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Exploring The Physiological Response of Three Barley (*Hordeum vulgare*) Cultivars to Cadmium Stress

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ÖZ

Arpa dünyanın en erken kültüre alımış ürünlerinden biridir ve mısır, pirinç ve buğdaydan sonra dördüncü sırada yer alır. Kadmiyum (Cd), hem bitki hem de hayvanlar için toksisiteyi gösteren, yaygın, esansiyelolmayan ve zehirli bir ağır metal kirleticisidir. Bu çalışmada çeşitli kadmiyum dozajları kullanılarak üç arpa çeşidinin tepkisini araştırmak için çabaladık. Farklı kadmiyum konsantrasyonları olarak 0, 25-50 µM Cd, 100 µM Cd ve 150 µM Cd konsantrasyonları kullanılmıştır. Bu çalışmanın sonuçları, kadmiyum dozunun artmasıyla sürgün uzunluğu ve ağırlığında azalmanın gözlemlendiğini ortaya koymuştur. Arpa çeşitleri arasında sırasıyla Sentosa ve Tarm 92 en duyarlı ve en dayanıklı çeşitler olarak bulunmuştur. Kadmiyum konsantrasyonunun artmasıyla birlikte klorofil ve prolin içeriklerinde azalma tespit edilmiştir. Kök dokularında daha yüksek kadmiyum birikimi bulunmuştur. Bu çeşitlerde çeşitli mineral içeriklerinde kadmiyum stresinin etkileri ve ayrıca kalsiyum içeriklerinde de artış gözlenmiştir. Düşük kadmiyum dozajına karşı fidelerin manganez, bakır ve çinko içerikleri arttı. Ancak daha yüksek Cd dozajına karşı bu minerallerin konsantrasyonunda azalma gözlenmiştir. Bu çalışmanın bulgularının, kadmiyum toksisitesinin mahsullerin büyümesini ve verimini nasıl etkilediğinin anlaşılmasında yardımcı olacağına inanıyoruz.

Anahtar kelimeler: *Hordeum vulgare*, Çeşitler, Kadmiyum, Abiyotik Stres, Büyüme

Üç Arpa (*Hordeum vulgare*) Çeşidinin Kadmiyum Stresine Fizyolojik Tepkisinin Araştırılması

ABSTRACT

Barley is one of the world's earliest domesticated crops, ranks fourth grain cereal after maize, rice and wheat. Cadmium (Cd) is a widespread, non essential and toxic heavy metal pollutant reflecting toxicity for both plant and animals. In this study, we made an effort to investigate the responce of three barley cultivars using various cadmium dosage. 0, 25-50 µM Cd, 100 µM Cd and 150 µM Cd were taken as different cadmium doses. Results of this study revealed that decrease in shoot length and weight was observed with the increase in cadmium dose. Sentosa and Tarm 92 were found most succeptible and resistant cultivars of barley respectively. Decrease in chlorophyll and proline contents were determined with an increase in cadmium dosage. Higher cadmimum accumulation was found in root tissues. Effects of cadmium stress were observed for various mineral contents in these cultivars and an increse in calcium contents was also observed. Manganese, copper and zinc content of seedlings increased against low cadmium dosage. However, decrease in the concentration of these mineral was observed against higher Cd dosage. We are confidant that findings of this study will be helpful for the understanding of how cadmium toxicity effects the growth and yield of crops.

Key words: *Hordeum vulgare,* Cultivars, Cadmium, Abiotic stress, Growth

INTRODUCTION

Plants can't move away and therefore face continous unfavorable environmental conditions. As a result of encountering an unexpected situation, their development and survival conditions are negatively affected and causes 'stress' (Shao et al., 2008; Çulha and Çakırlar 2011;Akkuş and Vural,2023;). Such as drought, salt, cold, heat and heavy matels are considered important environmental stresses, significantly effecting crop production (Gill and Tuteja, 2011). Cadmium (Cd) is a wide spread, non essential and extremely toxic element (Xu et al., 2011). As a non redox metal , Cd is unable to involve in Fenton-type reactions, however, results in oxidative stress by producing reactive oxygen species (ROS) (Garnieret et al., 2006) Cadmium (Cd) stress is an important agricultural problem with increasing environmental pollution and threatening whole living organisms. Cadmium is a heavy metal, which described in Di Toppi and Gabbrielli's review with a density higher than 5.0 g cm^{-3} (Di Toppi and Gabbrielli, 1999). It is found at the periodic table's 12th group with its +2 valance and 8,65 g cm^{-3} density (Kabata-Pendias and Mukherjee, 2007). Normally it takes place in the soil below 0.5 mg kg^{-1} of soil but human activities such as application of sewage sludge, phosphate fertilization, pesticides, industrial development, or metal smelting industry can significantly increase its concentration (Dresler et al., 2019). Cadmium doesn't have any metabolic importance for plant metabolism (Cherif et al., 2012).

Cadmium stress has lots of negative effects on plant metabolism and these effects depend on plant species, plant age, stress duration, time and concentration of metal (Gill and Tuteja, 2011). It suppresses growth and photosynthesis (Ahmad et al., 2011); increases carotenoid and superoxide dismutase activity which are indicators of oxidative stress (Li et al., 2008); increased proline contents (Siddiqui et al., 2012); decrease chlorophyll a and b content (Zhao et al., 2019); accumulates in plant root and shoot tissues and causes cell membrane damage (Li et al., 2013); negatively affects water use efficiency (Li et al., 2015); stomatal conductance (Marchiol et al., 1996), and nutrient intake (Koleva, 2010). It also decreases plant height, shoot diameter, thousand grain weight, bunch length in Sorghum plants (Yılmaz and Kökten, 2019).

Barley is considered one of the agriculture founder crop and archaeological remains of this crop has been found at various sites in Fertile Crescent region. Barley was domesticated from its wild relative *Hordeum spontaneum* 10000 years ago (Zohary and Hopf, 1994). It is an important crop belonging to *Poaceae* (*Gramineae*) family mainly grown as animal feed, malt production and human nutrition. Barley is fourthlarely cultivated cereal and among the top ten crop plants (Akar et al., 2004). In 2016, 148.6 million tones barley produced in the world and 6.7 million tones in Turkey (FAO, 2017).

