

EFFECTS OF CADMIUM AND HIGH TEMPERATURE ON CHLOROPHYLL AND MINERAL NUTRIENT CONTENTS IN *Triticum aestivum* L. SEEDLINGS

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Abstract. Wheat plays a particularly important role in the human diet. Environmental stresses negatively affect wheat development and yield. This study was conducted to determine the effects of high temperature-cadmium interactions on total chlorophyll content, chlorophyll a / chlorophyll b ratio as well as cadmium and mineral nutrient contents (potassium, magnesium, zinc, calcium) in wheat seedlings. Seedlings belong to two varieties of bread wheat (*Triticum aestivum* L.) named Dağdaş and ES-14 were treated with different concentrations of cadmium (Cd) (0, 15, 75 ppm), under different (24/16°C and 40/30°C daytime/night-time) temperature conditions. The total chlorophyll increased in the Dağdaş and decreased in the ES-14 in response to the high-temperature treatment (40/30°C). Cadmium caused a decrease in the Chlorophyll a/b ratio in varieties at high-temperature conditions. The accumulation of cadmium and uptake of other minerals in shoots differed depending on the wheat variety and cadmium-temperature interaction. Cadmium accumulation in shoots increased in response to the cadmium treatments. Cadmium accumulation decreased at the 40/30°C temperature compared to that at 24/16°C temperature. Calcium accumulation increased in shoots as a response to high temperature in both varieties. In both varieties, the potassium content increased in response to cadmium (15 µM) and high temperature.

Keywords: heavy metal, wheat, Dağdaş, ES-14, heat stress

Introduction

Climate change and global warming researchers have estimated that a dry and warm climate will affect many regions, including southern Europe recently (Kalefetoğlu and Ekmekçi, 2005). High temperatures can hamper grain production and quality (Gulli et al., 2005). Heat shock can also lead to the development of heavy metal resistance in wheat seedlings (Orzech and Burke, 1988). Soil contamination with heavy metals, such as cadmium (Cd), lead and mercury affects plant growth and development (Qiao et al., 2019). Microelement deficiency affects plant growth (Qiao et al., 2019). Cd can be absorbed easily by different plant parts (Gianazza et al., 2007). Previous research showed that both Cd and high temperatures caused stress in plants, leading to sizable production losses. High concentrations of Cd inhibited plant growth and development, as well as enzyme activity and photosynthetic organs, resulting in reduced photosynthesis (Di Toppi and Gabrielli, 1999). Besides, stress factors affect some transcription factor and genes. TaMYB73, TaERF1 and TaSRG genes' expression

levels increased in the seedlings of two wheat varieties (*Triticum aestivum* L. cv. Ç-1252 and Gün-91) exposed to chromium (Cr) and temperature stress (Ergün et al., 2014). Doğru and Ergün (2021) Dağdaş and Konya 2002 examined Cd and salt interactions in wheat varieties. The research result showed the highest increase in Dağdaş gene expression. High NaCl and Cd concentrations caused an increase in ERF1 expression in the Dağdaş variety. Increased TaSRG expression with Cd application in Konya 2002 variety, probably it may be associated with Cd resistance.

Wheat growth and development has declined worldwide because of agricultural areas becoming infertile due to various stresses, including high temperatures, salinity and heavy metal contamination (Öncel et al., 2000; Ergün et al., 2014). The increase in temperatures worldwide, in addition to heavy metal accumulation in the food chain, poses a threat to all living organisms on Earth. Research on the relation between heavy metal contamination and high temperature-induced stress is important for understanding tolerance mechanisms developed by plants growing in regions exposed to stressors. Such research on the mechanisms of heavy metal and high-temperature resistance in plants can aid the selection of heat-resistant and heavy metal-resistant varieties.

The present study aimed to detect the effects of different concentrations of Cd, temperature and temperature-heavy metal (Cd) interactions on the total chlorophyll (Chlorophyll a+b) (Chl) content, Chl a/b ratio, Cd accumulation and mineral (potassium [K], magnesium [Mg], zinc [Zn] and calcium [Ca]) contents in wheat plants.

