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The Responses of Antioxidant System against the Heavy Metal-Induced Stress in Tomato

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Heavy metal,
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Abstract: Plants maintain their life cycles under the various environmental conditions such as oxidative stress induced by heavy metals. Accumulation of metal ions in plants causes the formation of free radicals and stimulates the antioxidative defense systems. In this study, the activities of APX, POD, and SOD are investigated in the leaves and roots of tomato cultivated under the heavy metal-induced stress. The activities of APX, POD, and SOD exhibited remarkable induction with the treatment of Cd, Cu and Pb (10, 20 and 50 ppm) in the leaves of tomato compared to control plants except for 50 ppm Pb. In roots, APX activity changed depending on the heavy metal types and concentrations, while Cd clearly increased it with stress conditions, but Cu decreased in tomato compared to control. The activity of POD clearly exhibited that the all doses of heavy metals reduced the enzyme activity in roots polluted with heavy metals. The treatment of Cd (10, 20 and 50 ppm) significantly increased the activity of SOD, however, Cu showed the opposite effect which significantly decreased with increasing doses in roots compared to uncontaminated plants. Also, roots from plants grown on the high concentration of Pb (20 and 50 ppm) induced the activity of SOD. Briefly, it is clear responses which Cd significantly raised the activities of APX and SOD in leaves and roots of tomato. The decreases caused by these metals in the activity of POD and Cu in the activities of APX and SOD in roots of tomato can be clarified by the result of heavy metal-induced the over production of free radical.

Domateste Ağır Metal Kaynaklı Strese Karşı Antioksidan Sistem Yanıtları

Anahtar Kelimeler
Ağır metal,
APX,
Domates,
POD,
SOD

Özet: Bitkiler ağır metal kaynaklı oksidatif stres gibi çeşitli çevresel koşullar altında yaşamlarını sürdürürler. Ağır metallerin bitkilerde birikimi serbest radikallerin oluşmasına neden olur ve bu radikaller antioksidatif savunma sistemlerini uyarır. Mevcut çalışmada, ağır metal kaynaklı stres altında yetiştirilen domates bitkisinin kök ve yapraklarında ROS'u ortadan kaldırabilen APX, POD ve SOD enzim aktiviteleri araştırılmıştır. Bu enzimlerin aktiviteleri Cd, Cu ve Pb (10, 20 and 50 ppm) uygulaması sonucu yapraklarda 50 ppm Pb dışında kontrol grubuyla karşılaştırıldığında belirgin bir biçimde artış göstermiştir. Bitki köklerinde, APX aktivitesi ağır metal çeşit ve dozuna bağlı olarak değişmiştir. Kontrol grubuna kıyasla domateste, Cd uygulaması APX aktivitesini stres koşullarında artırırken, Cu enzimin aktivitesini azaltmıştır. Ağır metalle kirletilmiş köklerde POD aktivitesi tüm dozlarda açık bir şekilde azalmıştır. Köklerde kontrol grubuyla karşılaştırıldığında Cd (10, 20 and 50 ppm) uygulaması SOD aktivitesini önemli şekilde artırmıştır, fakat Cu ilavesi artan dozla birlikte ters etki göstererek SOD aktivitesini azaltmıştır. Ayrıca, yüksek konsantrasyonda Pb uygulaması (20 ve 50 ppm) bitki köklerinde SOD aktivitesini artırmıştır. Kısaca, kadmiyum uygulamasının domates kök ve yapraklarında APX ve SOD aktivitelerini artırması açık bir cevap olarak görülmüştür. Domates köklerinde her üç ağır metalin POD aktivitesinde, bakırın APX ve SOD aktivitelerinde azalmaya neden olması ağır metal kaynaklı aşırı serbest radikal üretiminin bir sonu olarak görülebilir.