Several reports had reflected that cadmium has toxic effects on barley seedlings. Demirevska-Kepova et al. (2006) stated that cadmium stress reduced plant length, biomass and pigment content. Wu et al. (2003) stated that Cd toxicity caused a concentration and genotype-dependent oxidative stress response in barley leaves, marked by an accumulation of MDA and the alternation pattern of antioxidative enzymes. Metwally et al. (2003) reported that salicylic acid mitigated Cd toxicity in barley seedling with different Cd detoxification mechanisms. Cadmium also affects plant nutrition balance that it decreased significantly calcium and manganese level in all plant tissues and also iron and zinc concentration in roots while it increased Cu concentration in roots and also decreased in leaves like other metals (Lachman et al., 2015).

The purposes of the present study were to systematically investigate the effects of cadmium on growth, pigment content, membrane and water situation of three different barley cultivars. It is also aimed to determine the relationship between cadmium and nutrient element uptakes and indicated resistant cultivar to cadmium. This would ensure new suggestions producers as they will have knowledge about resistant cultivars.

MATERIALS AND METHODS

Plant material, growth conditions and Cd Stress

Seeds of barley (*Hordeum vulgare* L. cvs. Finola, Sentosa and TARM 92) cultivars were obtained from ProGen seed company. Barley seeds were sterilized in 3% hypochloride solution for five minutes and rinsed. These seeds were sown in a perlite medium and watered with distilled water until germination in a controlled climate room (20/18 ^{0}C,380 µmolm-^{2}/s and 60% humidity (Tiryakioğlu et al., 2006). Each pot contains twenty seeds and a total of 15 pots for each cultivars were maintained. Four-days-old seedlings were watered with nutrient solution every day (700µM K_2O_4, 100 µM KCl, 2000 µM $Ca(NO_3)_2$, 750 µM $MgSO_4$, 200 µMKH2PO4, 100 µM FeEDTA, 1 µM H_3BO_3, 1 µM $MnSO_4$, 0,2 µM $CuSO_4$, 0,01 µM $(NH_4)6Mo_7O_{24}.4H_2O$, 1 µM $ZnSO_4$). At twoleafed-stage, different Cd concentrations (0, 25-50, 100 and 150 µM) were applied to seedlings and $CdCl_2$ was used as a source of Cd. Each application was replicated three times. Stress condition was continued until harvestingand plants were harvested at three-leafed-stage.

Determination of biomass

Three seedlings were selected randomly for each application and their roots were washed with 0,5 mmol L^{-1} $CaSO_4$ solution and distilled water (Tiryakioğlu et al., 2006). Roots and shoots were separated, both of their length and fresh weight recorded and dried at 65 C^0 for 72 hours in drying oven to determine dry shoot and root matter.

Determination of Chlorophyll Content

Chlorophyll content was measured with using the SPAD-502 chlorophyll meter and recorded as SPAD value (Chang and Robison, 2003) at harvest day. For this purpose youngest fully expanded leaf was preferred.

Proline Analysis

Proline content of barley seedlings was determined according to protocol suggested by Bates et al. (1973). Fresh leaf material (0,5 g) was homogenized with 3% sulphosalicylic acid and filtered. Following this, 2 ml homogenate put in a test tube; 2 ml ninhydrin solution (ninhydrin, orthophosphoric acid and acetic acid in it) and 2 ml acetic acid added. After incubation in boiling water and ice bath gradually, 4 ml toluene added on samples. Later, samples were read at 520 nm spectrophotometrically and proline concentration of samples were calculated with standard curve which drown with L-proline.

Determination of Relative Water Content

Relative water content (RWC) was calculated with some modifications of Smart’s method (Smart and Bingham, 1974). To determine RWC, fresh weight (FW) of four one-cm-length leaf pieces from each application were recorded and put in distilled water. Following day, the turgor weight (TW) of leaf samples were measured and the samples were dried at 65 C^0 for 72 hours to determine the dry weight (DW). RWC was calculated with the following formula.

RWC (%)= (FW-DW)/(TW-DW)*100

Determination of Membrane Damage

Membrane damage was estimated with lipid peroxidation and electrolyte leakage. For lipid peroxidation, Hodges et al.’s (1999) protocol was used. 0,5 g fresh leaf sample was homogenized with %80 ethanol and centrifuged 10 minutes at 3000 g. Analysis was continued with two stages with +TBA(%20 TCA , %0,01 BHT and %0,65 TBA in it) and TBA solutions (%20 TCA , %0,01 BHT). First stage samples read at 532 and 600 nm; second stage samples read at 440, 532 and 600 nm spectrophotometrically. The product of lipid peroxidation malondialdehyde (MDA) content was calculated with the following formula.

1. $[(ABS_{532}+TBA)-(ABS_{600}+TBA)-(ABS_{532}-TBA)-(ABS_{600}-TBA)]=A$
2. $[(ABS_{440}+TBA-ABS_{600}+TBA)*0.0571)]=B$
3. nmol MDA / ml =(A-B)/157 000)*10^6

Electrolyte leakage was determined with some modifications Campos et al.’s (2003) method. For this purpose, four one-cm-length leaf pieces from each were put in distilled water. The second day, the conductivity of samples were measured with a conductive meter (EC1). Leaf materials were incubated in boiling water and later put again in same samples. The third day, the conductivity of samples (EC2) were measured again and electrolyte leakage (ELC) was calculated with the following formula.

ELC (%)= (EC1/EC2)*100

Determination of Cadmium and Nutrient Elements

Three time randomly selected leaf and root samples from each genotype were used to determine various mineral contents in studied germplasm. For the removal of moisture, samples were firstly dried in an oven for 48 h at 65°C and then crushed to make powder form which was used for further analysis. 0.2g from each cultivar was used as sample and 5 ml concentrated nitric acid and 2 ml hydrogen per oxide was used for the digestion of these samples. Microwave digestion system (MARSxpress, CEM Corp. North Carolina, USA) was used for the digestion of these samples. Then mineral nutrient concentration in studied germplasm was determined through the inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Australia). Following the criteria suggested by Jackson (Jackson, 1962), P contents were determined, while K, Ca, Fe, Zn, Cu, Mg, and Mn concentrations were investigated through the atomic absorption spectrometry (Varian SpektrAA-300, Vienna, Austria) (Beaty and Kerber, 1993).

Calculation of Cadmium Tolerance Index

The cadmium tolerance index was calculated by using dry weight parameter with the following formula (Wilkins, 1978; Pourghasemian et al., 2019).

