

EFFECTS OF GA₃ HORMONE TREATMENTS ON ION UPTAKE AND GROWTH OF PEPPER PLANTS UNDER CADMIUM STRESS

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Abstract. The present study was conducted to identify the response of pepper plants under cadmium stress to gibberellic acid (GA₃) treatments. Plants were exposed to different cadmium chloride doses (0, 20, 40, 60 ppm) cadmium chloride (CdCl₂) and GA₃ (10 ppm) treatments. A resistance scale was used based on symptoms over the leaves and mineral element analyses (K, Cu, Zn, Fe, Mn, Mg and Cd) were performed. Experiments were conducted in hydroponic culture with Demre pepper cultivar under controlled conditions. Present results revealed significant effects of Cd treatments on Cd, Fe, Zn, Cu, Mn, Mg and K concentrations of the plants. Combined treatments of Cd and GA₃ (10 ppm) also increased ion accumulation especially in leaves. It was observed under stress conditions that GA₃ hormone treatments inhibited plant growth to prevent stress, increased ion uptake, well-adjusted ion balance and let the plants avoid from stressors.

Keywords: *cadmium, gibberellic acid, heavy metal stress, ion accumulation, pepper (Capsicum annuum L.)*

Introduction

Industrialization and urbanization bring about serious environmental pollution and exert significant threats on nature (Güven et al., 1999).

Even the trace amounts of heavy metals in air, soil and water resources may be dangerous for all living things. Agricultural and industrial activities may significantly increase especially cadmium (Cd) concentrations in the air surrounding us. The presence of heavy metals in trace amounts in soil, water and air can be dangerous to all living things, and the concentration of Cd in the environment is increasing due to agricultural and industrial factors (Foy et al., 1978). Such an increase is mostly resulted from anthropogenic sources and ultimately passed into the agricultural lands through sewage sludge treatments and fertilizer applications (Doğan and Saygıdeğer, 2009).

Cadmium may reduce germination, growth, development, yield and quality of various plants. Several studies were carried out to determine the threshold values and to investigate the morphological and physiological effects of Cd toxicity (Bertin and Averbeck, 2006).

Whether or not being essential element for plant growth, excessive heavy metal accumulation in plant tissues and organs negatively influence vegetative and generative organs of the plants (Gür et al., 2004). With toxic impacts, heavy metals may damage several physiological processes such as transpiration, stomatal conductance, enzyme activity, germination, protein synthesis, membrane stability and hormonal balance (Kennedy and Gonsalves, 1987). Toxicity may vary both from one metal to another and from one organism to another. Positive or negative (toxic) impacts not only depend on element type and concentration, but also closely related to genetic-based physiological responses of different species (Haktanır and Arcak, 1998).

Cadmium stress reduces water and ion uptake of plants and hinders root growth and development. Stomata also close under cadmium stress, thus water loss through transpiration is reduced and cadmium transport is hindered (Salt et al., 1995). Cd accumulation may have toxic impacts on plants and damage mineral nutrition and carbohydrate metabolism of the plants, and then ultimately limits plants growth and development (John et al., 2009). Cadmium also inhibits chlorophyll biosynthesis and reduces total chlorophyll content (Stobart et al., 1985). Zinc and iron are essential micro nutrients for several biochemical processes in plants (Marschner, 1995). Cadmium interacts with these nutrients and directly influences nutrient uptake. Such interactions ultimately influence nutrient distribution, results in nutrient deficiency/imbalance and recesses plant growth and development (Zhang et al., 2002).

There is a consensus on stimulation of internal hormone levels through external application of different growth regulators (El-Shahaby et al., 1992). Rodriguez et al. (2006) indicated that the hormones in EP (extracellular products) produced by Cyanobacteria developed salt-tolerance in paddy seedlings. It was also indicated in the same study that GA treatments stimulated abscisic acid (ABA) production as a response to salt stress and altered and reduced the ratios of growth regulators.