Materials and methods

Materials

This study was conducted at Hatay Mustafa Kemal University in Turkey. Two varieties of bread wheat (*Triticum aestivum* L. cv. Dağdaş and ES-14) were supplied by the Çukurova University Faculty of Agriculture in Turkey. The Dağdaş - 94 wheat variety has been reported to have higher salt tolerance than the ES-14 variety (Karanlık, 2001). The Dağdaş - 94 wheat variety is known to be resistant to drought, incubation and cold (Öztürk and Aydın, 2017).

Methods

Plant growth conditions

Seeds of the two varieties were germinated between two layers of filter paper in a plant growth cabinet at $24 \pm 2^\circ\text{C}$ for 48 h. At the end of this period, seedlings were transferred to pots containing sand and perlite and grown under 24/16°C (daytime/night-time) with 50% humidity in a Percival model plant growth cabinet for 5 d. The seedlings were then transferred to pots containing nutrient solution composed of half-strength Arnon and Hoagland (1940) nutrient solution (pH 5.8).

The experiments were designed according to a completely randomized design with 4 replications. Cultivars are a mean factor, temperature and cadmium doses are split plots on cultivars. The study was conducted under two different temperatures. In the first set of experiments, the plants were grown in nutrient solutions containing Cd at temperatures of 24/16°C (daytime/night-time), which are the optimum temperatures for wheat growth. Chlorine salt of Cd was used as a heavy metal stressor in this study, with three different concentrations (0, 15 or 75 μM) added to the nutrient solution. In the second set of experiments, the same Cd treatments were repeated, but the cabinet

temperature was increased to 40/30°C (daytime/night-time). The seedlings were grown in these solutions for 5 d. At the end of this period, the plants were harvested. Plant samples were cut from the zone where the root and offshoot parted, and the shoots and roots were harvested separately.

For analyses of chlorophyll content, the fresh samples were determined using a Shimadzu UV-VIS Spectrophotometer.

For analyses of heavy metal and mineral elements (Cd, Ca, K, Mg and Zn), the shoot samples were dried at 70°C for 48 h, and their dry weights were measured. The dry plant samples were digested with sulphuric acid, perchloric acid, nitric acid using the wet decomposition method. The Cd and mineral nutrient concentrations were determined using a Varian Liberty Series II model Inductively Coupled Plasma-Atomic Adsorption Spectrophotometer (Hatay Mustafa Kemal University Antakya Hatay Turkey).

After harvesting the samples, the Chl content in fresh shoots was investigated. The Chl a/b ratio and total Chl (a + b) contents (mg g⁻¹ fresh weight (F.W.)) in leaf tissues were determined, according to the method of Arnon (1949), and the corrected values were then calculated according to the method of Porra (2002).

Statistical analysis

The values obtained from the experiments were subjected to analysis of variance (ANOVA) using the general linear models procedure in the SPSS (SPSS Inc. Chicago, Illinois, USA) package program. Statistically significant results were subjected to a Least Significant Difference multiple comparison test ($p < 0.01$).

Results

Total chlorophyll (Chl) content

In the experiments, the total Chl content increased in the Dağdaş variety and decreased in the ES-14 variety in response to the high-temperature treatment (*Fig. 1*). Chl (a / b) ratio 15 µM Cd application, although both temperatures increased ES-14 varieties, did not increase in Dağdaş cultivar at 24/16°C day/night temperature. Dağdaş variety has the lowest total chl at 24/16°C 15 µM, while ES-14 variety increases the total Chl value at 15 µM Cd at both temperatures and decreases at 75 µM Cd.

The chlorophyll a/b ratio

However, the Chl a/b ratio in both varieties decreased in response to the 75 µM Cd treatment at 40/30°C. It also decreased in the ES-14 variety but not in the Dağdaş variety at 24/16°C. The Chl a/b ratio was low in Dağdaş seedlings grown under 24/16°C conditions at Cd concentrations of 15 and 75 µM (*Fig. 2*).

