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Abstract: Plants can sometimes be under the influence of more than one stress factor. In this case, combined stress factors can cause different responses in plants. In this study, the phytohormonal activity and hormone gene expressions of beans (*Phaseolus vulgaris* L.) were investigated in different drought levels and soils with different levels of cadmium accumulation. Plants were grown at 4 different cadmium (Cd) levels (Cd: 0, Cd1: 100, Cd2: 150, and Cd3: 200 mg kg^{-1}) and 3 irrigation levels (D0: 100%, D1: 75%, and D2: 50% of field capacity). Abscisic acid (ABA), indole acetic acid (IAA), gibberellic acid (GA), salicylic acid (SA), cytokinin, zeatin, and jasmonic acid contents and gene expressions related to them were investigated. As a result of the study, we determined that there was a significant decrease in the content of IAA, GA, SA, cytokinin, zeatin, and jasmonic acid with increasing doses of drought and Cd. Especially with the coexistence of both stress factors, this reducing effect (the reductions in these hormones were 86.6%, 64.1%, 71.9%, 66.7%, 45.5%, and 73.9% as compared to D0, respectively) on the measured parameters was greater. However, ABA content increased significantly with drought and/or Cd stresses, especially with Cd3D2, about a thousand times higher than the control. On the other hand, mRNA transcript levels of some hormone genes (ABA 8′-hydroxylase, adenylate isopentenyltransferase (AIPT3), auxin-related protein (AUX/IAA), MeJA biosynthesis and gibberellin 20-oxidase and NRAMP2 gene known to be involved in Cd transport, were detected. According to the results, while ABA 8′-hydroxylase, gibberellin 20-oxidase, and NRAMP2 genes were overexpressed in all stress treatments, AIPT3 and AUX/IAA genes were down-regulated and also, MeJA biosynthesis gene varied depending on dose and stress.

Key words: Bean, drought, heavy metal, stress, gene, hormone

1. Introduction

Agricultural plants are exposed to various abiotic stresses throughout their lifecycle that drastically reduce productivity and threaten global food security. Abiotic stresses such as salinity, drought, flood, heat, cold, freezing, excessive light, UV radiation, and heavy metal toxicity have a significant impact on reduced plant growth and compromised yield around the world (Rodziewicz et al., 2014). A combination of different abiotic stresses can act synergistically or cumulatively in terms of the effect on plant growth. Stress events that can occur at the same times such as salinity, drought, temperature, and heavy metals have been shown to be more detrimental to plant growth than either of these stresses. As a result, significant attention is currently paid to increasing plant tolerance to numerous abiotic stresses (Savvides et al., 2016; Ors et al., 2021). Abiotic stresses are factors that affect plant vital properties such as photosynthetic activity, chlorophyll pigments, enzyme activity, mineral element, and hormonal content, and therefore are very important in plant growth and development (Abdelgawad et al., 2014; Hashem, 2014; Shams et al., 2019).

Another important effect of stress in plants is the alteration of the hormone gene expressions during stress. ABA is known as a stress hormone, and it prevents excessive water loss by closing stomata, especially in osmotic stress, and stimulates the expression of stress-related genes under long-term stress conditions. ABA level is controlled by modulation of de novo biosynthesis and catabolism (Ma et al., 2018). Therefore, in order to understand the molecular mechanism that controls the ABA content in plant tissues, the genes and enzymes involved in the biosynthesis and catabolism of ABA must be studied in detail. The hydroxylation of ABA at the 8′ position is known as the