Tolerance ındex(%)= (Dry weight of Cd- treated plants/Dry weight of untreated plants)*100

Statistical Analysis

All analysis were performed by using the MSTAT-C statistical analysis program and XLSTAT (www.xlstat.com).

RESULTS AND DISCUSSION

The Effects of Cadmium Stress on Barley Cultivars’ Growth

Cadmium drastically affected on plant morphology as can be seen in Figure 1 and 2; Table 1.

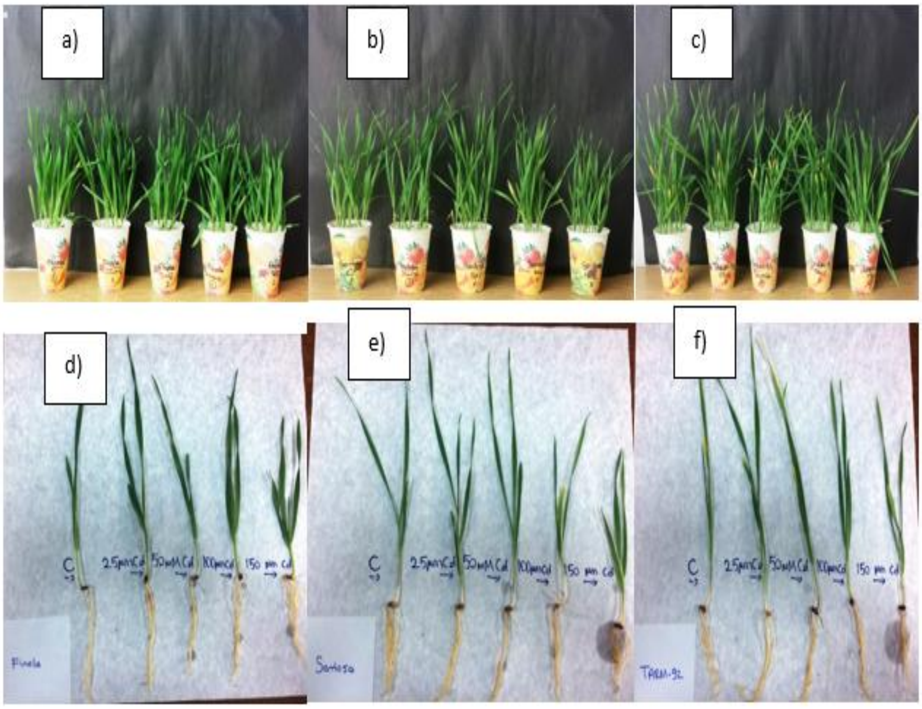

Figure 1. Effects of increasing cadmium concentrations on barley cultivars’ shoot and root morphology (a and d for Finola cv., b and e for Sentosa cv., c and f for Tarm 92; Control-25 µM Cd-50 µM Cd, 100 µM Cd and 150 µM Cd left to right).

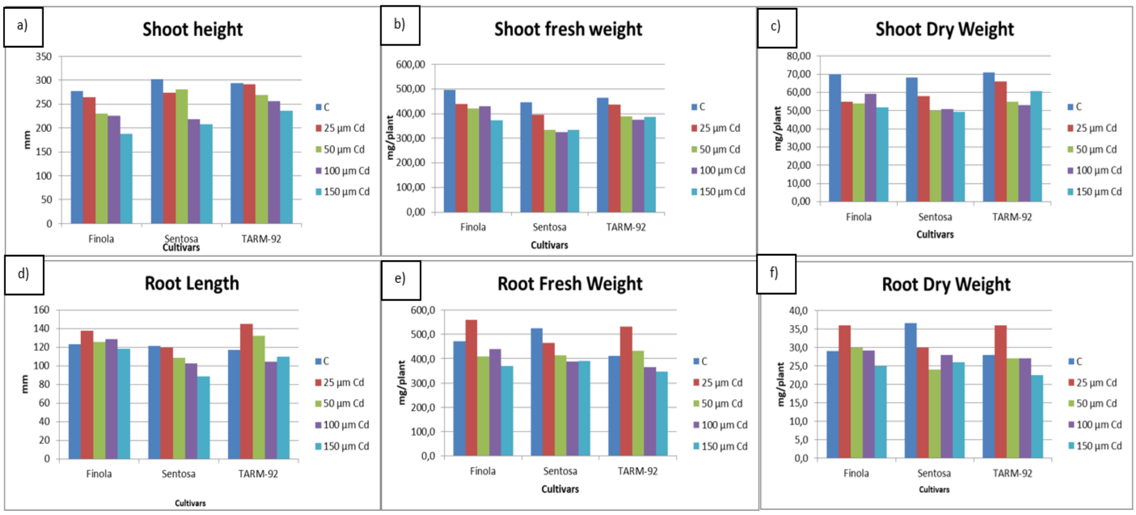

Figure 2. Effects of increasing cadmium concentrations on barley cultivars’ shoot and root growth (a, shoot height; b, shoot fresh weight; c, shoot dry weight; d,root length; e, root fresh weight and f, root dry weight).

Table 1. Effects of increasing cadmium concentrations on barley cultivars' growth and some physiological parameters.