1. Introduction

The environments have enclosed the plant life and the combination of external conditions influence the plant growth, development, and yield. Heavy metals are found in the agricultural fields and they cause undesirable effects reducing the quality of plant life cycles. The excess accumulation of heavy metals in

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plant cultivated medium is the results of diverse anthropogenic activities and restricts the plant productivity. Although some heavy metals like Cu, Fe, and Zn are accepted to be essential for plant growth serving as a cofactor of enzyme reactions, heavy metals like Cd and Pb are non-useful and toxic, reducing the plant growth. The toxicity of heavy metals in plants tissues varies with concentration, chemical form, soil textures and plant species [1-3]. The presence of heavy metals in plant can interfere with physiological and biochemical changes such as variations in enzyme activities, disturbances in water statues and formation of free radicals [4–7].

The excess uptake of metals can damage to plant cells stimulating the free radical generation and ROS is predominantly generated in chloroplast, mitochondria, and peroxisomes. Activated oxygen species are partially produced from the metabolized oxygen and ROS are closely controlled by the scavenging system in plant cells. Heavy metals influence the metabolic pathways interacting with organic compounds in the plant cell components when they enter the plant tissues. ROS generation is induced in response to metabolic deviation charged by stress factors. Heavy metals can cause the formation of free radicals and replace essential metals. These metals have the ability to generate reactive radicals such as superoxide, hydrogen peroxide, and lipid peroxides caused primarily imbalance in the cell homeostasis [2,8].

Plants have struggled to overcome the oxidative stress by a wide range of antioxidant molecules and enzymes protecting the cells against to the oxidative injuries to maintain the equilibrium between the oxidative and antioxidative capacity in plants. These antioxidants have capability to inhibit the electrons of the redox reactions. Antioxidant enzymes can terminate the oxidation reactions by scavenging the free radicals [9]. Plants have enzymatic mechanisms related with defense system such as catalase, glutathione reductase, glutathione *S*-transferase, peroxidase, and superoxide dismutase, which remove, neutralize and scavenge the free radicals [10]. Oxidative stress damage the equilibrium between ROS and antioxidant systems, and antioxidant enzymes react with the free radicals. These enzymes play the main protective role in the elimination of free radicals. Superoxide radical is scavenged with SOD and H_2O_2 decomposition is achieved by APX and CAT. Another well-known enzyme is POD related with stress, alterations in POD activity have been accepted to be associated with a wide range of physiological processes in response to environmental conditions. The coordinated function of these enzymes can help to maintain the oxidant and antioxidant status of plant cells [8,11,12].

Tomato (*Solanum lycopersicum*) is the widely grown and used for various purposes such as salad and sauces in all around the world and Turkey. The current study was set about to investigate the behavior of antioxidant enzymes like APX, POD, and SOD in tomato plants cultivated in different doses of heavy metals under greenhouse conditions.

2. Material and Method

2.1. Plant material and cultivation conditions

Tomato (*Solanum lycopersicum* cv. çiko F1) seedlings were cultivated in plastic boxes consist of the equal mixture of peat and garden soil (1:1) under glasshouse conditions. The plant cultivated soil include Mg: 6.85 (ppm), Ca: 54.95 (ppm), Fe: 10:90 (ppb), Mn: 417.80 (ppb), Zn: 19.70 (ppb) and Cu: 30.75 (ppb). All experiment was performed with 16:8 photoperiods, temperature at 25-27 ˚C, and N, P and K were utilized in 100 ppm doses for plant growth and development. After the three weeks of acclimatization, the heavy metal application was performed three times with an interval of 2 days and one time a day in the early hours of the day. The exposure concentration of Cd, Cu and Pb was 10, 20 and 50 ppm from $CdCI_2$, $CuSO_4$ and $Pb(NO_3)_2$ salts, respectively. Heavy metal concentration was adjusted according to their toxicity as described previously in plant nutrition. The seedlings were cultivated 10 trials with three times. The tomato leaves and roots were harvested two weeks after the applications and all samples were kept at -80˚C until the extraction of enzymes.

2.2. Enzyme extraction and assay

The samples of tomato (0.3 g) were grounded into a fine powder in liquid N_2. The obtained powder was then homogenized in 100 mM KH_2PO_4 buffer (pH=7.0) containing 1 mM EDTA and 1% (w/v) PVP. The homogenate were centrifuged at 15000 x g for 20 min in the cooled centrifuge (+4˚C) and supernatants of leaves and roots were used to determinate the enzyme activities of APX, POD, and SOD with spectrophotometrically (Carry 50 UV/VIS, Japan).