The present study was conducted with pepper plants of Solanaceae family, mostly produced as early grown or summer plant in Turkey, to investigate: a) the effects of cadmium on metabolic activities of the plants; b) the response of plants and adaptation mechanisms developed against this pollutant; c) stress relief through gibberellic acid treatments; d) correlations of plant growth regulators with ion uptake and accumulation in plants.

Materials and Methods

Experiments for stress factors and plant growth were conducted in hydroponic culture in a climate cabin with a split air-conditioner to provide normal atmosphere.

Pepper seeds were sown in pumice-filled plastic germination containers (40x25x5 cm). Following the sowing of 100 seeds to each container, irrigation was performed with tap-water. Containers had 9 holes (0.5 cm) beneath to drain the irrigation water. Pumice was thoroughly wetted and excess irrigation water was drained. Then germination containers were placed in climate cabin with 25°C temperature and 70% relative humidity. Containers were covered with moist paper and regularly controlled. Gradual tap water applications were continued to prevent the drying of pumice. Irrigations were started to be performed with Hoagland nutrient solution when the seedlings had horizontal cotyledon leaves and the first true leaves (Hoagland and Arnon, 1938). The seedlings with the 2nd true leaves were transplanted into hydroponic culture. Plastic cuvettes (25x25x18 cm) filled with Hoagland nutrient solution were used for hydroponic culture. Pepper seedlings were wrapped around with small sponge pieces and placed in specially designed platforms with holes over for each seedling. These platforms were placed over the cuvettes as to have the roots immersed in nutrient solution. Aeration was supplied through the nutrient solution with thin plastic hoses connected to two aquarium pumps.

Following the growth of seedling in hydroponic culture for two weeks, cadmium treatments were initiated. Experiments were conducted by completely randomized design with three replications with 20 plants in each replication. For cadmium treatments, 0, 20, 40, 60 ppm CdCl₂ doses were added to nutrient solution (1/2 Hoagland). Solutions were renewed weekly and care was taken to sustain the same

cadmium concentrations in each renewal. Together with cadmium, 10 ppm gibberellic acid was applied to plants. Sampling for measurements and analyses were made 15 days after cadmium treatments. Samples were used to determine some plant growth parameters (green herbage fresh weight, number of leaves, plant heights, cadmium resistance scales based on leaf symptoms) and to determine some physico-chemical parameters (Cd, K, Cu, Zn, Fe, Mn, Mg contents).

A total of 8 different treatments were performed (control, cadmium 20, 40, 60 ppm, cadmium 20, 40, 60 ppm + gibberellic acid GA₃ (10 ppm).

Mineral element analysis

Three leaves from tip to downward were taken and they were kept in deep freezer at –40°C. About 200 g samples were taken from the deep freezer and samples were supplemented with 10 ml 0.1 N HNO₃ (Nitric acid). They were then kept in plastic boxes at dark and room temperature for a week. Samples were shaken in a shaker for 24 hours and resultant extract was subjected to K⁺, Cu⁺, Zn⁺, Fe⁺, Mn⁺, Mg⁺, Cd ion analyses in flame photometer (Eppendorf flame photometer). Fresh leaf ion concentrations were expressed in µg/mg fresh weight (Taleisnik et al., 1997).

Experiments were conducted in randomized plots design with 3 replications. Statistical analyses for plant growth parameters, ion and enzyme data were performed with SAS (1985) software.

Results

Data about plant growth parameters are provided in *Table 1*. While the greatest root weight was obtained from the control treatment, the lowest value was obtained from Cd3+GA₃ treatment. The same treatments had the similar characteristics for root lengths. Control treatment had the greatest stem weight and it was followed by GA₃ treatment. As compared to the control treatment, the greatest decrease was observed in Cd+GA₃ treatment, but it was placed in the same statistical group.

