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The Abscisic Acid Levels of Wheat (*Triticum aestivum* L. cv. Çakmak 79) Seeds that were Germinated under Heavy Metal (Hg^{++}, Cd^{++}, Cu^{++}) Stress

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ABSTRACT
The purpose of this study was to investigate the level of endogenous abscisic acid of wheat seeds germinated in the presence of 60, 90 and 120 ppm of mercury, cadmium and copper salt solutions for 16 hours in order to see their effects on the germination. The levels of abscisic acid were analysed by High Performance Liquid Chromatography (HPLC). The results indicated that abscisic acid levels were affected by the kind and level of heavy metals used in the experiments. The effects of these toxic ions on abscisic acid content were found to be highest for mercury and lowest for copper. Regarding the correlation between the concentrations of the toxic elements, applied changes in endogenous abscisic acid levels in the seeds and germination development indicated that inhibition of germination was related with abscisic acid accumulation in the seeds.

Keywords: Abscisic acid, heavy metal, wheat seeds, germination

1. INTRODUCTION

A large area of land is contaminated with heavy metals due to the use of sewage sludge or municipal compost, pesticides, fertilizers and residues from metalliferous mines; and emissions from municipal wastes and residues from metalliferous mines; and emissions from municipal wastes incinerators, car exhausts, residues from metalliferous mines, and smelting industries. Excessive metal concentrations in the contaminated soils can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products [1, 2]. Heavy metal contamination of soils can be of concern for human and animal health, as the metals may be transferred and accumulated in the bodies of animals or human beings through the food chain [3].

Mn, Fe, Cu, Zn and Mo, unlike Al, Cd, As, Pb, Cr, Hg, Ag and Au, are required by biological systems as structural and catalytic components of proteins and enzymes as cofactors or prosthetic groups, which are essential to normal growth and development [4]. A common characteristic of heavy metals in general, regardless of whether they are biologically essential or not, is their high phytotoxicity at excess levels. Pollution by these metal ions initially inhibits growth and development, which is followed by the exertion of extreme toxicity, leading to the death of cells and organisms [5]. Heavy metals have inhibitory effects on nucleic acid and protein synthesis [6], chlorophyll synthesis [7], photosynthesis [8], and respiration [9], and also on seed germination, as expected [10].

Abscisic acid (ABA) is a plant hormone that plays important roles during many phases of the plant life cycle, including seed development and dormancy, and in plant responses to various environmental stresses. Because many of these physiological processes are correlated with endogenous ABA levels, the regulation of ABA biosynthesis is a key element facilitating the elucidation of these physiological characteristics [11]. Some studies reported that the abscisic acid content increased when the plants were exposed to copper and nickel pollution [12]. The application of nickel was also found to decrease the water potential and the stomatal conductance of the leaves while the abscisic acid levels were increasing [13]. It has been shown that the relative water uptake rate was decreasing, while the stomatal resistance and abscisic acid content was increasing after cadmium application to the leaves of ten day old bean seedlings [14].
It is well known that the quality of the water used in irrigation is very important for the plants grown. Wheat

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is an important field crop grown in irrigated fields in Turkey. Increasing the abundance of the wheat crop depends on the quality of the soil and water, besides other factors. One of the important factors which spoils the quality of the water is heavy metal pollution. In this study, the effects of the three heavy metals as copper, cadmium and mercury cations on abscisic acid levels of wheat seeds (*Triticum aestivum* L. cv. Çakmak 79) were investigated. We have tried to determine whether changes of endogenous abscisic acid levels are involved in the inhibitory effects of the above mentioned heavy metal ions on seed germination.

2. MATERIALS AND METHODS

In this study, wheat seeds (*Triticum aestivum* L. cv. Çakmak 79) were used. Seeds were obtained from Elazığ Agriculture Administrative Province Former Education and Training Office. The heavy metal salts used in this study included $CuCl_2$, $CdCl_2.H_2O$ and $HgCl_2$, of which stock solutions were prepared and further diluted to the needed experimental concentrations. In the preparation of the 60, 90, 120 ppm Cu, Cd, Hg and the control solutions double distilled H_2O (pH 6.4) was used.