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key step in ABA catabolism, and this reaction is catalyzed by ABA 8′-hydroxylase, a cytochrome P450. The ABA 8′-hydroxylase gene encodes (+)- ABA 8′-hydroxylase, which is related to the oxidative degradation of ABA (Bashir et al., 2019). Another hormone, cytokinin, is a class of phytohormones involved in many aspects of plant growth and development. During drought stress, cytokinin levels were found to decrease in plants such as soybean (Le et al., 2012), tobacco (Xu et al., 2017), rice (Bano et al., 1993) etc. Cytokinins are produced in plant tissues by two biosynthetic pathways: de novo synthesis and degradation of isoprenylated tRNA. The majority of isoprenoic cytokinins are synthesized due to the activity of ATP/ADP isopentenyl transferase (IPTs) (Kasahara et al., 2004; Miyawaki et al., 2006). The members of IPT genes are differentially expressed depending on the part of plants and according to kind of the stress (short-time/long-time) (Yang et al., 2012). Indole acetic acid (IAA), one of the auxin group hormones, contributes to the formation and development of plant roots. Aux/IAA, which is one of the early responding genes in the maintenance of auxin homeostasis, is a central regulatory gene. During stress, the increase or decrease of auxin is regulated by the change in the expression level of the Aux/IAA gene (Feng et al., 2019). Some reports are available on the role of MeJA in the protection of plants and the regulation of plant defense gene expression against stress (Ahmad et al., 2016; Shariatipour and Heidari, 2018). MeJA biosynthesis gene encodes 12-oxophytodienoic acid reductase involved in MeJA biosynthesis (Bashir et al., 2019). Another plant hormone, gibberellin, decreases and limits plant growth in abiotic stress conditions (Colebrook et al., 2014). Gibberellin 20-oxidase is thought to be the key enzyme of the GA biosynthetic pathway. Expression of this gene is subject to feedback inhibition by GAs further down the pathway, suggesting that GA biosynthesis has an auto-regulatory component (Jackson et al., 2000). Cadmium transport is associated with the activation of the NRAMP (Natural Resistance-Associated Macrophage Protein) gene family in plants. There are seven NRAMP families in the bean, and the transcriptional activities of these genes can be activated by external biotic and abiotic stresses during plant development (Ishida et al., 2018).

In recent years, molecular studies on the negative effects of abiotic stress factors have begun to be emphasized. Indeed, recent advances in genetics and molecular biology have enabled the identification of a complex signaling network that contributes to plant growth and development on the one hand, and the abiotic stress response on the other (Joshi et al., 2016). Under these additional developments, we aimed to investigate bean responses to both Cd and drought stress by determining the hormonal content, activity, and genetic level of the bean under different drought levels and different levels of cadmium accumulation in soils.

2. Materials and methods

2.1. Plant materials and treatments
The study was carried out in a controlled greenhouse (25 ± 2 °C/18 ± 2 °C day/night, humidity 40% ± 5%), in pots. Bean (*Phaseolus vulgaris* L. cv. Gina) seeds were used as plant material. The study was set up in 3 replications at 4 different levels of cadmium (Cd) (Cd: 0, Cd1: 100, Cd2: 150, and Cd3: 200 mg kg^{-1}) and 3 irrigation levels (D0: 100%, D1: 75%, and D2: 50%) (Kıymaz and Beyaz, 2019). In the study, the pots were filled with a mixture of garden soil: peat: sand (2:1:1 v:v:v), and the prepared medium (the soil is loamy with 31% sand, 36% silt, and 33% clay) was fertilized with the calculation of 80–120 kg ha^{-1} N, 100 kg ha^{-1} P$_2$O$_5$, and 100–120 kg ha^{-1} K$_2$O. For heavy metal stress, Cd was mixed into the medium at different concentrations and kept at the field capacity for 3 weeks of incubation. The amounts of water that the growing medium can hold at field capacity were determined with a soil moisture tracking device (ProCheck, Decagon Devices, Inc.). Bean seeds were sown at the end of the incubation period. After the true leaves were seen and the seeds started to emerge, drought applications were started. As a control (D0), the amount of irrigation water to reach field capacity was applied at each irrigation event. In water deficit applications, D1 and D2 of the water applied in the control treatment (D0) were applied at each irrigation event. Irrigations were practiced at three-day intervals until plant removal. After 50 days, measurements were made on fresh samples taken from the plants.

2.2. Hormone analysis
Extraction and purification of bean leaf samples were performed according to Kuraishi et al. (1991). One gram of fresh plant sample was combined with 80% methanol at a temperature of –40 °C. The prepared sample was homogenized for 10 min and then the solution was incubated in the dark ambient for 24 h. After, the prepared samples were dried at 35 °C with the help of evaporator pumps. Dried samples were dissolved in a pH 8 solution containing 0.1M KH$_2$PO$_4$. A Sep-Pak C18 cartridge produced by the Waters Company was used for further specific separation. Hormones adsorbed by cartridge were transferred to vials using 80% methanol. Hormone analysis was performed on Agilent 1200 HPLC device using Zorbax Eclipse-AAA C-18 column and the content of hormones was determined under UV (Turan et al., 2014).