Table 1. Plant growth and development parameters

Applications	Root weight	Root height	Stem weight	Stem height	Leaf weight	Number of leaves	Total plant weight
	(g)	(cm)	(g)	(cm)	(g)	(number)	(g)
Control	2.303 A	19.833 A	1.778 A	11.833 A	6.670 A	9.500 A	10.751 A
Cd1+GA ₃	1.220 C	10.550 CD	0.790 C	10.500 A	2.930 D	7.000 C	4.940 EF
Cd2+GA ₃	1.991 AB	14.167 BC	0.713 C	9.667 A	2.571 DE	6.833 C	5.275 DE
Cd3+GA ₃	1.075 C	9.333 D	0.615 C	10.167 A	1.900 E	6.000 C	3.590 F
Cd1	1.348 BC	15.833 AB	1.365 AB	11.000 A	4.483 C	7.333 BC	7.196 C
Cd2	1.510 BC	16.833 AB	1.323 B	11.000 A	3.733 C	7.333 BC	6.743 CD
Cd3	1.298 C	16.667 AB	1.311 B	10.167 A	2.898 D	7.066 C	6.908 CD
GA ₃	1.686 A-C	14.167 BC	1.725 A	11.667 A	5.686 B	9.166 AB	9.097 B

Means indicated with the same letters in the same column are not significantly different.

There were not any significant differences in stem lengths of the treatments. Control treatment had the greatest leaf weight. As compared to the control treatment, the greatest decrease was observed in Cd3+GA₃ treatment. The results for number of leaves were similar with the results for leaf weight. The greatest plant weight was observed in control treatment and the greatest decrease as compared to control treatment was observed in Cd3+GA₃ treatment. With regard to all growth parameters, it is remarkable that the lowest values were seen in Cd3+GA₃ treatment. Again as compared to the control treatment, leaf weight, number of leaves, total plant weight decreased with increasing cadmium doses.

As compared to control and single Cd treatments, Cd + GA₃ treatments increased root, stem and leaf Cd concentrations. The greatest leaf Cd accumulation in single Cd treatments was observed in leaves (*Table 2*). The greatest root cadmium concentrations were observed in Cd3+ GA₃, Cd2+ GA₃ treatments and they were followed by Cd1+ GA₃ treatment. The greatest stem cadmium concentration was observed in Cd3+ GA₃ treatment and it was followed by Cd2+ GA₃ and Cd1+ GA₃ treatments. The greatest root and stem cadmium concentrations were observed in Cd3+GA₃ treatments and increasing values were observed with increasing treatment doses. The same case was not valid for leaves; the greatest leaf cadmium concentration was obtained from Cd3 treatment and it was followed by Cd2 and Cd1 treatments.

Table 2. Root, stem and leaf Cd concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Cd	Cd	Cd
Control	1.17 D	0.786 E	0.629 E
Cd1+GA ₃	592.32 B	20.678 B	8.183 D
Cd2+GA ₃	737.52 A	21.895 B	19.896 C
Cd3+GA ₃	748.40 A	27.500 A	20.759 C
Cd1	438.16 C	5.373 D	27.895 B
Cd2	468.75 C	9.718 C	31.284 AB
Cd3	488.08 C	12.152 C	34.463 A
GA ₃	1.14 D	0.505 E	0.447 E

Means indicated with the same letters in the same column are not significantly different.

There were remarkable differences in Fe contents of plant organs in different treatments (*Table 3*). While the greatest root Fe content was obtained from the control treatment, the greatest stem Fe content was obtained from GA₃ treatment and the greatest leaf Fe content was obtained from the control and GA₃ treatments. The lowest root and stem Fe contents were obtained from Cd3+GA₃ treatment and the lowest leaf Fe content was obtained from Cd1 treatment.

Considering the Zn contents of the roots, stems and leaves of treated plants, the greatest values were obtained from the control and GA₃ treatments and the lowest values were obtained from single Cd treatments. As compared to control and single GA₃ treatments, Zn contents decreased with Cd + GA₃ treatments, but increased with single Cd treatments (*Table 4*).