The wheat seeds (10 g) were left at 24 ± 1 C in 200 ml heavy metal solution (or double distilled H_2O for the control) in a dark environment for 4 hours, which is used as a swelling up media. Swollen seeds were sown in Petri dishes, which were filled with two-layered filter paper and 9 ml heavy metal solution. After sowing, the seeds were left to germinate in darkness for 12 hours. Thus after a total of 16 hours heavy metal-treatment, the seeds were used for abscisic acid (ABA) analyses.

ABA extraction from these seeds was carried out according to Cabot et al. (1986) [15]. The dried extracts were solubilized in 2 ml methylene chloride aliquats for HPLC analysis. Injections were made in duplicate for each of the samples. The quantification was according to [15], utilizing maximum absorption of ABA at 254 nm. The HPLC separations were accomplished at room temperature with a Perkin-Elmer liquid chromatograph system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 µl sample-loop, an ultra-violet (UV) spectrophotometer detector (Cecil 68174), an integrator (HP 3395) and a Hi-Chrom ODS-1 packed (5µm particle) column (250 mm x 4.6 mm ID). Isocratic separation was performed with methanol at 1.5 ml min^{-1} flow rate.

For determining the content of ABA in the extracts, a series of standard solutions were prepared from standard ABA (+/- cis, trans, Sigma), which were diluted in methylene chloride (50-1400 ng) and 20 l aliquots were injected for the measurements. The retention time for ABA was approximately 1.5 minute. In the Figure 1 the standard chromatogram of the 100 ng ABA is presented. Calibration curves were obtained by measuring the peak height values of the ABA standards.

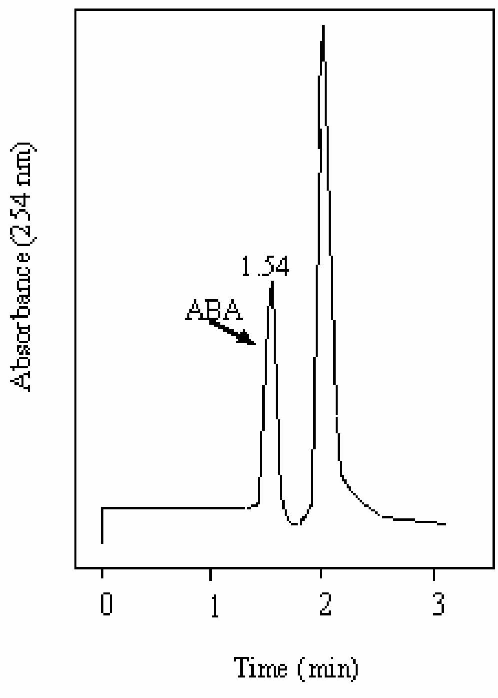

Figure 1. HPLC chromatogram of ABA (+/- cis, trans, Sigma). Chromatographic conditions were: Hi-Chrom ODS-packed (5µm particle) column (250 mm x4.6 mm ID) with a methanol mobile phase at room temperature, the flow rate was 1.5 ml.min^{-1}

All the results reported are the means of three replicates. The statistical analysis is based on SPSS (version 10.0) program. In order to detect the significant differences ($p<0.01$ or $p<0.05$), a multiple comparison (LSD) test was performed. All values are expressed as mean ± SEs.

3. RESULTS

Figures 2-5 present the effects of mercury, cadmium and copper on abscisic acid content in wheat seeds. Significant increases in the abscisic acid content in the seeds were detected after 16 h exposure to heavy metal ions.

3.1. Mercury

The abscisic acid contents in the wheat seed samples increased noticeably at 60, 90 and 120 ppm $HgCl_2$ concentrations (Fig. 2).