2.3. RNA extraction and Q-PCR analysis
The total RNA was isolated from frozen bean leaves using Tri Reagent (Sigma-Aldrich, St. Louis, MO, USA)

according to the manufacturer's instructions. The quality and quantity of RNA were evaluated by 1.2% agarose gel and spectrophotometrically (Thermo Multiskan GO 1510, Finland). For Q-PCR analysis, first-strand cDNA was synthesized using 1 µg of total RNA and RevertAid First Strand cDNA Synthesis Kit (Thermo). Q-PCR was conducted by using RT2 SYBR® Green qPCR Mastermixes (Qiagen). The primers were designed by Phytozom (https://phytozome.jgi.doe.gov/) (Table 1). Reaction conditions were performed for Q-PCR at universal cycling conditions (10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 60 s at 60 °C) in a total volume of 20 µL, containing 2 µL diluted cDNA sample, 10 µL SYBR green master mix (Qiagen) 0.1 µL forward and reverse primers (100 pmol) and RNase-free H_2O. The sample of each biological replicate was analyzed in three technical replicates. Data normalization was done with the β-actin reference gene. A threshold cycle was used for the relative quantification of input cDNA, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.4. Statistical analysis

The experiment was a completely randomized design with 3 replications at 4 different levels of Cd and 3 irrigation levels. A two-way ANOVA was employed for data analysis and the mean differences were compared by Duncan's Multiple Range Test using the SPSS 20.0 (SPSS Inc.).

3. Results

3.1. Hormones content

In the study, the effect of different levels of drought on the plant hormone content of beans grown under different doses of Cd medium was investigated and the results are given in Table 2. The effect of Cd and drought applications on plant hormone content was statistically significant ($p < 0.001$).

While the amount of plant hormones such as IAA, GA, SA, cytokinin, zeatin, and jasmonic acid decreased significantly, the amount of ABA increased with Cd application under drought stress.

In control (Cd0D0), IAA, GA, SA, cytokinin, zeatin, and jasmonic acid contents were determined as 5.43 ng mg^{-1}, 6.33 ng g^{-1}, 7.83 ng g^{-1}, 9.00 ng g^{-1}, 2.33 ng g^{-1} and 23.87 ng g^{-1}. The same parameters were 3.30, 5.80, 7.10, 8.57, 2.03, and 18.20, respectively in 50% water deficit (Cd0D2), and were 1.13, 2.80, 2.60, 3.70, 1.70, and 8.80, respectively in D0 treatment with 200 mg kg^{-1} Cd (Cd3D0) application.

The ABA content increased as the stress severity increased. The highest value (5480.00 ng g^{-1}) was obtained from 200 mg kg^{-1} Cd application with 50% irrigation treatment (Cd3D2). The amount of ABA, which was 353.33 ng g^{-1} in the control treatment, increased to 766.67 ng g^{-1} in 50% irrigation treatment (Cd0D2) and to 3850.00 ng g^{-1} in 200 mg kg^{-1} Cd treatment (Cd3D0). These stress factors caused a more significant negative effect on the measured IAA, GA, SA, cytokinin, zeatin, and jasmonic acid contents when both stress factors occurred together (Cd3D2).

3.2. mRNA expression levels

According to ABA 8′-hydroxylase gene mRNA expression analysis results, it was determined that this gene was up-regulated in all Cd and water restriction treatments

Table 1. The primers used in Q-PCR.

Gene		5' >3'	Gene ID
ABA 8′-hydroxylase	F	TGCATTTTCCTCTTCTTCTCATC	Phvul.003G292200.1
	R	TGGGAATGGGCTATATTGGA	
AIPT3	F	CAGCCTCTGAAAAACGTTCC	Phvul.001G149400
	R	TCTCCCAGAAGTGGAATTGG	
AUX/IAA	F	GCATCCACTCATCGGAATCT	Phvul.005G173000
	R	GGAACCCGAACATTTTGAAC	
MeJA biosynthesis	F	TTCCTAGTACAAACCAGCTTACC	PvTIFY10C
	R	TTCTTCATTTCAGTAGATTTGGC	
Gibberellin 20-oxidase	F	CAGGCTCCTACTCCAAACCA	PHAVU_010G087500g
	R	CCAAATTCCCTGAACTCTTCC	
NRAMP2	F	GCCTCTTGATCCAGCTTTTG	Phvul.009G069700.1
	R	TCCAGCAACAGTCCATGCTA	
β-Actin	F	GAAGTTCTCTTCCAACCATCC	PHAVU_011G064500g
	R	TTTCCTTGCTCATTCTGTCCG	

Table 2. Hormone content in bean leaf under cadmium and drought treatments.