Table 3. Root, stem and leaf Fe concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Fe	Fe	Fe
Control	2007.17 A	67.868 C	117.069 A
Cd1+GA ₃	1975.02 AB	70.118 C	83.241 B
Cd2+GA ₃	1732.66 C	52.837 D	83.141 B
Cd3+GA ₃	1234.88 E	37.097 E	51.767 D
Cd1	1456.66 D	31.742 E	35.582 E
Cd2	1535.60 D	31.872 E	47.357 D
Cd3	1728.47 C	37.438 E	60.662 C
GA ₃	1815.32 BC	92.548 A	123.345 A

Means indicated with the same letters in the same column are not significantly different.

Table 4. Root, stem and leaf Zn concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Zn	Zn	Zn
Control	55.601 A	9.5197 A	9.6410 BC
Cd1+GA ₃	50.569 AB	8.5043 B	8.3917 BC
Cd2+GA ₃	50.833 AB	8.9163 AB	8.7853 BC
Cd3+GA ₃	48.915 AB	7.0087 C	8.3247 BC
Cd1	33.755 DE	4.6890 D	5.9730 D
Cd2	39.789 CD	4.2420 D	5.9220 D
Cd3	35.123 DE	4.9230 D	5.7107 D
GA ₃	56.944 A	9.7750 A	9.2063 AB

Means indicated with the same letters in the same column are not significantly different.

With regard to Cu accumulation in roots, stems and leaves of pepper plants, similar with Fe and Zn, the greatest values were observed in control and GA₃ treatments without Cd treatments. As compared to control treatment, decrease was observed with Cd + GA₃ treatments; however Cu accumulation levels were lower in single Cd treatments (Table 5).

Considering Mn accumulation in roots, stems and leaves of pepper plants, the greatest values were observed in control and GA₃ treatments. As compared to control treatment, Cd + GA₃ treatments generally decreased Mn contents of roots and leaves, but yielded higher Mn contents than single Cd treatments. Cd+GA₃ treatments yielded the same stem Mn contents with the control treatment, but higher than Cd treatments (Table 6).

Table 5. Root, stem and leaf Cu concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Cu	Cu	Cu
Control	13.379 A	3.6810 A	3.8047 A
Cd1+GA ₃	10.021 BC	1.8233 CD	3.0947 AB
Cd2+GA ₃	11.060 B	2.3680 BC	2.6833 B
Cd3+GA ₃	9.781 BC	2.8907 B	2.7610 B
Cd1	8.319 C	1.4003 DE	2.7590 B
Cd2	8.962 BC	1.1940 E	2.3107 C
Cd3	8.660 BC	1.6737 DE	2.1593 C
GA ₃	12.549 A	3.7523 A	3.7723 A

Means indicated with the same letters in the same column are not significantly different.

Table 6. Root, stem and leaf Mn concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Mn	Mn	Mn
Control	265.12 A	10.4447 A	25.054 A
Cd1+GA ₃	180.62 BC	10.7663 A	22.349 B
Cd2+GA ₃	256.66 A	10.3053 A	23.168 B
Cd3+GA ₃	202.31 B	10.3833 A	22.392 B
Cd1	98.69 D	5.8190 B	11.960 D
Cd2	109.17 D	6.8473 B	11.721 D
Cd3	98.34 D	6.8153 B	15.042 C
GA ₃	243.89 A	10.8620 A	27.505 A

Means indicated with the same letters in the same column are not significantly different.

Control treatments had the greatest root Mg contents. However, contrary to other ion concentrations, single GA₃ treatments had the least Mg accumulation levels. Cd + GA₃ treatments also had lower Cd accumulations than single Cd treatments. With regard to stem Mg contents, Cd+GA₃ treatments were placed in the same statistical group with control treatments, but single Cd treatments had lower stem Cd contents and were places in different statistical group. As it was in other ions, the differences in root, stem and leaf Mg contents of different cadmium doses were not found to be significant (Table 7).

Table 7. Root, stem and leaf Mg concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	Mg	Mg	Mg
Control	61.354 A	30.467 A	27.889 A
Cd1+GA ₃	24.376 CD	29.546 AB	29.322 A
Cd2+GA ₃	22.798 D	30.160 AB	27.485 A
Cd3+GA ₃	23.237 D	26.924 B	23.255 B
Cd1	38.136 B	13.390 D	21.836 B
Cd2	31.969 BC	17.091 C	22.644 B
Cd3	29.839 BC	16.864 C	23.536 B
GA ₃	19.182 D	29.389 AB	28.706 A

Means indicated with the same letters in the same column are not significantly different.

