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Full Length Research Paper

Effects of some heavy metals and heavy metal hormone interactions on wheat (*Triticum aestivum* L. cv. Gun 91) seedlings

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In this study, effects of heavy metals which are lead (Pb), zinc (Zn) and cadmium (Cd), alone, and their interactions with abscisic acid (ABA) and gibberellin (GA_3) hormones on soluble proteins, soluble phenolics and the amount of free proline have been investigated with respect to time (5th and 10th) days in wheat seedlings. At seedlings on which heavy metals and heavy metals + hormones applied, the amount of free proline and soluble phenolics have increased. Amount of soluble protein has increased at the seedlings on which Pb and Cd applied but has decreased at the 10th day samples of the seedlings on which Zn, Zn + GA_3, Cd + ABA and Cd + GA_3 were applied. Among the parameters worked with, Cd has shown the most toxic effects and lead and zinc followed.

Key words: Wheat, heavy metal stress, proline, soluble protein, soluble phenolics.

INTRODUCTION

Those heavy metals that can be assimilated easily by plants at high concentrations inhibit the plants development and growth. Additionally, the metals damage cell membranes within the plants, decreasing transpiration, destroying protein synthesis, damaging the organelles related to photosynthesis - thus inhibiting photosynthesis and affecting enzyme activation - and increasing lipid peroxidation (Foy et al., 1978; Sanitá di Toppi and Gabrielli, 1999). These heavy metals accumulate in plant tissue and threaten human health further along food chain. The protection mechanisms of the plants are affected by environmental stress, as is the ability of plants to accumulate proline (Chen et al., 2003). Plant growth regulators especially ABA are needed for plants in different stress conditions (Zeevart and Creelman, 1988). It is also well known that plant hormones play important roles in the movement of ions. The aim of this study was the heavy metals (Cd, Zn and Pb) and interactions between the heavy metals and the plant hormones, GA_3 and ABA on free proline, soluble proteins and soluble phenolic content in the wheat (*Triticum aestivum* L. Gun 91) seedlings.

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MATERIALS AND METHODS

The wheat seedlings used in this study (*T. aestivum* cv. L. Gun 91) were provided by Tarla Bitkileri Merkez Arastirma Institute (Ankara). Cadmium, zinc and lead ($CdCl_2$, $ZnCl_2$, $[Pb(NO_3)_2]$) are used as heavy metals in this study. The seeds germinated in Petri dishes under sterile conditions at a temperature of 25 ± 2°C for 48 h. They were transfered to pots containing a mixture of sand-perlite (1:1, v:v) and irrigated with distilled water. The seedlings were grown 5 days in greenhouse conditions. The wheat seedlings were taken into the Arnon and Hoagland (1940) solutions (pH 6) and they were incubated for eight days in greenhouse conditions by ventilation. The nutrition solution was refreshed once every eight days. Heavy metals (Pb, Zn, Cd) and hormones (ABA, GA_3) were been introduced into the nutrient solution at concentrations of control (0), 100, 200 and 300 µM. In hormone treatments with heavy metals, hormones were applied into the nutrient solution as 14 µM (GA_3) and 19 µM (ABA) (Moya et al., 1995). The plants used in this study were grown from June to August under green house conditions where the temperature was between 20 to 35°C, relative humidity was 65 to 80% and the luminous intensity was about 13,000 to 34,000 lux. The seedlings treated with heavy metals and hormones were cropped at day 5 and day 10. Twigs of seedlings were prepared for analysis drying through lyophilization. Free proline extraction and determination were carried out on freeze dried material (Bates et al., 1973). Total soluble phenolics extraction and determination were performed spectrophotometrically against a chlorogenic acid standard (Ferraris et al., 1987). Soluble protein extraction (Jordan et al., 1992) and determination were performed spectrophotometrically against bovine serum albumin (Lowry et al., 1951).