Cd	Irrigation	IAA	GA	SA	ABA	Cytokine	Zeatin	Jasmonic acid
		ng mg tissue^{-1}	ng g DW^{-1}	ng g DW^{-1}	ng g DW^{-1}	ng g DW^{-1}	ng g DW^{-1}	ng g DW^{-1}
Cd0	D0	5.43 a	6.33 a	7.83 a	353.33 j	9.00 a	2.33 a	23.87 a
	D1	4.77 b	6.27 a	7.40 b	491.00 j	8.70 b	2.13 ab	21.93 b
	D2	3.30 e	5.80 b	7.10 b	766.67 ı	8.57 b	2.03 bc	18.20 c
Cd1	D0	4.50 c	5.10 c	6.30 c	1386.67 h	8.03 c	2.17 ab	15.83 d
	D1	3.83 d	4.73 d	6.13 cd	1766.00 g	7.37 d	1.97 bcd	14.93 de
	D2	3.33 e	4.30 e	5.83 d	1970.00 f	7.30 d	1.90 cde	13.80 e
Cd2	D0	2.93 f	3.77 f	4.67 e	2433.33 e	5.23 e	2.17 ab	14.97 de
	D1	1.80 g	3.60 fg	4.10 f	2733.33 d	5.17 e	1.87 cde	11.57 f
	D2	1.23 h	3.43 g	4.07 f	2900.00 d	5.13 e	1.80 de	11.07 f
Cd3	D0	1.13 h	2.80 h	2.60 g	3850.00 c	3.70 f	1.70 e	8.80 g
	D1	0.77 ı	2.43 ı	2.40 gh	4600.00 b	3.50 f	1.40 f	7.22 gh
	D2	0.73 ı	2.27 ı	2.20 h	5480.00 a	3.00 g	1.27 f	6.23 h

There is no statistical difference between means shown with the same letters in the same column (p < 0.001). Cd0: 0 mg kg^{-1}, Cd1: 100 mg kg^{-1}, Cd2: 150 mg kg^{-1} and Cd3: 200 mg kg^{-1}, D0: 100%, D1: 75% and D2: 50%

compared with the control. While the highest expression rate was reached in 200 mg kg^{-1} Cd and 50% irrigation treatment (Cd3D2) (6.28), the lowest expression rate was reached in 150 mg kg^{-1} Cd treatment (1.12) (Cd2D0) according to Cd0D0 (Figure 1).

The cytokinin biosynthesis gene AIPT3 was down-regulated in all treatments according to the control (Cd0D0). The lowest expression rate was reached at Cd3D2 (0.05) and the highest expression rate was reached in the highest Cd application (Cd2D0) (0.76) alone (Figure 2).

The AUX/IAA gene was down-regulated in all Cd and water restriction treatments compared to the control. While the lowest expression rate was reached in CD0D1 treatment (0.04), the highest expression rate was reached in Cd3D0 treatment (0.88). The down-regulation level of this gene varied considerably between other treatments and doses (Figure 3).

Significant differences were observed in the expression of the MeJA biosynthesis gene under different Cd and drought treatments (Figure 4). The expression of this gene was significantly up-regulated after exposure to Cd and irrigation treatments applied individually and also in treatments both Cd1D1 and Cd1D2. The highest transcript level was observed under Cd0D2 treatment (5.35). However, this gene was down-regulated in both Cd2 and Cd3 as well as in drought treatments (D1 and D2). The lowest transcript level was seen at Cd2D2 treatment (0.61) (Figure 4).

The gibberellin 20-oxidase gene was up-regulated to higher levels in all treatments of Cd and drought as well as

their interactive applications compared to the control. A higher expression rate was observed in Cd1D2 (3.48). The lowest expression rate was obtained in Cd1D0 treatment (Figure 5).

The NRAMP2 gene had the highest expression level among the genes used in this study. The up-regulation rate of the Cd2D0 treatment was the highest (7.28), followed by Cd0D2 treatment (6.39). Therewithal, the lowest expression rate was reached in Cd3D1 treatment (1.18) (Figure 6).