Table 8. Root, stem and leaf K concentrations (μ g/mg T.A.)

APPLICATIONS	ROOT	STEM	LEAF
	K ⁺	K ⁺	K ⁺
Control	306.82 A	339.97 A	317.73 A
Cd1+GA ₃	157.68 B	327.60 A	284.20 AB
Cd2+GA ₃	164.41 B	334.99 A	278.43 AB
Cd3+GA ₃	158.21 B	325.24 A	278.09 AB
Cd1	126.95 CD	230.64 B	259.45 BC
Cd2	134.78 C-D	224.87 B	214.04 C
Cd3	132.69 C-D	239.86 B	216.80 C
GA ₃	160.34 B	331.72 A	331.36 A

Means indicated with the same letters in the same column are not significantly different.

Potassium (K) contents of root, stem and leaf samples are provided in Table 8. The greatest root K content was obtained from the control treatment and it was respectively followed by Cd+GA₃ treatments and single GA₃ treatment. The least root K content was obtained from single Cd treatments, but the differences between Cd doses were not significant. The differences in stem and leaf K contents of different Cd doses were not also found to be significant. Only the differences between Cd and GA₃ treatments were found to be significant.

Discussion

Current findings revealed that cadmium significantly hindered root, stem and leaf growth of pepper seedlings. However, differences in plant growth parameters of different cadmium doses were not found to be significant. Despite the insignificant differences in stem lengths, Cd + GA₃ treatments inhibited plant growth and development. Current findings about the effects of cadmium comply with results of various earlier studies carried out with different plants. It was previously reported that cadmium inhibited root and stem growth of *Vigna unguiculata* L. var. Pusa falguni plants (Nagor, 1997); cadmium, copper, lead and zinc reduced root and shoot development of *Sorghum bicolor* L. plants (Pandit and Prasannakumar, 1999). Cadmium and nickel reduced root and stem lengths of *Oryza sativa* L. cv. Bahia plants (Moya et al., 1993); zinc reduced stem lengths of *Brassica juncea* seedlings (Prasad et al., 1999); cadmium also reduced stem and root lengths of *Zea mays* L. Dekalp cv. 73 Sponsor plants (Rascio et al., 1993). Similarly, Zengin and Munzuroğlu (2003) investigated the effects of cadmium (CdCl₂.H₂O) on root, stem and leaf growth of bean seedlings and reported that cadmium significantly hindered root, stem and leaf growth and inhibitions were parallel to increasing cadmium doses. As compared to control treatment, plant growth was slow in Cd+GA₃ treatments. Yasar et al. (2016) applied GA₃ to eggplant seedlings under salt stress and reported selective ion uptake of plants. As reported by Ashraf et al (2001), GA₃ might have reduced nitrogen uptake of the plants. Combined application of GA₃ and cytokine-like growth regulators may provide positive contribution in elimination of salt stress (Xiong et al., 2002). Lin and Kao (1995) and Rodriguez et al. (2006) also reported that GA₃ treatments reduced the growth inhibition of paddy and some other plants.

Cd treatments increased root, stem and leaf Cd concentrations as compared to control and single GA₃ treatments. Cd accumulation in roots and stems were higher in Cd + GA₃ treatments than in single Cd treatments. However, an inverse case was valid for leaves. It was observed that GA₃ prevented toxic ion transport to leaves to prevent heavy metal toxicity. Several researchers reported increasing plant Cd concentrations with increasing Cd doses. Bachir et al. (2004) reported increasing plant Cd concentrations with Cd treatments in cotton; Safarzadeh et al. (2013) reported the same case in paddy plants. Tiryakioğlu et al. (2006) reported that increasing Cd doses increased green herbage and especially root Cd concentrations, majority of cadmium taken up by the plants accumulated in roots and slight amounts were transported to green herbage.