Cultivars	Applications	Shoot height (mm)	Shoot Fresh weight (mg)	Shoot Dry weight (mg)	Root length (mm)	Root Fresh weight (mg)	Root Dry weight (mg)	Chlorophyll	RWC	ELC	Proline	MDA
Finola	Control	277.00	495.83	70.00	123.67	471.67	29.17	42.27 ab	94.09 a	9.63	21.73	0.71
Finola	25 µM Cd	264.33	439.17	55.00	138.00	559.17	35.83	41.30 a-c	91.63 ab	11.23	20.89	0.78
Finola	50 µM Cd	230.00	420.00	54.17	126.00	408.33	30.00	39.80 b-d	76.38 c	13.31	31.04	1.07
Finola	100 µM Cd	225.00	430.83	59.17	128.67	439.17	29.17	39.53 cd	92.82 ab	8.90	29.74	1.06
Finola	150 µM Cd	188.00	372.50	51.67	118.33	369.17	25.00	38.13 d-f	94.81 a	12.15	30.41	1.01
Sentosa	Control	302.67	445.83	68.33	121.67	524.17	36.67	42.50 a	73.40 c	7.80	27.39	0.35
Sentosa	25 µM Cd	274.00	395.83	57.50	119.67	464.17	30.00	38.50 de	91.26 ab	8.52	34.73	0.88
Sentosa	50 µM Cd	281.00	333.33	50.00	108.67	413.33	24.17	37.37 d-f	90.95 ab	8.45	50.22	1.52
Sentosa	100 µM Cd	219.00	324.17	50.83	102.67	387.50	28.33	34.20 gh	94.40 a	82.65	31.71	1.12
Sentosa	150 µM Cd	208.00	334.17	49.17	89.00	390.83	25.83	33.27 gh	97.70 a	9.10	41.96	1.32
Tarm 92	Control	293.67	464.17	70.83	117.67	411.67	27.50	37.00 ef	82.48 bc	9.18	24.53	0.28
Tarm 92	25 µM Cd	291.67	435.83	65.83	145.33	533.33	35.83	37.33 d-f	81.59 bc	10.01	34.57	1.15
Tarm 92	50 µM Cd	269.00	389.17	55.00	132.33	432.50	26.67	35.73 fg	90.77 ab	13.65	33.06	0.92
Tarm 92	100 µM Cd	256.00	375.83	52.50	104.67	364.17	26.67	32.87 h	88.41 ab	11.79	33.12	1.19
Tarm 92	150 µM Cd	236.67	385.83	60.83	110.00	346.67	22.50	34.17 gh	88.33 ab	12.54	27.09	1.66
	F	ns	ns	ns	ns	ns	ns	*	**	ns	ns	ns
	LSD	-	-	-	-	-	-	2.476	11.33	-	-	-
	CV (%)	6.86	13.13	12.30	12.00	17.34	16.76	4.00	7.77	22.35	26.96	65.00

Cadmimum application resulted in decreased shoot length and weight for all three cultivars. Very recently, Didwania et al. (2019) found a gradual shoot length decrease in onion with an increase in Cd dosege. Bahmani et al.(2012) recorded 66,3% decrease in shoot length due to Cd application in bean genotypes. Interestingly, it was obserable that lower Cd dosage didn't affect root length and resulted an increase in root parameters in Finola and Tarm 92 cultivars (as seen Figure 2). Our findigs were found in line with the reported by Tamas et al. (2015), as they stated that lower level of Cd dosage results in increased barley root length. Song et al. (2017) also found an increase in root length due to Cd application and same was also found in this study. Among all three cultivars, Sentosa was was found most susceptible one because it was severely effected even at lowest Cd dosage. Tarm 92 was less affected because its dry weight raised with the lowest Cd concentration, and at highest concentration it had maximum tolerance index Table 2). Wu et al. (2004) stated that Cd application results in lower plant biomass.

Table 2. Tolerance Index (%) of barley cultivars against cadmium.

	25 µm Cd	50 µm Cd	100 µm Cd	150 µm Cd
Finola	92	85	89	77
Sentosa	83	71	75	71
TARM_92	103	83	81	85

The Effects of Cadmium Stress on Barley Cultivars' on Chlorophyll Content

Cadmium showed sever effects related to chlorophyll contents in barley. A rapid decrease in chlorophyll content was observed with an increase in Cd dosage. Most visible effect was seen in Sentosa cultivar (Figure 3.a). Cd can inhibit net photosynthesis by causing changes in chloroplast structure and ultimately results in decreased chlorophyll content (Gallego et al., 1996). Our findigs were confirmed by Zhao et al. (2017) as they also found decreased chlorophyll contents in maize seedlings during Cd traetment. Decrease in the chlorophyll contents occurs due to negative effects of Cd on chlorophyll fluorescence and photosystem 2 activity together. Dobrikova and Apiostolova (2019) comprehensively explained how Cd effects the chlorophyll contents. According to them, cadmium ions induced changes in the functionality of photosynthetic membranes by inhibiting the lipid composition,plastid structure, chlorophyll metabolism and ultimately performance of photosystems. Therfore, it can be assumed that decline in growth of barley seedlings, may be associated with photosynthesis inhibition in this study.

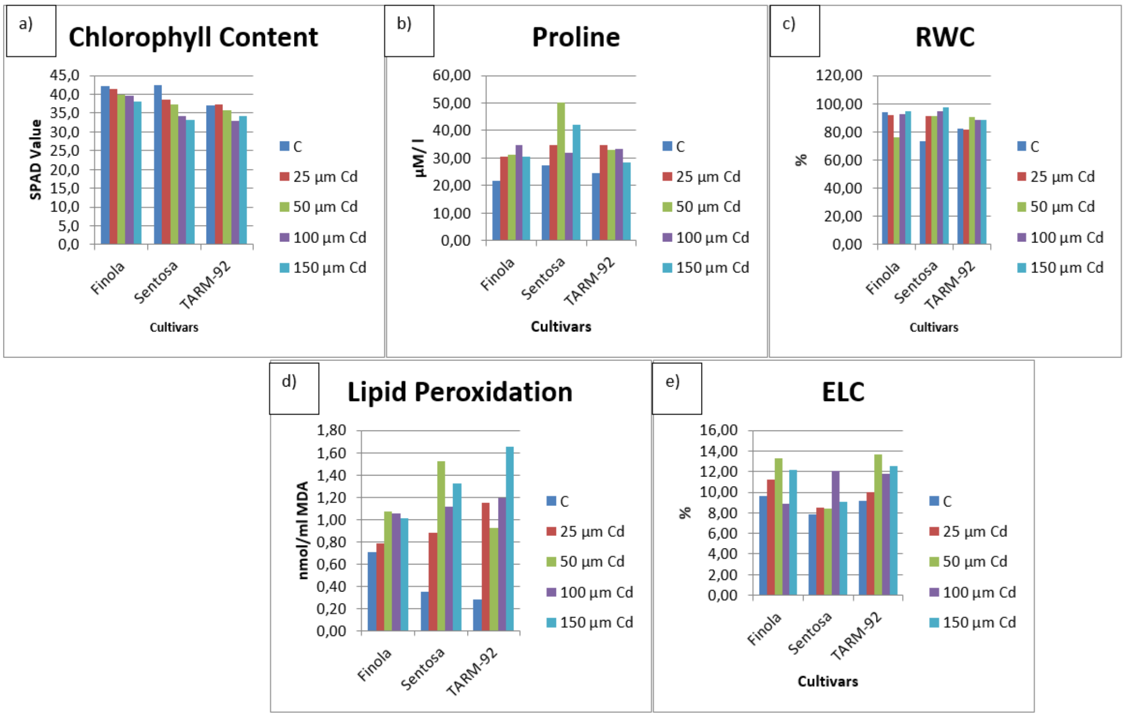

Figure 3. Effects of increasing cadmium concentrations on barley cultivars' physiological parameters (a, chlorophyll content; b, proline concentration; c, relative water content; d, Lipid Peroxidation; e,electrolyte leakage).