4. Discussion

Excessive accumulation of heavy metals in the soil is life-threatening for all organisms, including plants. Different metals and metalloids such as arsenic, lead, cadmium, and mercury are thought to be nonessential and harmful to plants and animals (Gall et al., 2015; Rizwan et al., 2016). Drought is another important abiotic stress factor that causes serious damage to plant growth, development, and crop losses. In previous studies, it has been reported that changes in the level of plant hormones such as ABA, IAA, SA occur in plants exposed to stress (Jia et al., 2017; Emamverdian et al., 2021; Raza et al., 2022). In our study, under drought and heavy metal stress separately or together, significant damage occurred in plant growth in beans. The effects of stress on plant growth have also been clearly observed on hormonal levels. Abass and Mohamed (2011) stated that drought stress causes significant decreases in growth parameters, photosynthetic pigments, total carbohydrates, and phytohormones in beans. In
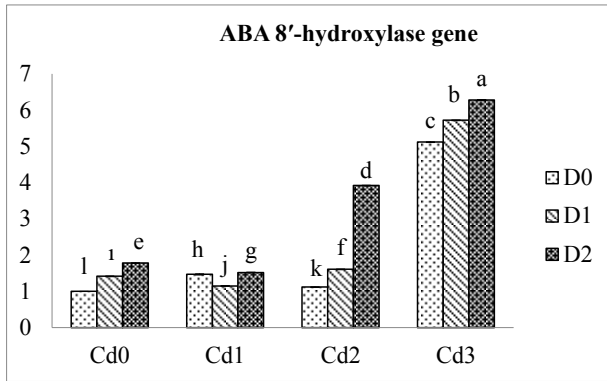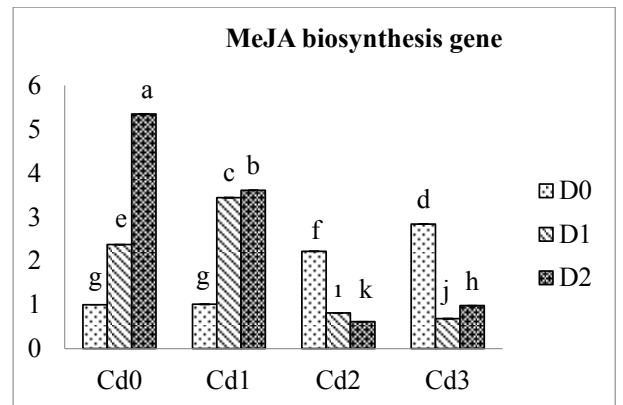
Figure 1. ABA 8′-hydroxylase gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters on the bars (p < 0.001). Cd0: 0 mg kg⁻¹, Cd1: 100 mg kg⁻¹, Cd2: 150 mg kg⁻¹ and Cd3: 200 mg kg⁻¹, D0: 100%, D1: 75% and D2: 50%.
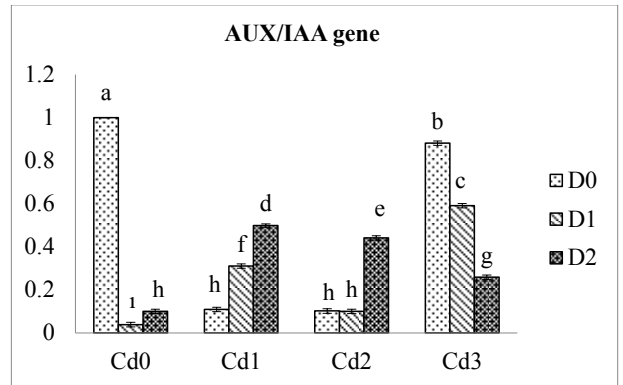
Figure 3. AUX/IAA gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters on the bars (p < 0.001). Cd0: 0 mg kg⁻¹, Cd1: 100 mg kg⁻¹, Cd2: 150 mg kg⁻¹ and Cd3: 200 mg kg⁻¹, D0: 100%, D1: 75% and D2: 50%.
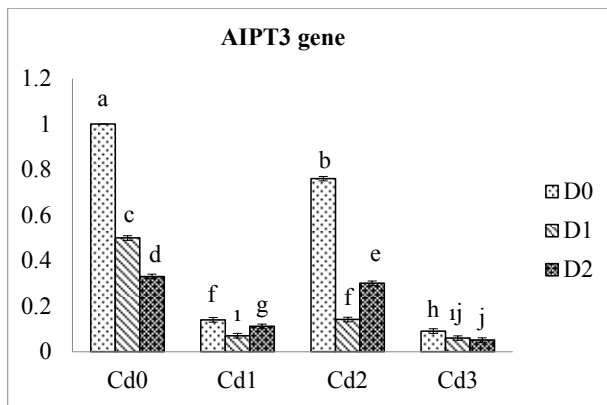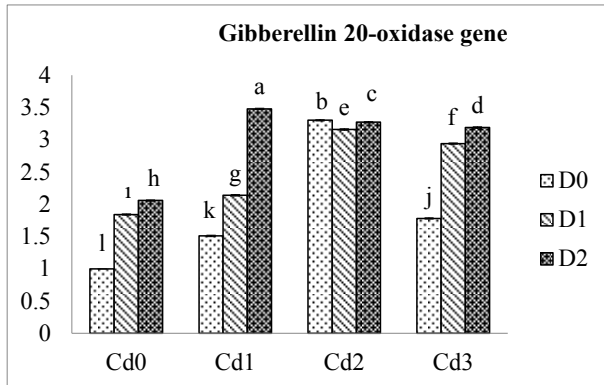