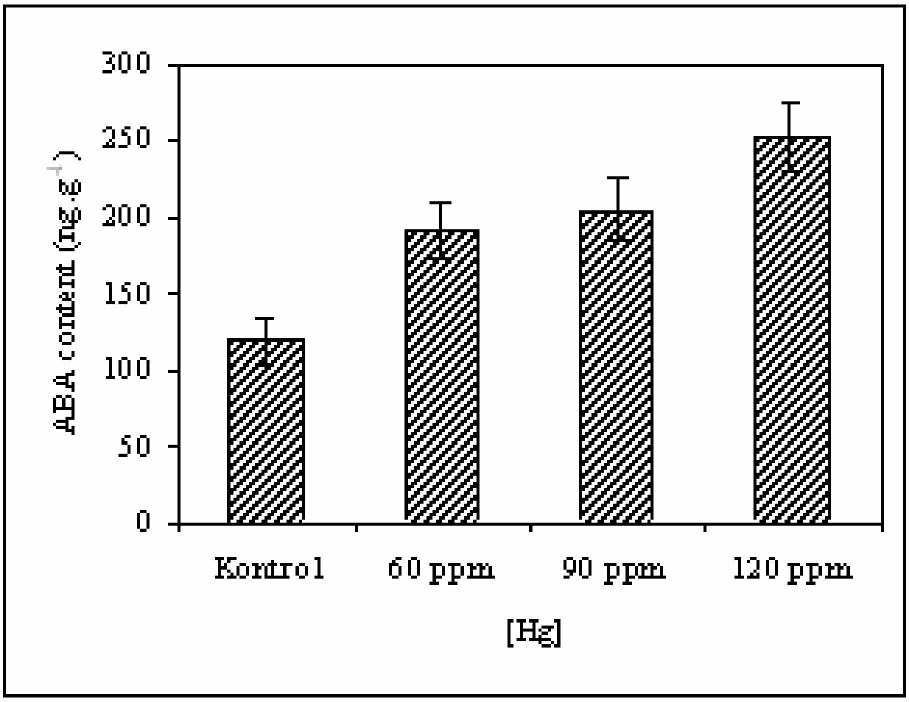

Figure 2. ABA ($ng.g^{-1}$ FW) content in wheat seeds treated with different concentrations of mercury ($HgCl_2$).

As can be seen, the mercury stress increased the abscisic acid contents of the wheat seeds ($p<0.01$). In the treatments with 60, 90 and 120 ppm mercury, the abscisic acid content of the seeds increased within 16 h by 62.1, 73.0 and 118.1 %, respectively, as compared to the untreated control ($p<0.01$).

After 16 h incubation, no significant ($p>0.05$) difference in the ABA content was observed between the 60 and 90 ppm-treatments. At the concentration of 120 ppm, However, mercury caused the abscisic acid content to increase significantly ($p<0.05$).

3.2. Cadmium

The endogen abscisic acid contents of the wheat seeds, which were treated with different concentrations of cadmium, are given in Figure 3.

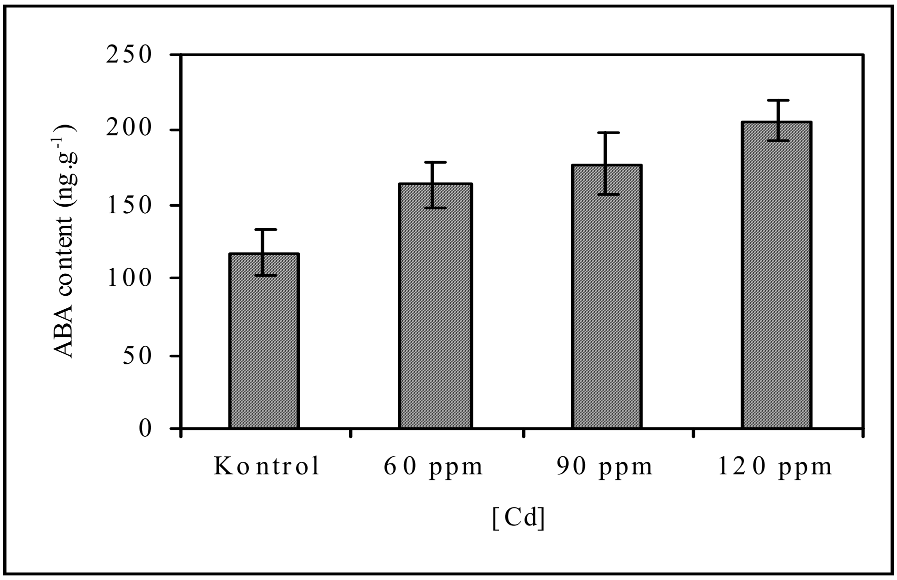

Figure 3. ABA (ng.g^{-1} FW) content in wheat seeds treated with different concentrations of cadmium ($CdCl_2$ H_2O).

The cadmium salt solutions have caused a great increase in the endogen abscisic acid content of the wheat seeds ($p<0.01$). The values of ABA in the samples treated with 60, 90 and 120 ppm $CdCl_2H_2O$ increased by 37.8%, 49.5% and 73.5%, respectively, compared to the control seeds. All of these differences were significant in the 60 ppm and 90-120 ppm concentrations of Cd at the level of $p<0.05$ and $p<0.01$, respectively.