Effects of Cadmium Stress on on Proline Content in Barley Cultivars'

Compared with control plants, proline synthesis was induced by cadmium applications (3.b.). Changes in proline contents were observed with the Cd application. An increase in proline level was observed with an increase in Cd dosage. Yuanjie et al.'s (2019) found that under higher Cd application, plant is undergoing to stress and produce higher proline contents. Proline takes part as an osmoprotectant by aiding to membrane regulation and ROS scavenging. In many plant species, ascent of proline content may show retaining of energy and also play roles as a stress signal molecule against water deficiency (Hare et al., 1996; Tamás et al., 2008). Wu et al. stated that proline performed in detoxification of heavy metals by its direct activity or by way of biosynthesis of chelating compounds (Wu et al., 2004).

The Effects of Cadmium Stress on Barley Cultivars' on Relative Water Content

All three cultivars exhibited different stances on relative water content against cadmium (Figure 3.c.). Decrease in relative water content was observed in Finola's cultivar at 50 µM Cd dose. However, relative water contents increased with all doses in Sentosa cultivar. Vassilev et al. (1998) found that Cd didn't have a significant effect on the RWC of Cd treated young barley plants. Yordanova et al. (2017) stated that Cd did not effect RWC. De Maria et al. (2013) determined vaiations against cadmium application in sunflower RWC.

The Effects of Cadmium Stress on Barley Cultivars' on Membrane Damage

Membrane damage was tested with two parameters; lipid peroxidation and electrolyte leakage. Both parameters, increased with cadmium applications as seen in Figure 3.d and e. Due to cadmium, malondialdehyde, which is the last compound of lipid peroxidation, was increased and this rise damaged cell membranes lost their permeability. Following this, electrolyte leakage was increased as shown in Figure 3.e.

Anjum et al. (2015) found that electrolyte leakage and MDA content increased as a response to cadmium in maize plants likewise to our results. They also found that, Cd could be promoting the production of reactive oxygen species (ROS), inhibiting electron transport and basic reactions in PS2 (Sandalio et al., 2001). Also, Yordanova et al. (2017) stated that Cd application caused a higher level of electrolyte leakage in maize plants, too. It is expressed that, MDA produced as the final compound of membrane lipids peroxidation, so the MDA level is identified as an indicator of lipid peroxidation against stress (Chaoui et al., 1997; Shamsi et al., 2010).

The Effects of Cadmium Stress on Barley Cultivars' Cadmium and Mineral Content

Cadmium application resulted in metal accumulation in barley root and shoot tissues. All cultivars reflected higher Cd accumulation in root tissues. Higher cadmium doses resulted in higher metal accumulation can be seen in Table 3 and 4. In addition to these, Finola cultivar had the highest cadmium concentrations in all cadmium applications. Our results showed similarity with the findings of Akhter et al. as they cames to know that barley seedlings retained cadmium in their roots and there is a big difference between root and shoot cadmium concentration (Akhter et al., 2014). Earlier studies revealed that roots are main area of Cd accumulation in barley (Tamás et al., 2008) and other plants like sunflower (De Maria et al., 2013), tomato (López-Millán et al., 2009), soybean (Shamsi, 2010), wheat and maize (Zhao, 2011). Cadmium is easily taken by plants from solutions of cadmium compounds applied to the soil (Shacklette, 1972) and reaches to other parts of plant via vascular system in 24 hours.

Table 3 Effects of increasing cadmium concentrations on barley cultivars' shoot nutrient element and cadmium concentrations.

Cultivar	Treatment	Plant tissue	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Ca (%)	Mg (%)	K (%)	P (%))	Cd (%)
Finola	Control	Shoot	26,27±0,12	35,00±0,26	191,88±1,95	53,76±0,11	1,83±0,04	1,57±0,00	4,87±0,01	0,58±0,00	0,00±0,00
Finola	25 µM Cd	Shoot	29,55±0,11	42,74±0,44	115,62±2,68	56,37±0,03	1,37±0,01	1,52±0,01	5,17±0,01	0,65±0,00	9,88±0,10
Finola	50 µM Cd	Shoot	35,29±0,07	53,18±0,00	73,20±1,07	59,47±0,31	1,54±0,01	1,54±0,00	5,16±0,01	0,74±0,01	40,09±0,01
Finola	100 µM Cd	Shoot	31,78±0,78	47,34±2,94	68,99±1,12	55,80±0,39	1,47±0,04	1,52±0,04	4,96±0,12	0,76±0,06	70,00±0,18
Finola	150 µM Cd	Shoot	27,31±0,06	44,96±0,14	71,19±2,29	52,12±0,16	1,14±0,00	1,35±0,01	4,61±0,01	0,70±0,01	73,51±0,05
Sentosa	Control	Shoot	30,07±0,04	52,39±0,22	69,79±1,37	56,07±0,11	1,55±0,00	1,56±0,00	5,21±0,00	0,55±0,01	0,00±0,00
Sentosa	25 µM Cd	Shoot	31,08±0,91	50,31±2,27	69,63±1,10	56,44±0,23	1,16±0,34	1,49±0,05	5,09±0,11	0,64±0,08	5,59±4,84
Sentosa	50 µM Cd	Shoot	31,09±0,00	48,01±0,04	74,18±0,68	54,69±0,09	1,23±0,20	1,55±0,00	4,81±0,00	0,64±0,00	36,86±0,02
Sentosa	100 µM Cd	Shoot	25,13±0,03	42,02±0,17	74,63±1,02	47,08±0,11	1,30±0,00	1,49±0,00	4,73±0,01	0,56±0,01	51,57±4,77
Sentosa	150 µM Cd	Shoot	24,99±0,10	45,68±0,07	72,91±0,49	46,25±0,31	1,28±0,00	1,42±0,01	4,86±0,00	0,64±0,01	65,82±1,76
TARM 92	Control	Shoot	29,16±0,05	42,50±0,15	72,07±0,73	41,69±0,16	1,48±0,01	1,61±0,01	4,63±0,00	0,69±0,00	0,00±0,00
TARM 92	25 µM Cd	Shoot	34,79±0,02	52,08±0,04	71,74±2,24	44,59±0,18	1,48±0,00	1,56±0,00	4,81±0,01	0,67±0,00	7,10±0,09
TARM 92	50 µM Cd	Shoot	32,76±0,03	48,08±0,18	72,81±0,20	42,70±0,31	1,58±0,01	1,61±0,00	4,56±0,01	0,60±0,00	18,39±1,75
TARM 92	100 µM Cd	Shoot	28,97±0,03	43,85±0,04	68,60±0,65	39,79±0,26	1,51±0,00	1,55±0,00	4,61±0,00	0,68±0,01	43,66±0,02
TARM 92	150 µM Cd	Shoot	28,99±0,07	33,99±0,26	70,48±0,58	39,39±0,04	1,66±0,00	1,56±0,00	4,65±0,01	0,79±0,01	57,39±0,01