Data obtained from measurements and biochemical analyses have been evaluated statistically at factorial level by means of variance analyses (ANOVA) and their significance levels ($P < 0.05$ or 0.01) have been determined. To determine whether there was any statistical difference between the compared groups in the variance analysis, one of the multiple comparison techniques – the Turkey Test – was used to determine the different groups.

RESULTS AND DISCUSSION

The effects of heavy metals-hormone interactions on the amount of free proline

Comparing day-5 samples of seedlings treated with Pb, and Pb with ABA and GA_3, the amounts of free proline is higher than those of the seedlings treated with only Pb in the same concentration. Similarly, at day 10, in samples with a combined treatment of ABA and Pb, the amount of free proline increased (Table 1). Poschenrieder et al. (1989) observed that the amount of ABA was increased in *Phaseolus vulgaris* seedlings treated with Cd. On the other hand, they did not notice any significant change in the amount of stress proteins. It is known that proline is accumulated as a response to environmental stress (Öncel et al., 2000). However, aspartic acid and some derivatives of aspartic acid such as asparagine, isoleucine, leucine and valine amino acids are also accumulated in plants (Hsu and Kao, 2003).

In our study, the reason for reduced proline in seedlings treated with only Pb and Pb, ABA, and GA_3 together might be the accumulation of amino acids which have hydrophilic characteristics. When the samples from seedlings treated only with Zn and Zn with ABA and GA_3 together were compared at day 5 and 10, it was observed that the amount of free proline in the seedlings treated with Zn + GA_3 at high concentrations was higher than the seedlings treated with only Zn and Zn + ABA. This might be caused by GA_3 that increases the accumulation of free proline to prevent toxic effects of Zn. The amount of free proline in seedlings treated with 300 µM Zn + ABA was about 1.5 times more than the seedlings treated with 300 µM Zn (Table 1). When the day 5 samples of seedlings treated with only Cd and seedlings treated with Cd, ABA, and GA_3 were compared, in terms of the amount of free proline, it was determined that free proline levels were higher in the seedlings treated with Cd, ABA, and GA_3 together than the seedlings treated with only Cd.

It was determined that ABA and GA_3 hormones caused the amount of free proline to increase in the seedlings treated with high levels of the Cd concentration, compared to those seedlings treated only with Cd during the 10 day experiment period. Talanova et al. (2000) stated that the amount of ABA, free proline, and soluble proteins increase in seedlings that are treated with Pb and Cd. They also state that an increase in the amount of ABA triggers protein synthesis (Table 1).

The effects of heavy metals-hormone interactions on the amount of soluble protein

When samples of seedlings treated only with Pb and seedlings treated with Pb, ABA, and GA_3 are compared at day 5, those treated with the combined solution showed an observable increase in the amount of soluble protein at lower concentrations (Table 2). The amount of protein decreased in the seedlings treated with 300 µM Pb + ABA, when compared, those treated with 300 µM Pb and 300 µM Pb + GA_3, particularly in the day-10 samples. The decrease in soluble proteins in the seedlings with high levels of Pb and ABA, like 300 µM, might be caused by the effect of ABA to promote degradation of existing proteins or inhibit total protein synthesis. This situation might be due to the degradation of proteins to amino acids or new protein synthesis. When samples of seedlings treated only with Zn and seedlings treated with ABA, GA_3 and Zn together were compared at day 5, the amount of soluble protein was higher in the seedlings treated with the highest concentrations of Zn, rather than the seedlings treated with ABA, GA_3 and same amount of Zn together. However, depending on the length of the treatment duration, particularly in the seedlings treated with 300 µM Zn + ABA, the amount of protein was observed to be higher when compared to other treatments. It is known that plants increase ABA synthesis to enhance strength in stressful conditions. The rise in the soluble proteins depended on the length of the treatment duration in seedlings treated with 200 and 300 µM Zn and ABA, compared to the seedlings treated with GA_3, and may be caused by the ABA that is used with Zn at these concentrations, since ABA causes the synthesis of stressed proteins to increase the deactivation of toxic effects of Zn (Table 2). We agree with the researchers that it is important to monitor the changes of specific proteins instead of monitoring total or soluble protein content under stress conditions.