3.3. Copper

The abscisic acid contents of the wheat seeds with copper treatment are given in Figure 4.

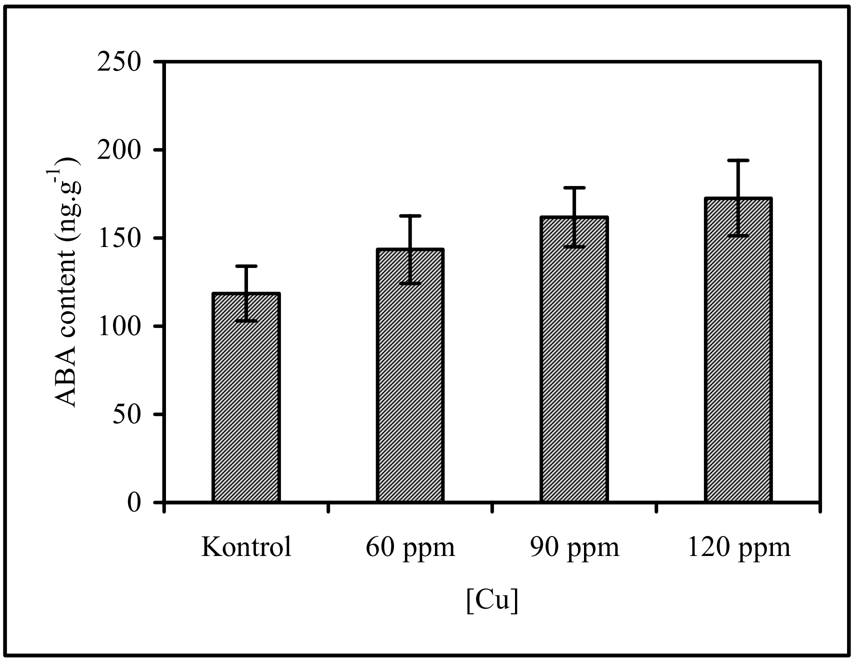

Figure 4. ABA (ng.g^{-1} FW) content in wheat seeds treated with different concentrations of copper ($CuCl_2$).

The ABA content of the seeds increased with increasing concentration of copper in the treatment solutions. The content of abscisic acid in the wheat seeds to which 60, 90 and 120 ppm $CuCl_2$ was applied were increased by

21%, 36.6% and 45.7%, respectively, compared to the control seeds. No differences in the abscisic acid contents were observed at 60 ppm concentration when compared to control seed ($p>0.05$), whereas with 90 and 120 ppm Cu, significant ($p>0.05$) differences in the abscisic acid contents were observed. Significant differences were observed, however, between the 60 ppm and 120 ppm copper treatment ($p<0.05$).

Copper, cadmium and mercury are among the most common heavy metals found in our environment. The selection of these heavy metal ions and their concentrations were based on the concentrations of these metals found in the environment, as described in the previously published studies on various aspects of the topic. Each of these heavy metals increased the ABA content of the samples in the present study at all three tested concentrations.

The amounts of these increases were related with the kind of heavy metal ions and their concentrations used in the study. The evaluation of the overall results that mercury was the most and copper the least effective ion on the endogenous abscisic acid levels (Figure 5).

4. DISCUSSION

It is well known that the exposure of plants to heavy metals ions induces the generation of active oxygen species (AOS), which are harmful to plants [16]. The injury of plant cells caused by heavy metals is, to a great extent, related to the destruction of the balance between the generation and detoxification of AOS. Plants possess protective mechanisms to scavenge these toxic AOS and abscisic acid is one of these protective substances [17]. It has been suggested that AOS played an intermediatory role as the activities of antioxidant enzymes, such as superoxide dismutase, ascorbate peroxidase and glutathione reductase, were enhanced by ABA treatments [18].