Effects of Cd application on the mineral concentrations in barley can be seen in Table 3 and 4. In cadmium polluted soils, it is a high possibility of its competition with nutrient elements. Calcium and cadmium had many physical similarities with a similar charge and ionic radius (Lachman et al., 2015). Generally, the competition between calcium and cadmium causes the decrease of calcium content but this is in contrast to our results. In roots there is no winner of this struggle; in shoots, except for Tarm 92 cv, cadmium affected accumulation of calcium.

Another mineral competes with cadmium is magnesium. It has same charge to cadmium and calcium. In a previous study in barley, magnesium nutrition prevented cadmium translocation to shoots (Kudo et al., 2015). In the present study, magnesium content of roots wasn't affected with cadmium but in shoots, cadmium caused decreasing of magnesium level. Fe content in shoots wasn't affected by cadmium (except Finola cv.), but in roots, Fe didn't show a regular increasing or decreasing regime. Brune and Dietz (1995), stated that transportation of Fe in barley seedlings was hardly affected and this may cause inhibition in the chlorophyll biosynthesis pathway. In the previous work, the Fe content of Finola and Tarm 92 cvs', had a similar trend with chlorophyll level. Same researches found no or little changes in potassium and phosphorus content in roots as our results, but the phosphorus level in shoots of them didn't change while potassium decreased against cadmium. Potassium level of barley shoots in our study, increased in Finola and Tarm 92 cv., but decreased in

Sentosa cv. Also phosphorus content of barley shoots increased against cadmium except for Tarm 92 cv's lower applications.

The shoot manganese, copper and zinc content of the barley seedlings in the present study showed similar trends against cadmium. The concentration of these microelements increased against low cadmium applications, later decreased again in higher doses. Zinc content of roots was decreased with increasing cadmium levels Copper concentration in roots, increased in Finola and Sentosa cvs., but decreased in Tarm 92. In roots, cadmium prevented accumulation of mangan.

Table 4 Effects of increasing cadmium concentrations on barley cultivars' root nutrient element and cadmium concentrations.

Cultivar	Treatment	Plant tissue	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Ca (%)	Mg (%)	K (%)	P (%)	Cd (%)
Finola	Control	Root	43,09±0,06	31,87±0,04	72,00±1,22	32,63±0,36	1,32±0,00	1,67±0,00	2,43±0,01	0,51±0,00	0,00±0,00
Finola	25 µM Cd	Root	35,82±0,13	36,83±0,36	71,94±0,20	34,99±0,25	1,67±0,00	1,70±0,00	2,36±0,00	0,57±0,00	251,59±0,82
Finola	50 µM Cd	Root	31,75±0,06	34,15±0,04	70,06±0,54	31,47±0,40	1,56±0,00	1,67±0,00	2,47±0,01	0,53±0,01	440,87±0,40
Finola	100 µM Cd	Root	32,97±0,01	36,54±0,11	71,65±1,07	30,21±0,31	1,60±0,00	1,67±0,00	2,35±0,00	0,62±0,01	547,40±0,53
Finola	150 µM Cd	Root	39,07±0,08	39,88±0,04	74,05±0,20	31,99±0,14	1,57±0,00	1,62±0,00	2,32±0,01	0,72±0,01	861,71±0,28
Sentosa	Control	Root	46,54±0,59	35,74±0,29	73,76±2,78	37,67±0,03	1,52±0,00	1,62±0,01	2,79±0,00	0,66±0,01	0,00±0,00
Sentosa	25 µM Cd	Root	33,33±0,39	34,11±0,04	75,54±0,52	33,59±0,20	1,23±0,00	1,67±0,01	2,79±0,00	0,70±0,01	153,84±0,02
Sentosa	50 µM Cd	Root	28,39±0,12	43,56±0,04	74,63±1,41	31,42±0,09	5,07±0,18	1,62±0,00	2,22±0,02	0,67±0,01	240,76±0,45
Sentosa	100 µM Cd	Root	28,35±0,06	42,14±0,15	74,05±1,32	30,03±0,16	0,99±0,04	1,59±0,01	2,37±0,01	0,74±0,01	408,04±5,12
Sentosa	150 µM Cd	Root	33,90±0,43	47,46±0,18	75,38±0,73	64,82±0,52	4,60±0,01	1,35±0,01	2,49±0,00	0,69±0,00	675,57±0,58
TARM 92	Control	Root	48,92±0,10	42,24±0,26	75,96±0,97	13,86±0,21	1,00±0,00	1,55±0,01	2,31±0,01	0,72±0,00	0,00±0,00
TARM 92	25 µM Cd	Root	46,24±0,29	28,53±0,04	75,51±0,73	9,12±0,26	1,85±0,01	1,67±0,01	2,01±0,02	0,66±0,00	134,05±0,47
TARM 92	50 µM Cd	Root	38,23±0,02	41,83±1,04	74,79±1,12	9,79±0,22	1,48±0,00	1,61±0,00	1,77±0,01	0,70±0,01	275,67±0,31
TARM 92	100 µM Cd	Root	37,36±0,03	25,25±0,58	78,17±0,00	8,39±0,15	1,82±0,01	1,62±0,01	1,92±0,00	0,58±0,00	454,24±0,29
TARM 92	150 µM Cd	Root	40,46±0,01	26,36±0,40	74,66±1,17	6,66±0,04	1,63±0,00	1,50±0,00	1,77±0,01	0,44±0,01	663,55±0,07

CONCLUSIONS

This study comprehensively explained the harmful effects of Cd on the root, shoot and various mineral concentrations. It was observed that a rapid decrease in chlorophyll contents was observed with an increase in Cd concentrations. Proline level and cell membranes (which indicated with MDA ontent and electrolyte leakege) were also afffected by Cd. An increase in the concentartions of manganese, copper and zinc content were observed with lower concentartions of Cd. As a result of these, Cd had toxic effects on barley seedlings' growth, chlorophyll content, biochemical structure and mineral uptake.