It is known that heavy metal stress causes the increase

Table 1. Illustrations of the differences in the amount of free proline in wheat seedlings when it was grown under heavy metal and combined heavy metal-hormone treatments (n = 3) (upper case letters represent concentration differences and lower case letters represent the differences between days).

Heavy metal	Concentration (µM)	Free prolıne (µmol/g DW)	
		5th day	10th day
Pb	Control	19.94±1.05A[a]	25.67±1.21A[b]
	100	23.38±0.86A[a]	35.29±0.74B[b]
	200	25.25±0.72A[a]	38.11±0.68B[b]
	300	41.13±2.16B[a]	47.47±0.18C[b]
	Control +ABA	72.92±1.84B[b]	56.56±1.16B[a]
	100+ABA	62.05±0.29A[b]	26.05±0.55A[a]
	200+ABA	71.36±1.05A[b]	26.79±0.92A[a]
	300+ABA	78.50±0.58C[b]	58.88±1.73B[a]
	Control + GA_3	33.26±1.02A[b]	29.74±0.74B[a]
	100+GA_3	54.03±2.77B	62.55±1.68D
	200+GA_3	44.33±2.77B	48.10±0.77C
	300+GA_3	52.21±2.13B[b]	20.07±1.24A[a]
Zn	Control	19.94±1.05A[a]	25.67±1.21A[b]
	100	23.73±0.79AB[a]	73.88±3.46C[b]
	200	25.97±0.87B[a]	63.02±0.40C[b]
	300	32.76±0.70C	40.64±3.69B
	Control +ABA	72.92±1.84C[b]	56.56±1.16C[a]
	100+ABA	42.33±1.54A[b]	12.56±0.18A[a]
	200+ABA	68.74±0.52C[b]	19.85±0.93B[a]
	300+ABA	59.57±1.38B	60.03±0.58C
	Control +GA_3	33.26±1.02A[b]	29.74±0.74A[a]
	100+GA_3	80.66±0.86B[b]	35.19±2.07B[a]
	200+GA_3	83.84±0.73B[b]	46.18±0.92C[a]
	300+GA_3	93.01±1.01C[b]	70.80±0.31D[a]
Cd	Control	19.94±1.05A[a]	25.67±1.21[b]
	100	26.60±0.14B	26.32±0.69
	200	30.29±0.37C	28.45±1.47
	300	38.17±0.31D[b]	28.94±2.46[a]
	Control + ABA	72.92±1.84C[b]	56.56±1.16B[a]
	100+ABA	68.15±2.22C[b]	40.33±0.77A[a]
	200+ABA	54.38±0.26B[a]	60.02±0.58B[b]
	300+ABA	30.94±0.92A[a]	58.71±0.66B[b]
	Control + GA_3	33.26±1.02A[b]	29.74±0.74A[a]
	100+GA_3	35.56±1.15A[b]	30.01±0.14A[a]
	200+GA_3	58.64±0.23C[b]	32.14±1.11A[a]
	300+GA_3	51.72±0.23B[a]	67.58±1.68B[b]

of phytochelatin and metallothionein in plants (Steffens, 1990). When the seedlings treated only with Cd and the seedlings treated with Cd, ABA, and GA_3 for a short time were compared, the amount of soluble protein decreased in the seedlings treated with higher concentrations of Cd in the GA_3 solution, relative to the seedlings treated with

Table 2. Illustration of the differences in the amount of soluble protein wheat seedlings when it was grown under heavy metal and combined heavy metal-hormone treatments (n = 3) (upper case letters represent concentration differences and lower case letters represent the differences between days).