Figure 2. AIPT3 gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters on the bars (p < 0.001). Cd0: 0 mg kg⁻¹, Cd1: 100 mg kg⁻¹, Cd2: 150 mg kg⁻¹ and Cd3: 200 mg kg⁻¹, D0: 100%, D1: 75% and D2: 50%.
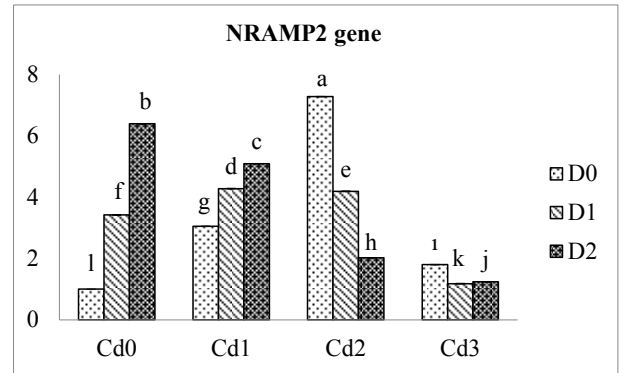

Figure 4. MeJA biosynthesis gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters on the bars (p < 0.001). Cd0: 0 mg kg⁻¹, Cd1: 100 mg kg⁻¹, Cd2: 150 mg kg⁻¹ and Cd3: 200 mg kg⁻¹, D0: 100%, D1: 75% and D2: 50%.

addition, under increased drought stress levels, an increase in antioxidant-compatible solutes, polyamine contents, and ABA contents was observed in the shoots of beans. Similarly, in our study, we determined that Cd and drought stress significantly decreased the content of IAA, GA, SA, cytokinin, zeatin, and jasmonic acid, while the content of ABA increased. The ABA content increased by 989% with the highest Cd application (Cd3D0), and by 117% under the most severe drought (Cd0D2). Under the highest stress conditions (Cd3D2) which both stress factors occurred together, we determined that there was a thousand-fold increase compared to the control. Increased levels of stress severity of both Cd and drought significant decreases

occurred in plant hormone contents except ABA. In the highest Cd application (200 mg kg⁻¹), the content of IAA, GA, SA, cytokinin, zeatin, and jasmonic acid decreased by 79.2%, 5.8%, 66.8%, 58.9%, 27.0%, and 63.1% compared to the control (D0). On the other hand, under the most severe drought conditions (D2), the reductions in these hormones were 39.2%, 8.4%, 9.3%, 4.8%, 12.9%, and 23.8%, respectively as compared to the control treatments (D0). Under the highest level of stress factors (Cd3D2) which both stresses occurred together, the reductions in IAA, GA, SA, cytokinin, zeatin, and jasmonic acid were 86.6%, 64.1%, 71.9%, 66.7%, 45.5%, and 73.9% as compared to control treatment (Cd0D0) (Table 2). Similarly, Abdalla

Figure 5. Gibberellin 20-oxidase gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters in the same bar ($p < 0.001$). Cd0: 0 mg kg^{-1}, Cd1: 100 mg kg^{-1}, Cd2: 150 mg kg^{-1} and Cd3: 200 mg kg^{-1}, D0: 100%, D1: 75% and D2: 50%.