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REFERENCES

Ahmad, P., Nabi, G., Ashraf, M. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern.&Coss.] plants can be alleviated by salicylic acid. *South African Journal of Botany*, 77:36-44.

Akar, T., Avcı, M., Dusunceli, F. 2004. Barley. Post harvest operetions: http://www.fao.org/3/a-au997e.pdf. Acessed on 11.02.2019.

Akhter, M.F., Omelon, C.R., Gordon, R.A., Moser, D., Macfie, S.M. 2014. Localization and chemical speciation of cadmium in the roots of barley and lettuce. *Environmental And Experimental Botany*, 100: 10-19.

Akkuş, H., & Vural, H. (2023). Mevsimlik Çiçeklerin (*Impatiens balsamina, Zinnia elegans*) Tuz ve Su Stresine Karşı Dayanıklılığının Belirlenmesi. Türk Tarım ve Doğa Bilimleri Dergisi, 10(4), 933-943.

Anjum, S.A., Tanveer, M., Hussain, S., Bao, M., Wang, L., Khan, I., Shahzad, B. 2015. Cadmium toxicity in Maize (*Zea mays* L.): consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environmental Science and Pollution Research*, 22(21): 17022-17030.

Bahmani, R., Bihamta, M.R., Habibi, D., Forozesh, P., Ahmadvand, S. 2012. Effect of cadmium chloride on growth parameters of different bean genotypes (*Phaseolus vulgaris* L.). *ARPN Journal of Agricultural and Biological Science,* 7: 35-40.

Bates, L.S., Waldern, R.P., Teare, I.D. 1973. Rapid determination of free prolin for water-stress studies. *Plant and Soil*, 39: 205-207.

Beaty, R.D., Kerber, J.D. 1993. Concepts, instrumentation and techniques in Atomic Absorption Spectrophotomerty, Perkind Elmer. *Inc.: Shelton, CT*.

Brune, A., Dietz, K.J. 1995. A comparative analysis of element composition of roots and leaves of barley seedlings grown in the presence of toxic cadmium, molybdenum, nickel, and zinc concentrations. *Journal of Plant Nutrition*, 18(4): 853-868.

Campos, P.S., Quartin, V., Ramalho, J.C., Nunes, M.A. 2003. Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of Coffea sp. Plants. *Journal of Plant Physiology*, 160: 283–292.

Chang, S.X., Robison, D.J. 2003. Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. *Forest Ecology and Management*, 181(3): 331-338.

Chaoui, A., Mazhoudi, S., Ghorbal, M.H., El Ferjani, E. 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Science*, 127(2): 139-147.

Cherif, J., Derbel, N., Nakkach, M., Berhman, H., Jemal, F., Ben Lakhdar, Z. 2012. Spectroscopic studies of photosynthetic responses of tomato plants to the interaction of zinc and cadmium toxicity. *Journal of Photochemistry and Photobiology B: Biology*, 111: 9-16.

Çulha, Ş. ve Çakırlar, H. 2011. Tuzluluğun Bitkiler Üzerine Etkileri ve Tuz Tolerans Mekanizmaları. AKU J. Sci., 11 (2), 11-34.

De Maria, S., Puschenreiter, M., Rivelli, A.R. 2013. Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. *Plant, Soil and Environment*, 59(6): 254-261.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyonova, Z.P., Feller, U. 2006. Cadmium Stress in Barley: Growth, Leaf Pigment, and Protein Composition and Detoxification of Reactive Oxygen Species. *Journal of Plant Nutrition*, 29: 451–468.

Di Toppi, L.S., Gabbrielli, R. 1999. Response to cadmium in higher plants. *Environmental and Experimental Botany*, 41(2): 105-130.

Didwania, N., Jain, S., Sadana, D. 2019. In-vitro phytotoxic effects of cadmium on morphological parameters of allium cepa. *Biological*, 12(1): 137.

Dobrikova, A.G., Apostolova, E.L. 2019. Damage and Protection of the Photosynthetic Apparatus Under Cadmium Stress. In Cadmium Toxicity and Tolerance in Plants (pp. 275-298). Academic Press.

Dresler, S., Hawrylak-Nowak, B., Kovacik, J., Pochwatka, M., Hanaka, A., Strezemski, M., Sowa, I. and Wójciak, K. 2019. Allantoin attenuates cadmium-induced toxicity in cucumber plants. *Ecotoxicology and Environmental Safety*, 170: 120-126.

FAO Food Outlook Report, (2017). http://www.fao.org/3/a-i7343e.pdf. Acessed on 11.02.2019

Gallego, S.M., Benavides, M.P., Tomaro, M.L. 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science*, 121(2): 151-159.

Garnieret, J., Cébron, A., Tallec, G., Billen, G., Sebilo, M., Martinnz, A. 2006. Nitrogen behaviour and nitrous oxide emission in the tidal Seine River estuary (France) as influenced by human activities in the upstream watershed. *Biogeochemistry*, 77: 305-326.

Gill, S.S., Tuteja, N. 2011. Cadmium stress tolerance in crop plants: probing the role of sulfur. *Plant Signaling & Behavior*, 6(2): 215-222.

Hare, P.D., Du Plessis, S., Cress, W.A., Van Staden, J. 1996. Stress-induced changes in plant gene expression. Prospects for enhancing agricultural productivity in South Africa. *South African Journal of Science* (South Africa).

Hodges, D.M., Delong, J.M., Forney, C.F., Prange, R.K. 1999. Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207: 604-611

Jackson, M.L. 1962. Soil Chemical Analysis. Constable and Company, UK.

Kabata-Pendias, A., Mukherjee, A.B. 2007. *Trace elements from soil to human*. Springer Science & Business Media.

Koleva, L. 2010. Mineral Nutrients Content In Zinc- And Cadmium-Treated Durum Wheat Plants With Similar Growth Inhibition. *General and Applied Plant Physiology*, 36 (1–2): 60-63.

Kudo, H., Kudo, K., Uemura, M., Kawai, S. 2015. Magnesium inhibits cadmium translocation from roots to shoots, rather than the uptake from roots, in barley. *Botany*, 93(6): 345-351.