Mineral content

In the present study, there was a close relationship between the Cd concentration and its accumulation in shoots in both varieties ($p < 0.01$) (*Table 1*). More Cd accumulated in the ES-14 variety as compared with that in the Dağdaş variety under 24/16°C conditions ($p < 0.05$). In a previous study on wheat seedlings treated with Cd, more Cd accumulated in root parts than in shoots and seeds. The role of temperature in increasing toxic responses to heavy metals is well known (Li et al., 2011). Roots are the first

organs to be affected by the accumulation and retention of heavy metals. Heavy metal accumulation together with increasing temperature, affects membrane lipids in the roots and leads to significant inhibition of root growth (Fritioff et al., 2005). In this study, Cd accumulation in shoots increased in the Cd treatments and decreased at high temperature. These findings may be due to Cd accumulation in roots inhibiting root growth, with a subsequent decrease in metal transport to shoots.

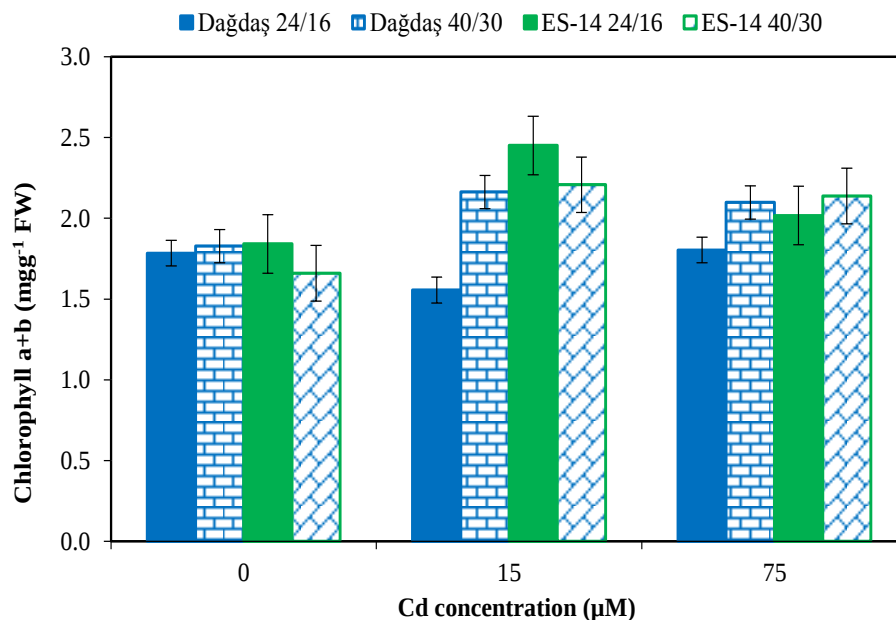


Figure 1. Effects of cadmium-temperature (24/16°C and 40/30°C) interactions on total chlorophyll (Chl) content (mg g⁻¹ FW) in Dağdaş and ES-14 wheat seedlings

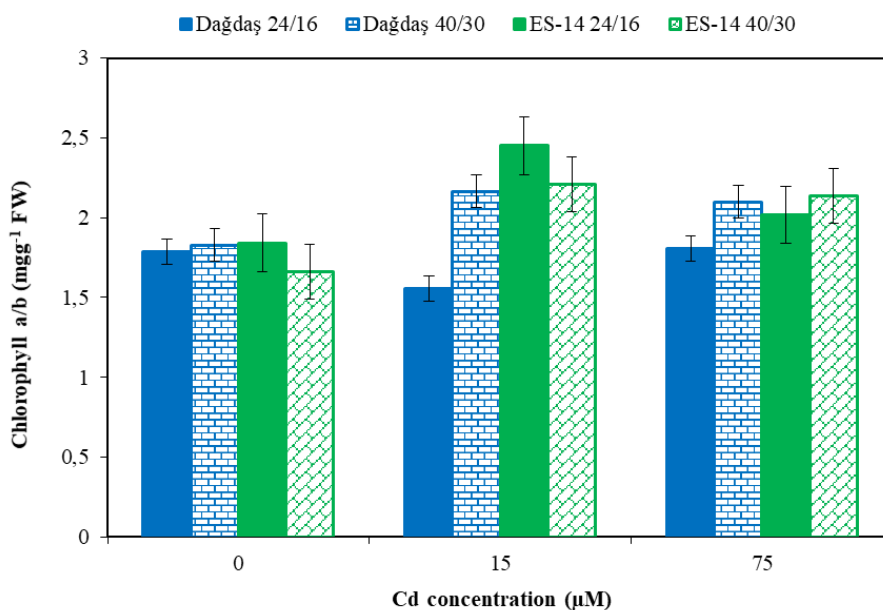


Figure 2. Effects of cadmium-temperature (24/16°C and 40/30°C) interactions on the chlorophyll a/b ratio in Dağdaş and ES-14 wheat seedlings