Heavy metal	Concentration (µM)	Soluble protein (mg/g DW)	
		5th day	10th day
Pb	Control	35.07±0.74B^b	29.07±0.71A^a
	100	26.93±1.48A^a	32.40±1.29AB^b
	200	38.13±1.07B	35.73±1.16B
	300	33.87±1.16B^a	40.40±0.40C^b
	Control +ABA	26.27±1.27A	29.67±1.45B
	100+ABA	35.47±0.27B^b	29.87±0.96B^a
	200+ABA	33.33±1.62B	37.07±1.07C
	300+ABA	30.98±0.51AB^b	17.33±0.71A^a
	Control + GA_3	16.00±0.80A^a	21.07±1.16A^b
	100+GA_3	31.67±1.10D^a	44.47±0.29C^b
	200+GA_3	21.50±0.22B^a	40.00±1.00B^b
	300+GA_3	26.13±1.16C^a	43.37±0.43BC^b
Zn	Control	35.07±0.74C^b	29.07±0.71C^a
	100	17.87±0.71A^a	29.33±1.16C^b
	200	24.93±0.74B^b	18.17±0.42A^a
	300	36.67±0.48C^b	21.73±0.58B^a
	Control +ABA	26.27±1.27B	29.67±1.45BC
	100+ABA	18.80±0.23A^a	24.40±0.23A^b
	200+ABA	33.73±0.93C	36.91±0.82C
	300+ABA	28.27±1.31B	28.83±0.44B
	Control +GA_3	16.00±0.80A^a	21.07±1.16B^b
	100+GA_3	30.27±0.74B^b	23.20±0.92B^a
	200+GA_3	33.47±0.35B^b	16.80±0.46A^a
	300+GA_3	18.80±0.83A^b	15.37±0.57A^a
Cd	Control	35.07±0.74B^b	29.07±0.71A^a
	100	30.88±1.40A	37.47±0.35B
	200	33.20±0.61AB^a	39.33±0.13B^b
	300	34.07±0.07AB^a	42.00±0.23C^b
	Control + ABA	26.27±1.27B	29.67±1.45B
	100+ABA	30.70±0.27C	28.80±0.80B
	200+ABA	19.20±0.46A	19.87±0.13A
	300+ABA	26.13±0.48B^a	27.86±0.35B^b
	Control + GA_3	16.00±0.80A^a	21.07±1.16B^b
	100+GA_3	26.67±0.53C^a	31.73±0.27C^b
	200+GA_3	26.53±0.13C^a	30.67±0.27C^b
	300+GA_3	22.40±0.46B^b	8.67±0.13A^a

300 µM Cd + ABA. The amount of soluble protein in the seedlings treated with only Cd rises with the length of the treatment duration, and more so than the seedlings treated with Cd, ABA and GA_3 (Table 2).

The effects of the heavy metal-hormone interaction on the amount of soluble phenolic compounds

When day 5 samples of seedling treated only with Pb and

Table 3. Illustration of the differences in the amount of soluble phenolics wheat seedlings when it was grown under heavy metal and combined heavy metal-hormone treatments (n = 3) (upper case letters represent concentration differences and lower case letters represent the differences between days).