The amount of ABA is determined by the dynamic balance between biosynthesis and degradation, and these two processes are influenced by development, environmental factors such as light, water stress, and other growth regulators [19]. ABA level was higher in plants growing under salinity stress and also in heavy metals pollution sites [20]. Many kinds of environmental stresses are known to stimulate the ABA synthesis, and ABA has been suggested to have a central function in cross-adaptation [21]. For example, as a response to water deficit, there is an increase in the endogenous ABA levels that rapidly limits water loss through transpiration, by inducing closure of stomatal aperture. ABA is also involved in other aspects of stress adaptation such as cold acclimation and changes in root morphogenesis in response to stress [22]. In general, plants possess physiological mechanisms that enable them to resist elevated heavy metal concentrations in their substrate [23]. Resistance to heavy metal ions can be based on either avoidance or tolerance mechanisms, as they can tolerate external heavy metal ion supplies through their specific physiological tolerance mechanisms [23]. One of these mechanisms is hindering the transportation from roots to shoots. There are a number of reports indicating the operation of a hindering mechanism, which ceases the translocation of some nutrients to the shoots by increasing the ABA levels in the roots of the plants under salt stress [24]. Such increases in endogenous ABA levels caused by heavy metal ions in the environment can be used in explanation of ceasing water uptake or hindering the translocation of the heavy metals from roots to shoots.

The number of studies on the mechanisms involved in the interrelationships between heavy metal ion stresses and hormone levels are limited, because this aspect of the subject attracted the attention of physiologists very recently. For instance, Talanova et al. reported [25] that the ABA content increased in the *Cucumis sativus* L. seedlings following the application of 1-500 M lead and 5-1000 M cadmium on the 1^{st}, 4^{th}, and 7^{th} days of the treatment. Supporting evidence was also found in *Phaseolus vulgaris* L. cv Contender plants [14]. It was reported that the content of ABA increased in roots and leaves, which led to increased stomatal resistance values, thus the water content of the leaves decreased in bean plants which were left in 3 M cadmium solutions for 144 h. It was found that the ABA contents in chickpea seeds germinated at all of the Pb or Zn concentrations used, increased significantly [26]. In another experimental study the stomatal conductance decreased and the level of ABA, which is known to regulate the water status of the plants, increased two-fold in the bean leaves when the plants were exposed to Ni ions [13]. The ABA content increased as *Empetrum nigrum* L. leaves were exposed to the highest heavy metal treatment used in the study [12].

The results of the present study showed that Cu, Cd and Hg solutions applied to the wheat seeds during germination increased the endogenous abscisic acid levels, and the changes were related to the kind of heavy metals ions and their concentrations in the solutions applied. In general, the increase in the mercury, cadmium and copper concentrations resulted in an increase in the abscisic acid levels of the seeds.

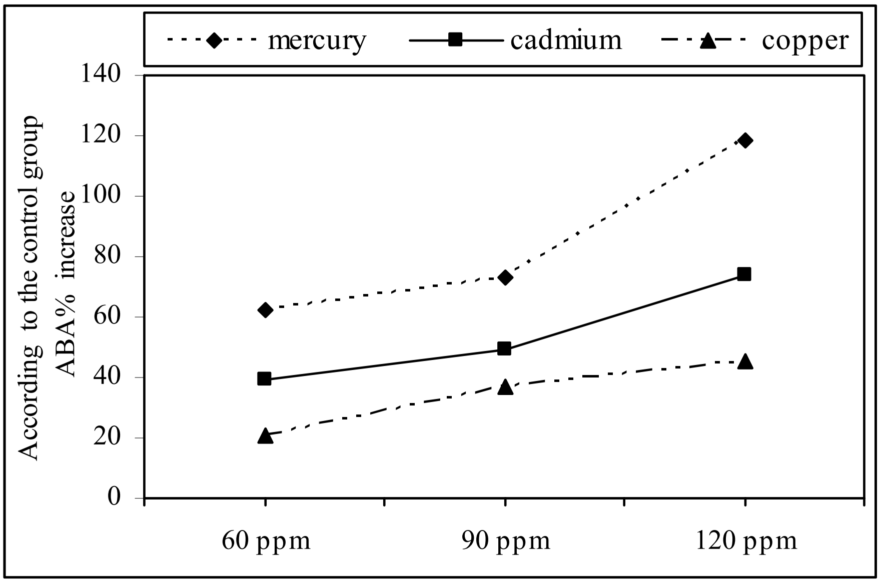

Figure 5. Effects of different concentrations of mercury, cadmium and copper ions as compared to the controls on endogenous abscisic acid levels in the germinated wheat seeds.

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