Lachman, J., Kotikova, Z., Zámečníková, B., Miholová, D., Száková, J., Vodičková, H. 2015. Effect of cadmium stress on barley tissue damage and essential metal transport into plant. *Open Life Science*, 10: 30-39.

Li, F., Qi, J., Zhang, G., Lin, L., Fang, P., Tao, A., Xu, J. 2013. Effect of Cadmium Stress on the Growth, Antioxidative Enzymes and Lipid Peroxidation in Two Kenaf (*Hibiscus cannabinus* L.) Plant Seedlings. *Journal of Integrative Agriculture*, 12(4): 610-620.

Li, M., Zhang, L.J., Tao, L., Li, W. 2008. Ecophysiological responses of Jussiaea rapens to cadmium exposure. *Aquatic Botany*, 88: 347-352.

Li, S., Yang, W., Yang, T., Chen, Y., Ni, W. 2015. Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of elsholtzia argyi—a cadmium accumulating plant. *International Journal of Phytoremediation*, 17(1): 85-92.

López-Millán, A.F., Sagardoy, R., Solanas, M., Abadía, A., Abadía, J. 2009. Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environmental and Experimental Botany*, 65(2-3): 376-385.

Marchiol, L., Leita, L., Martin, M., Peressotti, A., Zerbi, G. 1996. Physiological responses of two soybean cultivars to cadmium. *Journal of Environmental Quality*, 25: 562-566.

Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, 132: 272-281.

Pourghasemian, N., Landberg, T., Ehsanzadeh, P., Greger, M. 2019. Different response to Cd stress in domesticated and wild safflower (*Carthamus* spp.). *Ecotoxicology and Environmental Safety*, 171: 321-328.

Sandalio, L.M., Dalurzo, H.C., Gómez, M., Romero-Puertas, M.C., Río, L.A. 2001. Cadmium-induces changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, 52: 2115–2126.

Shacklette, H.T. 1972. Cadmium in plants (No. 1314). US Government Printing Office.

Shamsi, I.H., Jiang, L., Wei, K., Jilani, G., Hua, S., Zhang, G.P. 2010. Alleviation of cadmium toxicity in soybean by potassium supplementation. *Journal of Plant Nutrition*, 33(13): 1926-1938.

Shao, H-B., Chu, L-Y., Jaleel, C.A. ve Zhao, C-X. 2008. Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus Biologies, 331(3), 215-225.

Siddiqui, M.H., Al-Whaibi, M.H., Sakran, A.M., Basalah, M.O., Ali, H.M. 2012. Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. *International Journal of Molecular Sciences*, 13: 6604-6619.

Smart, R.E., Bingham, G.E. 1974. Rapid Estimates of Relative Water Content. *Plant Physiology*, 53: 258-260.

Song, J., Feng, S.J., Chen, J., Zhao, W.T., Yang, Z.M. 2017. A cadmium stress-responsive gene AtFC1 confers plant tolerance to cadmium toxicity. *BMC Plant Biology*, 17(1): 187.

Tamás, L., Dudíková, J., Ďurčeková, K., Halušková, L.U., Huttová, J., Mistrík, I., Ollé, M. 2008. Alterations of the gene expression, lipid peroxidation, proline and thiol content along the barley root exposed to cadmium. *Journal of Plant Physiology*, 165(11): 1193-1203.

Tamás, L., Mistrík, I., Alemayehu, A., Zelinová, V., Bočová, B., Huttová, J. 2015. Salicylic acid alleviates cadmium-induced stress responses through the inhibition of Cd-induced auxin-mediated reactive oxygen species production in barley root tips. *Journal of Plant Physiology*, 173: 1-8.

Tiryakioğlu, M., Eker, S., Özkutlu, F., Husted, S., Cakmak, İ. 2006. Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. *Journal of Trace Elements in Medicine and Biology*, 20: 181-189.

Vassilev, A., Berova, M., Zlatev, Z. 1998. Influence of Cd2+ on growth, chlorophyll content, and water relations in young barley plants. *Biologia Plantarum*, 41(4): 601-606.

Wilkins, D.A. 1978. The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist*, 80: 623–633.

Wu, F., Zhang, G., Dominy, P. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environmental and Experimental Botany*, 50: 67-78.

Wu, F.B., Chen, F., Wei, K., Zhang, G.P. 2004. Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. *Chemosphere*, 57(6): 447-454.

Xu, W.F., Shi, W.M., Yan, F., Zhang, B., Liang, J.S. 2011. Mechanisms of cadmium detoxification in cattail (*Typha angustifolia* L.). *Aquatic Botany*, 94: 37-43.

Yılmaz, H. Ş., & Kökten, K. (2019). Kadmiyum (Cd) uygulamasının tane sorgumda (Sorghum bicolor L.) bazı morfolojik özellikler üzerine etkisinin belirlenmesi. Türk Tarım ve Doğa Bilimleri Dergisi, 6(3), 447-456.

Yordanova, R., Baydanova, V., Peeva, V. 2017. Nıtrıc oxıde medıates the stress response ınduced by cadmıum ın maıze plants. *Genetics and Plant Physiology*, 7(3–4): 121–134.

Yuanjie, D., Weifeng, C., Xiaoying, B., Fengzhen, L., Yongshan, W. 2019. Effects of exogenous nitric oxide and 24-epibrassinolide on the physiological characteristics of peanut seedlings under cadmium stress. *Pedosphere*, 29(1): 45-59.

Zhao, L.J., Xıe, J.F., Zhang, H., Wang, Z.T., Jıang, H.J., Gao, S.L. 2017. Enzymatic activity and chlorophyll fluorescence imaging of maize seedlings (*Zea mays* L.) after exposure to low doses of chlorsulfuron and cadmium.

Zhao, Y. 2011. Cadmium accumulation and antioxidative defenses in leaves of *Triticum aestivum* L. and *Zea mays* L. *African Journal of Biotechnology*, 10(15): 2936-2943.

Zhao, Y., Hu, C., Wu, Z., Liu, X., Cai, M., Jia, W., Zhao, X. 2019. Selenium reduces cadmium accumulation in seed by increasing cadmium retention in root of oilseed rape (*Brassica napus* L.). *Environmental and Experimental Botany*, 158: 161-170.

Zohary, D., Hopf, M. 1994. Domestication of plants in the Old World, 2nd edn. Clarendon.