Cadmium treatments significantly reduced Fe, Zn and Cu concentrations of all plant organs. However, as compared to single Cd treatments, Cd+GA₃ treatments increased Fe, Zn and Cu accumulations in all three plant organs. Single GA₃ treatments also increased stem and especially leaf Fe, Zn and Cu accumulations as

compared to single Cd treatments. Similarly, Bachir et al. (2004) reported that 0.1 and 1 μ M Cd treatments increased Fe, Zn and Cu concentrations of cotton plants and indicated a potential synergic effect of Cd on Fe and Zn uptake. Köleli et al. (2004) reported under Zn-deficient conditions that increasing Cd treatments reduced green herbage Fe concentrations; however, Cd treatments did not have significant effects on green herbage Fe concentrations under Zn-sufficient conditions. Safarzadeh et al. (2013) also reported that Cd treatments significantly reduced green herbage Zn and Fe concentrations. Wu et al. (2004) carried out a study about the effects of cadmium on micro element uptake and transport in cotton plants and reported that 0, 0.1 and 1 μ M Cd treatments did not change leaf Fe, Zn and Cu concentrations significantly, but 10 μ M Cd treatment significantly increased leaf micro element concentrations. Amal et al. (2014) applied GA₃ to barley plants under salt stress and reported decreasing Zn, Fe, Co, Pb, Cr, Cd and Mn ion accumulation under salt stress and indicated that GA₃ treatments relieved the impacts of salt stress and increased ion uptake levels. Akman (2009) also reported similar results for Fe, Zn and Cu ion accumulation in wheat.

As it was in other ion accumulations, Cd and GA₃ treatments yielded similar results for K, Mn and Mg accumulations. Cd treatments reduced the accumulation levels of all ions, except for Cd; GA₃ treatments also yielded slight decreases in accumulation of all ions again except for Cd as compared to control treatment, but increased ion accumulation as compared to single Cd treatments. Mohamed and Gomaa (2012) and Amal et al. (2014) carried out a study with barley plants under salt stress and reported that GA₃ treatments reduced K, Mn and Mg ion accumulation levels in plants and GA₃ treatments also reduced the impacts of salt stress and increased ion uptakes. Similarly, Schachtman and Lio (1999) in barley and Iqbal and Ashraf (2013) in wheat plants under salt stress, reported that salt stress reduced ion uptake in GA₃ treatments, but GA₃ treatments improved ion uptake of plants under salt stress.

It was concluded based on current findings that combined GA₃ and Cd treatments did not have positive contributions to plant growth, but plant under these treatments behaved selectively in ion uptakes to prevent from toxic impacts of Cd. Such impacts of growth regulation hormone GA were also reported by previous researchers. When applied to plants under abiotic stressors, GA inhibited plant growth through synthesis of DELLA proteins. GA treatments increase the activity of this protein and may have positive contributions to stress tolerance of the plants since it inhibits plant growth under abiotic stress conditions (Achard et al., 2006; Achard et al., 2008a; Magome et al., 2008). Increased DELLA activity with GA treatments limits the accumulation of reactive oxygen species (ROS) under Cd stress, thus prevents cell deaths (Achard et al., 2008b). DELLA proteins were reported as behaved like inner-cell suppressors of GA-induced metabolic activities (Peng et al., 1997; Silverstone et al., 1998; Ogawa et al., 2000; Ikeda et al., 2001; Chandler et al., 2002).

Conclusion

When we evaluate the data we obtained without working, we found that the GA₃ application increased ion uptake in plants, GA₃ treatment with Cd inhibited plant growth, but Cd and GA₃ treatments did not cause damage to plants caused by strthenogenesis, even though Cd stress was applied to plants. The reason for this is that it may be originated from DELLA proteins that inhibit enzyme activity when GA₃ is

applied to plants at the time of stress. Enzyme activities and DELLA proteins should be looked for in order to be fully illuminated. In addition, after application of GA₃ at the time of stress, the levels of organic acid in the cell should be monitored.

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