2.3. Ascorbate peroxidase (APX) activity

The reaction mixture for the APX consists of 50 mM KH_2PO_4 buffer (pH=7.0), 2.5 mM ascorbate, H_2O_2 and 30 µl enzyme extract. The enzyme reaction was initiated by addition of H_2O_2, and the APX activity was determined by measuring the oxidation of ascorbate at 290 nm (E= 2.8 mM^{-1} cm^{-1}) [13].

2.4. Peroxidase (POD) activity

The activity of POD was defined according to oxidation of guaiacol previously described [14]. The activity of POD was initiated with 30 µl enzyme extract into the mixtures of 50 mM KH_2PO_4 buffer (pH= 6,5), 30 mM guaiacol, 10 mM H_2O_2 and the rise in the absorbance at 470 nm was recorded (E= 6.26 mm^{-1} cm^{-1}).

2.5. Superoxide dismutase (SOD) activity

Total SOD activity was defined by the reduction of NBT (nitroblue tetrazolium) at 560 nm previously described the method with a slight modification [15]. The assay is based on creation farmazon by superoxide anion and reaction mixture consist of 50 mM KH_2PO_4 buffer (pH= 7), 13 mM methionine, 60 µM NBT, 0.1 mM EDTA and 50 µl enzyme extract. Riboflavin (2 µM) was last added to the mixture and sample tubes were incubated for 30 min under light (4000-5000 lux). One unit of SOD was accepted as the activity of enzyme that inhibited 50% formazan formation.

2.6. Statistical analysis

Data analysis of the results was carried with SPSS software 20.0 by using one-way ANOVA followed by Duncan multiple tests. The results are exhibited as mean ± sd and significant differences related to the control groups were accepted at $P < 0.05$.

3. Results

3.1. The effect of heavy metals on the APX activity

The activity of ascorbate peroxidase in the leaves and roots of tomato changed with the application of heavy metals, and all results are shown Fig 1. APX activity significantly increased with application of heavy metals except for 50 ppm Pb in leaves ($p<0.05$). The highest activities were seen in the addition of 20 ppm of Cu, and 10 ppm of Pb and Cd, but there was no significant change in the 50 ppm of lead. The activity of APX in the extract of the roots was changed depending on the heavy metal types and concentration. The application of Cd significantly raised the APX activity in roots compared to control plants. However, the all doses of Cu and 10 ppm doses of Pb decreased the enzyme activity, but it remained unchanged in the other doses in tomato roots polluted with Cu and Pb.

3.2. The effect of heavy metals on the POD activity

The treatment of Cu, Cd, and Pb to the plant cultivated soil significantly increased the activity of POD in all applications into the tomato leaves. The analysis of POD clearly showed that these metals decreased the enzyme activity in roots contaminated with metal ions compared to control plants ($p<0.05$). The results of POD activities are shaped in Fig 2. and the biggest inducing heavy metal in POD activity is Cd, especially 10 and 20 ppm doses in leaves of tomato.

3.3. The effect of heavy metals on the SOD activity

The activity of SOD in the leaves and roots of tomato cultivated in polluted pots is affected by the heavy metals and the results are shown in Fig 3. SOD activity was shown to be significantly raised by all application and doses of Cu, Cd, and Pb in leaves of tomato according to control plants ($p<0.05$). The activity of SOD in roots changed heavy metal types and doses and the application of cadmium clearly increased the activity with increasing doses of Cd. However, the activity of SOD significantly reduced with increasing doses of Cu in the roots compared to unpolluted plants. Also, roots from plants grown on the low concentration of Pb (10 ppm) exhibited SOD activity below the control levels, but it induced the activity of SOD at high doses (20 and 50 ppm) in roots compared to control groups of tomato. The highest SOD activity was determined in the application of Pb (10 and 20 ppm) and Cd (50 ppm) in the leaves and roots, respectively and the low activity of SOD was observed in the addition of Pb (10 ppm) into tomato breeding medium.