Heavy metal	Concentration (µM)	Soluble phenolıc compounds (mg/g DW)	
		5th day	10th day
Pb	Control	51.83±0.44A^b	46.67±0.67A^a
	100	56.83±0.44C^b	47.33±1.45A^a
	200	54.66±0.33B	55.00±0.58B
	300	55.33±0.33BC	55.33±0.88B
	Control +ABA	66.33±0.88A^b	56.00±1.53^a
	100+ABA	71.17±0.73B^b	55.33±0.88^a
	200+ABA	67.78±0.39A^b	57.66±0.88^a
	300+ABA	72.90±0.49B^b	57.08±1.10^a
	Control + GA_3	65.67±0.67D^b	54.67±1.67^a
	100+GA_3	54.77±0.39A	54.67±0.33
	200+GA_3	57.87±0.47B	56.77±1.89
	300+GA_3	60.47±0.29C^b	57.82±0.91^a
Zn	Control	51.83±0.44BC	46.67±0.67A
	100	39.17±1.59A^a	53.33±0.67B^b
	200	48.00±2.00B^a	55.20±1.10B^b
	300	56.00±1.15C	55.73±0.37B
	Control +ABA	66.33±0.88C^b	56.00±1.53B^a
	100+ABA	48.00±1.15A	49.33±1.20A
	200+ABA	56.67±1.76B	52.00±1.00AB
	300+ABA	57.08±1.10B^a	65.83±0.83C^b
	Control l+GA_3	65.67±0.67D^b	54.67±1.67A^a
	100+GA_3	54.77±0.39A	54.67±0.33A
	200+GA_3	57.87±0.47B	58.67±0.67A
	300+GA_3	60.47±0.29C^a	66.67±0.42B^b
Cd	Control	51.83±0.44A^b	46.67±0.67A^a
	100	54.58±1.82A	54.00±0.58B
	200	78.67±0.67B^b	74.00±1.53C^a
	300	80.67±0.67B^b	74.33±2.03C^a
	Control + ABA	66.33±0.88B^b	56.00±1.53^a
	100+ABA	51.67±0.33A	52.00±1.15
	200+ABA	55.00±1.53A	55.33±1.76
	300+ABA	63.00±0.58B^b	56.00±1.15^a
	Control + GA_3	65.67±0.67C^b	54.67±1.67AB^a
	100+GA_3	41.00±0.58A^a	49.33±1.33A^b
	200+GA_3	53.00±0.58B	56.30±1.33B
	300+GA_3	56.00±1.15B^a	59.33±0.33B^b

those treated with Pb, ABA and GA_3 were compared, in terms of the amount of soluble phenolic compounds, it was noticed that the treatment with ABA and GA_3 hormones together with the high concentrations of Pb caused an increase of soluble phenolic compounds. It was determined that the treatment of ABA and GA_3 with Pb in low concentrations for a longer time causes the increase of soluble phenolic compounds in the seedlings (Table 3).

Diaz et al. (2001) have found that heavy metals induce enzymatic activities and cause soluble phenolic compounds and lignin to accumulate. It has been stated that polyphenols are responsible for the deactivation of the toxic effects of heavy metals (Lavid, 2001a, b). When day five samples of the seedlings treated only with Zn and seedlings treated with Zn, ABA, and GA_3 are compared, it was observed that the amount of soluble phenolic compounds in the seedlings treated with high concentrations of Zn + GA_3 was higher than the seedlings treated with 300 µM Zn. In the 10-day limited treatments, the increase in the amount of soluble phenolic compounds in the seedlings treated with 300 µM Zn, ABA and GA_3 was higher than the seedlings treated with only 300 µM Zn. This situation might be related to the ABA and GA_3 which induce the accumulation of soluble phenolic compounds to remove the stresses caused by the heavy metal, Zn and it might also be related to immobilization of Zn with soluble phenolic compounds.

When day five samples of the seedlings treated with only Cd and the seedlings treated with Cd, ABA and GA_3 were compared in terms of the amount of soluble phenolic compounds, the highest increase was observed in the seedlings treated with 300 Cd. The amount of soluble phenolic compounds in the seedlings treated with 200 µM and 300 µM Cd, ABA and GA_3 decreased compared to the seedlings treated with 200 and 300 µM Cd. It was observed that at day 5 and 10, in the samples of seedlings treated with Cd, ABA and GA_3, the soluble phenolic compounds were lower than the samples of seedlings treated with only Cd. This might be caused by Cd, ABA and GA_3 that prevent the increase of phenolic compounds. According to the literature, heavy metals and the hormones that are treated with heavy metals might have completely different effects on plants depending on plant type, its development stage, concentration of the heavy metals and the treatment time. Consequently, GA_3 when treated together with Pb for a long time affected the amount of soluble protein in a positive way. However, we think that further research should be done in this issue.

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