Figure 6. NRAMP2 gene expression changes in beans under cadmium and drought treatments ($2^{-\Delta\Delta Ct}$). There is no statistical difference between means shown with the same letters in the same bar ($p < 0.001$). Cd0: 0 mg kg^{-1}, Cd1: 100 mg kg^{-1}, Cd2: 150 mg kg^{-1} and Cd3: 200 mg kg^{-1}, D0: 100%, D1: 75% and D2: 50%.

and El-Khoshiban (2007) stated that drought stress significantly decreased the amounts of auxin, gibberellin, and cytokinin while increasing the concentration of ABA. ABA is the main plant hormone that signals abiotic stresses, especially under drought (Wilkinson and Davies, 2002). ABA concentration of the plant increases under drought stress, which triggers ion flow from the guard cells and causes the closure of stomata (Mori and Murata, 2011). ABA is critical in determining the balance between productivity and water loss (Traub et al., 2017). However, it has been stated that the survival and productivity of a plant under drought stress depend on the maintenance of ABA homeostasis (Sreenivasulu et al., 2007).

Phytohormones play a vital role in many processes related to stress. Furthermore, up- or down-regulation of genes related to phytohormones regulates plant responses to stress (Ullah et al., 2018). It is known that changes in the expression level of genes associated with these hormones and signaling molecules are not fully associated with changes in the level of these compounds and that there is a complex network of other genes/proteins/molecules that affects hormone level and status. In this study, the expression level of the ABA 8'-hydroxylase gene, which catalyzes the hydroxylation at the 8' position of ABA and the key step of ABA catabolism, was investigated during drought and cadmium applications in the bean. According to the results of our study, we determined that while the ABA content increased gradually depending on the application severity (Table 2), the ABA 8'-hydroxylase gene overexpressed in all treatments (Figure 1). The reason why the expression of this gene increases at high application levels with increased ABA content is due to ensure of ABA homeostasis. The main function of ABA

is to regulate plant water balance and osmotic stress tolerance (Zhu, 2002). It is thought that stomatal closure and drought-related gene expression are induced by ABA accumulation, thus providing an important adaptation by improving the tolerance of the plant to drought stress (Nambara and Marion-Poll, 2005). Ji et al. (2011) mentioned that the overexpression of genes responsible for ABA degradation decreases ABA content. Similarly, in a study, HvABA8'OH-1 (is ABA catabolic gene), is up-regulated under drought stress in barley (Seiler et al., 2011). Kushiro et al. (2004) reported that a major ABA catabolic enzyme (hydroxylase enzyme) was induced by drought stress in Arabidopsis.

The state of cytokinin levels is regulated by mechanisms such as the protection of photosynthetic machinery, strengthening of antioxidant systems, and improvement in the regulation of water balance, modulation of plant growth and differentiation, and modulation of the activities of stress-related phytohormones. It is stated that cytokinin regulates plant growth and development as well as mediates plant tolerance against adverse factors such as drought stress (Hai et al., 2020). The AIPT3 gene is a gene associated with the first and rate-limiting step of cytokinin metabolism where an isopentenyl group is transferred to AMP, ADP, or ATP. While the cytokinin content decreased gradually according to stress doses, the AIPT3 gene was repressed in drought and Cd treatments (Figure 2). Plants respond by reducing cytokinin levels by modulating the expression status of cytokinin receptor genes under drought conditions. The negative cytokinin regulatory function in plants exposed to stress is modified by loss of the isopentyl transferase and other biosynthesis genes (Werner et al., 2010; Nishiyama et al., 2011). Therefore, the down-regulation of this gene in our study can be assumed as a lock of this regulatory system. On the other hand,

overexpression of IPT was found to cause decreased root growth (Medford et al., 1989). Therefore, the system may prevent the AIPT3 gene from being overexpressed, so the roots grow excessively under drought conditions. Similar to our study, the expression of IPT genes decreased under combined drought and aluminum stress (Yang et al., 2012).