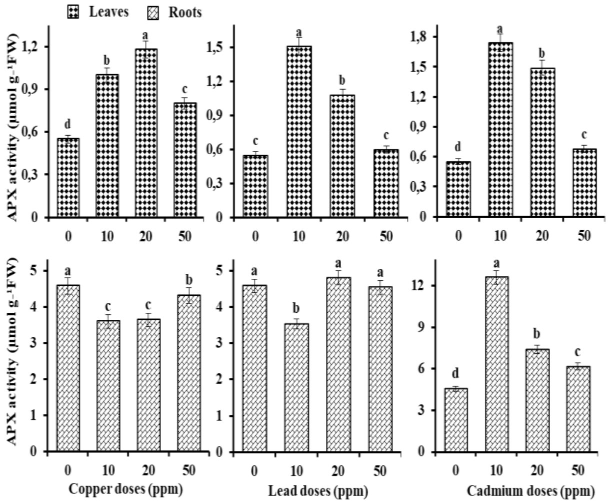

Figure 1. The effect of heavy metals on the enzyme activity of APX and the results are presented as µmol g^{-1} FW of leaves and roots of tomato under 0 (control), 10, 20, 50 ppm of Cu, Pb and Cd. Mean values with the different letters show significantly differences at p ≤0.05 from each other's.

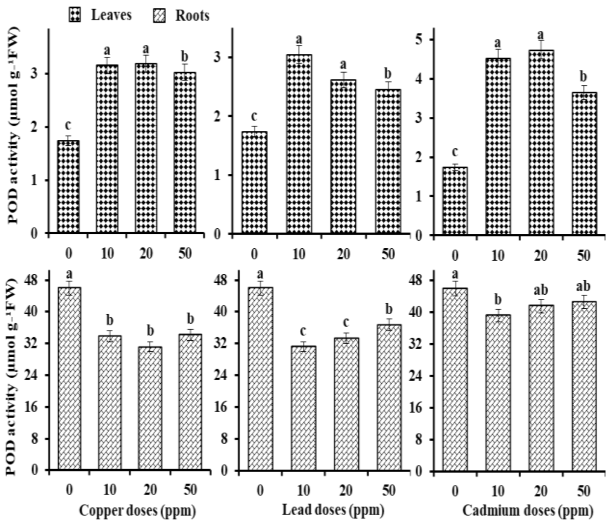

Figure 2. The effect of heavy metals on the enzyme activity of POD and the results are presented as µmol g^{-1} FW of leaves and roots of tomato under 0 (control), 10, 20, 50 ppm of Cu, Pb and Cd. Mean values with the different letters show significantly differences at p ≤0.05 from each other's.

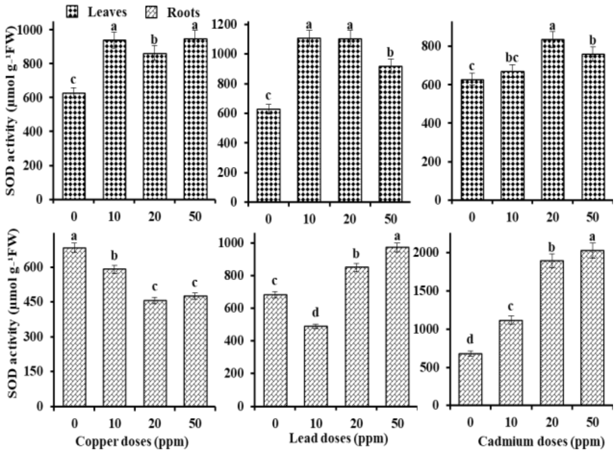

Figure 3. The effect of heavy metals on the enzyme activity of SOD and the results are presented as µmol g-1 FW of leaves and roots of tomato under 0 (control), 10, 20, 50 ppm of Cu, Pb and Cd. Mean values with the different letters show significantly differences at p ≤0.05 from each other's.