Table 1. The effects of genotype and temperature on the minerals (Ca, Cd, K, Mg and Zn) content

Genotype	Temperatures (°C)															
	Ca (%D.W)			Cd (ppm)			K (%D.W)			Mg (%D.W)			Zn (ppm)			
	24/16	40/30	Avr.	24/16	40/30	Avr.	24/16	40/30	Avr.	24/16	40/30	Avr.	24/16	40/30	Avr.	
Dağdaş	0.34	c	0.51	B	0.43	B	37.4	b	15.3	D	26.3	b	0.89	b	0.90	B
ES-14	0.54	ab	0.55	A	0.55	A	41.7	a	22.1	C	31.9	a	1.11	a	0.93	b
Average	0.44	B	0.53	A			39.5	A	18.7	B			1.00	A	0.92	B
G	*			*			**			ns			ns			
T	***			***			**			*			ns			
GxT	0.04	***			1.2	*		0.06	**			0.04	*			ns
CV	6.07				5.03			5.87				11.9				6.82

***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$ and ns: non-significant

In the present study, Ca accumulation in both varieties increased at high temperature. However, the Cd treatment did not appear to influence the Ca content, irrespective of the concentration. More Ca accumulated in the ES-14 variety under optimum temperature conditions as compared with that in the Dağdaş variety under the same conditions ($p < 0.05$). The Ca content in the Dağdaş variety seedlings increased in the high Cd/high-temperature treatment ($p < 0.01$) (Table 1). In a previous study, Ca accumulation in shoots of wheat seedlings treated with Cd increased as compared with that in a control Lii 667 variety and decreased in Huabei 45-4 and E81513 varieties (Zhang et al., 2002).

In the present study, the K content in wheat seedlings of the ES-14 variety in the 16/24°C treatment increased as compared with that in the Dağdaş variety under the same temperature conditions ($p < 0.01$) (Table 2). The K content in the ES-14 seedlings in the high-temperature/high Cd treatment decreased as compared with that in the control treatment. In contrast, the K content in the Dağdaş variety seedlings increased in the high Cd/high temperature ($p < 0.01$) (Table 3). Similarly, Zhang et al. (2002) reported that K accumulation in shoots of wheat seedlings treated with Cd decreased as compared with that in a control Lii 667 variety, whereas it increased in Huabei 45-4 and E81513 varieties.

Table 2. The effects of cadmium (Cd) and genotype on the minerals (Ca, Cd, K, Mg and Zn) content

Genotype	Cadmium doses (µM)																										
	Ca (%D.W)			Cd (ppm)			K (%D.W)			Mg (%D.W)			Zn (ppm)														
	0	15	75	0	15	75	0	15	75	0	15	75	0	15	75												
Dağdaş	0.39	c	0.45	b	0.44	b	0.06	e	22.9	d	56.1	b	0.87	d	0.92	cd	0.91	d	0.22	0.23	0.23	27.3	b	26.3	c	26.2	bc
ES-14	0.55	a	0.53	a	0.56	a	0.06	e	27.0	c	68.5	a	1.07	a	1.02	ab	0.98	bc	0.24	0.22	0.21	30.7	a	29.8	a	25.9	bc
Average	0.47		0.49		0.50		0.06	C	25.0	B	62.3	A	0.97	0.97	0.94				0.23	0.23	0.22	29.0	A	28.0	AB	26.1	B
Cd	ns			1.27 ***			ns			ns			1.61 **														
GxCd	0.04 **			1.79 ***			0.07 *			ns			2.27 **														
CV	6.07			5.03			5.87			11.9			6.82														

