep* 2023;13:5285
- Wahab A, Abdi G, Saleem MH, et al. Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. *Plants* 2022;11:1620.
- Seleiman MF, Al-Suhaibani N, Ali N, et al. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* 2021;10:259.
- Nandy S, Mandal S, Gupta SK, et al. Role of polyamines in molecular regulation and cross-talks against drought tolerance in plants. *J Plant Growth Regul* 2022;1-17.
- Napieraj N, Janicka M, Reda M. Interactions of polyamines and phytohormones in plant response to abiotic stress. *Plants* 2023;12:1159.
- Sharma A, Shahzad B, Kumar V, et al. Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules* 2019;9:285.
- Muhammad Aslam M, Waseem M, Jakada BH, et al. Mechanisms of abscisic acid-mediated drought stress responses in plants. *Int J Mol Sci* 2022;23:1084.
- Hai NN, Chuong NN, Tu NHC, et al. Role and regulation of cytokinins in plant response to drought stress. *Plants* 2020;9:422.
- Gujjar RS, Roytrakul S, Chuekong W, Supaibulwatana K. A synthetic cytokinin influences the accumulation of leaf soluble sugars and sugar transporters, and enhances the drought adaptability in rice. *3 Biotech* 2021;11:1-14.
- Pérez-Llorca M, Pollmann S, Müller M. Ethylene and jasmonates signaling network mediating secondary metabolites under abiotic stress. *Int J Mol Sci* 2023;24:5990.
- Chen K, Li GJ, Bressan RA, et al. Abscisic acid dynamics, signaling, and functions in plants. *J Integr Plant Biol* 2020;62:25-54.
- Shao J, Huang K, Batool M, et al. Versatile roles of polyamines in improving abiotic stress tolerance of plants. *Front Plant Sci* 2022;13:1003155.
- Cao ZY, Sun LH, Mou RX, et al. Profiling of phytohormones and their major metabolites in rice using binary solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry. *J Chromatogr A* 2016;1451:67-74.
- Savchenko T, Kolla VA, Wang CQ, et al. Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. *Plant Physiol* 2014;164:1151-60.
- Kienow L, Schneider K, Bartsch M, et al. Jasmonates meet fatty acids: functional analysis of a new acyl-coenzyme A synthetase family from *Arabidopsis thaliana*. *J Exp Bot* 2008;59:403-19.
- Ribot C, Zimmerli C, Farmer EE, et al. Induction of the *Arabidopsis* PHO1; H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. *Plant Physiol* 2008;147:696-706.
- Ali MS, Baek KH. Jasmonic acid signaling pathway in response to abiotic stresses in plants. *Int J Mol Sci* 2020;21:621.
- Iqbal S, Wang X, Mubeen I, et al. Phytohormones trigger drought tolerance in crop plants: outlook and future perspectives. *Front Plant Sci* 2022;12:3378.
- Per TS, Khan MIR, Anjum NA, et al. Jasmonates in plants under abiotic stresses: Crosstalk with other phytohormones matters. *Environ Exp Bot* 2018;145:104-20.
- Salvi P, Manna M, Kaur H, et al. Phytohormone signaling and crosstalk in regulating drought stress response in plants. *Plant Cell Rep* 2021;40:1305-29.
- Wang X, Li Q, Xie J, et al. Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. *Crop J* 2021;9:120-32.
- Alsharafa K, Vogel MO, Oelze ML, et al. Kinetics of retrograde signalling initiation in the high light response of *Arabidopsis thaliana*. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130424.

26. Yi R, Du R, Wang J, et al. Dioxygenase JID1 mediates the modification of OPDA to regulate jasmonate homeostasis. *Cell Discov* 2023;9:39.
27. Arnold MD, Gruber C, Floková K, et al. The recently identified isoleucine conjugate of cis-12-oxo-phytodienoic acid is partially active in cis-12-oxo-phytodienoic acid-specific gene expression of *Arabidopsis thaliana*. *PLoS One* 2016;11:e0162829.
28. Schulze A, Zimmer M, Mielke S, et al. Wound-induced shoot-to-root relocation of JA-Ile precursors coordinates *Arabidopsis* growth. *Mol Plant* 2019;12:1383-94.
29. Wasternack C, Song S. Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *J Exp Bot* 2017;68:1303-21.
30. Yang J, Duan G, Li C, et al. The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. *Front Plant Sci* 2019;10:1349.
31. Mahmud S, Ullah C, Kortz A, et al. Constitutive expression of JASMONATE RESISTANT 1 induces molecular changes that prime the plants to better withstand drought. *Plant Cell Environ* 2022;10:2906-22.
32. Pandey S, Chakraborty D. Salicylic acid and drought stress response: biochemical to molecular crosstalk. In: Tripathi B, Müller M (eds) *Stress Responses in Plants*. Springer, Cham; 2015.
33. Sidhu GPS, Bali AS. Plant responses to drought stress: role of brassinosteroids. In: *Brassinosteroids in plant developmental biology and stress tolerance*. Cambridge: Academic Press; 2022. pp. 201-16.
34. Singh PK, Srivastava D, Tiwari P, et al. Drought tolerance in plants: molecular mechanism and regulation of signaling molecules. In: *Plant signaling molecules*. Woodhead Publishing; 2019. pp. 105-23.
35. Zhang Y, Li Y, Hassan MJ, et al. Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. *BMC Plant Biol* 2020;20:150.
36. Verma V, Ravindran P, Kumar PP. Plant hormone-mediated regulation of stress responses. *BMC Plant Biol* 2016;16:86.
37. Hallmark HT, Rashotte AM. Cytokinin isopentenyladenine and its glucoside isopentenyladenine-9G delay leaf senescence through activation of cytokinin-associated genes. *Plant Direct* 2020;4:e00292.
38. Shohat H, Cheriker H, Kilambi HV, et al. Inhibition of gibberellin accumulation by water deficiency promotes fast and long-term 'drought avoidance' responses in tomato. *New Phytol* 2021;232:1985-98.
39. Zhang C, Huang Z. Effects of endogenous abscisic acid, jasmonic acid, polyamines, and polyamine oxidase activity in tomato seedlings under drought stress. *Sci Hortic* 2013;159:172-7.
40. Skowron E, Trojak M. Effect of exogenously-applied abscisic acid, putrescine and hydrogen peroxide on drought tolerance of barley. *Biologia* 2021;76:453-68.