IAA, either alone or together with other hormones, is involved in plant responses to abiotic stresses, including drought, cold, salt, etc. Aux/IAA is one of the three major gene families of early response in the auxin signaling pathway. Also, it is known to function as a transcriptional repressor (Shani et al., 2017). Most of the genes that respond to auxin are suppressed during stress after a mechanism called systemic acquired resistance (Verma et al., 2016). This clearly demonstrates that auxin promotes disease susceptibility and that increased resistance to disease will require suppression of auxin signaling. According to our results, the auxin gene was down-regulated in both drought and cadmium treatments (Figure 3). This can be explained by the fact that auxin acts as an essential component of the signaling network of hormones that mediate the regulation of the defense response.

Methyl jasmonate (MeJA) plays an important role in plant development and in the regulation of plant defense gene expression in response to various stresses (Shariatipour and Heidari, 2018). According to the results we obtained from our study, it is quite interesting that the MeJA biosynthesis gene was up-regulated in drought and cadmium treatments individually, while it was down-regulated in the treatments that drought and cadmium were combined and this gene was differentially expressed according to the severity of the applications (Figure 4). MeJA is known to inhibit stomatal opening (Anjum et al., 2011; Yan et al., 2015). Therefore, it can be interpreted that this gene is up-regulated during drought, promoting stomatal closure. It has also been reported that MeJA activates genes in cadmium-related signaling pathways (Per et al., 2016). This indicates that the MeJA gene cooperates with other regulatory hormones and signals.

Gibberellin provides energy to explants by promoting seed germination and degradation of storage compounds. It also cross-reacts with many other hormones to regulate plant growth and development in response to stresses (Emamverdian et al., 2020). Our results showed that the gibberellin 20-oxidase gene was up-regulated in all treatments. The highest up-regulation rates were achieved in drought and cadmium treatments when applied together (Figure 5). Recalibrating growth responses and gaining improved tolerance levels to stresses is key to plant survival. At the molecular level, these are facilitated by the presence of multiple signaling intermediates for each hormone and their ability to interfere at various signal levels. Although an increasing number of potential components of molecular mechanisms in GA signaling, such as Ca^{2+}, calmodulin, protein kinases, various cis-elements, and transcription factors have been identified, their relationship with GA has not been fully elucidated (Zhu et al., 2012). GA reduces heavy metal accumulation and alleviates the damaging effect of Cd in plants (Chen et al., 2010). The reason for the decrease in the amount of endogenous GA in our study, as the stress dose increased may be due to the inability to repair the damage caused by multiple stress. However, the increase in the mRNA expression level of the gibberellin 20-oxidase gene may be a resistance developed by the plant to cope with multiple stresses.

The NRAMP gene belongs to the natural resistance-associated macrophage protein gene family. The NRAMP family mediates the transport of divalent metal ions across cellular membranes. Qin et al. (2017) determined that the GmNRAMP gene family is quite sensitive to Cd toxicity. Similarly, in our study, the NRAMP2 gene was up-regulated in all treatments, the highest rate was reached in 150 mg kg^{-1} Cd application (7.28), while the lowest expression rate was reached in 200 mg kg^{-1} Cd + 75% irrigation treatment (1.18) (Figure 6). Accordingly, it is thought that the NRAMP gene mediates Cd transport and may play a role in the regulation of Cd and other metal ions uptake and homeostasis. Also, Kozak et al. (2022) noted that NRAMP encodes proteins with low substrate specificity to maintain metal cross-homeostasis in the cell. In addition, the effect of NRAMP gene on Cd transport in drought stress is unknown.

5. Conclusion

In conclusion, our results demonstrated that compared to the effects of stress individually, combined drought and cadmium stresses showed strong changes in both the hormone content and the expressions of the hormone genes in bean. There was a decrease in the content of IAA, GA, SA, cytokinin, zeatin, and jasmonic acid with increasing doses of drought and Cd. On the other hand, ABA content increased with drought and/or Cd stresses. It is noteworthy that three genes ABA 8′-hydroxylase, gibberellin 20-oxidase and NRAMP2 were commonly up-regulated, two genes AIPT3 and AUX/IAA were down-regulated under combined stress factors, indicating that these genes could play extremely important roles in combined stress tolerance. However, MeJA gene is differentially expressed in alone and combined stress treatments. In the future, the mechanisms of above-mentioned genes involved in stress deserve further investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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