***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$ and ns: non-significant

Table 3. The effects of cadmium (Cd) and temperature on the minerals (Ca, Cd, K, Mg and Zn) content in Dağdaş and ES-14 seedlings

Genotype	Temperature (°C) (Day/night)	Ca (% D.W)					Cd (ppm)			K (% D.W)				Mg (% D.W)			Zn (ppm)											
		Cd (µM)					Cd (µM)			Cd (µM)				Cd (µM)			Cd (µM)											
		0	15	75	0	15	75	0	15	75	0	15	75	0	15	75	0	15	75									
Dağdaş	24/16	0.34	d	0.33	d	0.35	d	0.06	28.6	83.5	0.90	de	0.84	e	0.95	cd	0.17	0.20	0.22	27.3	cd	20.6	f	30.8	b			
	40/30	0.43	c	0.57	ab	0.53	ab	0.07	17.3	28.6	0.90	de	0.99	bc	0.86	de	0.27	0.27	0.24	27.3	cd	32.0	bc	21.6	ef			
ES-14	24/16	0.52	b	0.53	ab	0.57	ab	0.05	30.9	94.0	1.30	a	1.06	b	0.98	bc	0.24	0.22	0.21	27.2	cd	35.3	a	23.9	e			
	40/30	0.58	a	0.53	ab	0.55	ab	0.06	23.0	43.1	0.80	e	0.97	bc	0.98	bc	0.23	0.22	0.21	34.1	a	24.3	de	27.9	bc			
Average	24/16	0.43		0.43		0.46		0.05	e	29.7	c	88.8	a	1.10	a	0.95	b	0.97	b	0.20	0.21	0.21	27.3	b	27.9	b	27.3	b
	40/30	0.51		0.55		0.54		0.07	e	20.2	d	35.8	b	0.80	c	0.98	b	0.92	b	0.25	0.24	0.22	30.7	a	28.1	bc	24.8	c
TxCd		ns					1.79			**		0.07			**		ns			2.27					**			
GxTxCd		0.06					**		ns			0.01			**		ns			3.22					**			
CV		6.07								5.03			5.87						11.9			6.82						

** : p < 0,01; * : p < 0,05 and ns: non-significant

Discussion

It is known that heavy metal and heat stress decreased the chlorophyll content in plants. According to Ergün et al. (2014), total Chl in wheat decreased under combined heat and heavy metal stress, whereas carotenoid levels slightly increased. Chl contents decreased in *T. aestivum* L. seedlings treated with high Cd concentrations under low and high-temperature conditions (Öncel et al., 2000). Hsu and Kao (2003) reported a reduction in Chl in *Oryza sativa* L. TN 1, a Cd-sensitive variety, as compared with that in *O. sativa* L. TNG 67 variety, a Cd-tolerant variety, pointing to Cd-induced toxicity. Shukla et al. (2003) found that the amount of Chl decreased in *T. aestivum* L. seedlings treated with Cd.

Cd causes oxidative damage in plants and competes for cofactors of basic metal ions involved in Chl synthesis (Di Toppi and Gabrielli, 1999). Stobart et al. (1985) reported that Cd inhibited Chl biosynthesis due to its toxic effects. In their study, decreases in the total Chl content were closely related to the type and concentration of heavy metals, with dramatic reductions observed at higher heavy metal concentrations.

In our study, Cd concentrations in both wheat varieties caused a decrease in the amount of Chl. Previous research reported that Cd caused chlorosis by inhibiting the uptake of elements, such as Mg, K, Fe and Ca, which are basic cofactors of the enzymes of photo-system (PS) I and PSII (Shukla et al., 2003). In the present study, Cd caused a distinct decrease in the Chl a/b ratio in both varieties, especially at temperatures of 40/30°C (Fig. 2). In a previous study, although the Chl a/b ratio decreased significantly after Cd treatment, the effect was greater on PSII than PSI (Weigel, 1985). In a study on *Phragmites australis* plants treated with Cd, the total Chl content decreased, and Cd-related damage of PSII was higher than that of PSI (Pietrini et al., 2003). In another study, Cd had unfavourable effects on Chl, especially Chl b, causing an increase in the Chl a/b ratio (Ekmekçi et al., 2008).

In this study, the increase in Ca content due to the 40/30, Cd increase in Dağdaş variety wheat seedlings is statistically significant ($p < 0.01$). There is a significant increase in Cd content in both wheat varieties depending on the temperature and the increase in Cd content ($p < 0.01$). While a decrease in K content was observed due to the increasing Cd in the presence of ES-14 at 24/16°C, it is observed that the K content remained the same in Cd (75 µM) content at both temperatures. In Dağdaş variety, Cd (15 µM) dependent increase ($p < 0.01$) at 40/30°C is important, while increasing Cd value at 24/16°C causes a decrease in K content and a significant increase in Cd (75 µM) concentration has been ($p < 0.01$). While the Zn content of the Dağdaş variety decreases at 24/16°C due to the increase in Cd, it increases in the high Cd concentration ($p < 0.01$), but it was found to increase first and then decrease at 40/30°C ($p < 0.01$). It was stated that the highest Zn content was at Cd (15 µM) application at ES-14 24/16°C, and Cd (15 µM) at 40/30°C in Dağdaş variety ($p < 0.01$). In the present study, there was no significant variation in the Mg content of the wheat seedlings treated with Cd and exposed to different temperatures. However, Zhang et al. (2002) observed that Mg accumulation in shoots of wheat seedlings of E81513 and Huabei 45-4 varieties treated with Cd increased as compared with that in a control and that Mg content decreased only in Lii 667 variety.

In the present study, the highest Zn concentration was detected in ES-14 seedlings treated with 15 µM of Cd at 24/16°C, whereas the lowest concentration (20.6 ppm) was observed in the Dağdaş variety under these conditions ($p < 0.01$) (Table 3). However, there was no statistically significant difference in the Zn concentration under the

different Cd and temperature treatments. Zhang et al. (2002) found that Zn accumulation increased in offshoots of wheat seedlings treated with Cd in Lii 667 and Huabei 45-4 varieties and that it decreased only in an E81513 variety.

Cd affects the permeability of plasma membranes, thereby affecting the nutrient intake of the affected plant (Zhang et al., 2002). Similarly, in this study, the accumulation of mineral nutrients altered, depending on the wheat variety, as well as the Cd concentration and temperature. In previous research, high concentrations of applied Cd reduced concentrations of essential macro- (Mg and S) and micronutrients (Zn, Fe, Mn and Cu) in stems of *Pfaffia glomerata* (Gomes et al., 2013). Besides, interactions between Cd and other nutrients resulted in reduced nutrient uptake and reduced fertility (Zhang et al., 2002). High Cd concentrations caused a significant reduction in K, Mg, Ca, Fe and Zn concentrations in roots and stems of *Juncus effusus* L. (Najeeb et al., 2011).

Conclusion

As a result, it is concluded that high temperature caused a decrease in Cd accumulation in the shoot when caused an increase in Ca and K accumulation. It is concluded that this decrease in accumulation of Cd under the high-temperature stress cause increase in Ca and K uptake. Dağdaş variety used in the study is known that resistant to drought, cold and salinity. In the study, total chlorophyll content was found to be higher in Dağdaş cultivar than ES-14 cultivar. In this case, it is concluded that Dağdaş variety is more resistant to high temperature than the ES-14 variety.

Since the Cd uptake varies considerably at different temperatures, it is necessary to choose species and varieties resistant to Cd toxicity in agriculture, especially in regions with Cd pollution. Crop production has decreased worldwide because agricultural areas have become infertile due to various environmental factors, such as high temperatures, salinity and heavy metal contamination. Understanding physiological and molecular mechanisms is essential to tolerate stress conditions. In today's world where the world population is increasing rapidly, it is necessary to increase new efficiency-enhancing studies to prevent food shortage. Regarding this issue, we think that new studies are needed to take measures against stress and to identify the relevant genes and increase their usability in wheat breeding studies.

New physiological, biochemical and molecular studies on the relationship between heavy metal and high temperatures are needed to improve crop productivity. The present study revealed that interactions between heavy metals (Cd) and temperature may result in the accumulation of Cd and other minerals in shoots, depending on the wheat variety.

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