4. Discussion and Conclusion

Plants have constantly subjected to environmental stress and heavy metals remain in the agricultural soil for a long time, exchanging the characteristic of the natural cultivation field of the plant. Activated oxygen species are generated as by product of various metabolic pathways in the plant cell in the course of plant life; however, serious oxidative stress can disturb the cellular balance between the oxidant and antioxidant compounds in cells [16]. Antioxidant scavenging systems concerned with the detoxification of ROS endeavor to achieve the defense capacity of plant tolerating the oxidative stress in plant tissues [17].

In the present study, it is investigated the activity of APX, POD, and SOD globally accepted as ones of the main antioxidant defense enzymes. The results of APX in the current study indicated that the activity of enzymes generally increased in the leaves of tomato grown heavy metal containing soils. The treatments of Cd induced the activity of APX, while Cu caused the decrease in the enzyme activity. Moreover, Pb showed that the enzyme activity in the extract of roots depending on the applied doses. The study on *Solanum lycopersicum* reported that the oxidative stress induced by Cd caused the major increases in the activity of CAT extract from the leaves exposed to 0.1 and 0.2 mM $CdCI_2$, whereas POD didn't show significantly change in the leaves and roots of tomato. Also, SOD activity in the same study didn't exhibit any significant changes, apart from a slight reduction at concentration above 0.2 mM $CdCI_2$ [16]. It was declared that the activity of APX demonstrate remarkable induction with increase in the doses of heavy metals (Hg, Cd, Pb, and Cr) applied on *Allium cepa* cultivated medium [18]. Also, the activity of APX remained unchanged in pea plants cultivated in the $CdCI_2$ containing soils [19]. In the current study, it was declared that the activity of POD was induced in leaves and whereas it was reduced in roots by the addition of Cu Cd and Pb compared to uncontaminated plant growth pots. It was previously noticed that the activity of POD raised transiently as an early antioxidant response against Cu induced oxidative stress in the leaves of tomato. However, this antioxidant defense mechanism broke down and activity of enzyme reduced at the higher $CuSO_4$ concentration [20]. The study carried on rice exposed to $Cd(NO_3)_2$ indicated that the activities of POD and SOD raised, and the increase in POD activity was greater than SOD in the rice seedlings [21].

The results from leaves extract from tomato in the current study showed that the activity of SOD wholly increased by the application of Cu, Cd, and Pb compared to control groups. However, obtained result from roots showed that Cd induced the activity of enzyme and Cu decreased the quantity of SOD with increasing doses of them compared to control plants. Also, activities analyzed from roots of tomato cultivated in the Pb-containing soils changed depending on the lead doses which it caused the reduced at the 10 ppm, and enhancement at the 20 and 50 ppm compared to unpolluted plant soils. It was observed that Cd-treated tomato plants had significantly higher activity of SOD, and the activities of CAT and APX were suppressed with the treatment of Cd. However, Zn supplementation restored and improved the functional activities of the enzymes as compared to Cd-alone applied tomato leaves [22]. It was declared that the activities of SOD and APX enhanced with increasing concentration of $Cd(NO_3)_2$ for two maize cultivars [23]. Another study on poplar stated that the activity of POD decreased in the young fine roots of *Populus nigra* and *P. deltoids* grown in heavy metal polluted environment. Heavy metals induced the over production of free radicals in fine roots [24]. These results from the study are consistent with previous oxidative stress-induced studies that have been mentioned above sentences.

In the current study, exposure of *Solanum lycopersicum* to three heavy metals caused to behave differently in the activity of enzymes depending on the tissue assayed; the application of Cd, Cu, and Pb globally enhanced the activities of APX, POD and SOD which are clear responses given by the leaves of tomato, while their activities fluctuated depending on the heavy metal types and concentration in roots. For example, the activity of POD was reduced by all application of these metals. The cause of the decrease in activity of some enzymes in the roots can be expressed by the fact that heavy metals triggered the over production of free radical in fine roots which is the first tissue to encounter the heavy metals in the plants. Therefore, roots are the most endangered of the plant's tissues and the first line of defense against toxicity in agricultural area [25]. This study elucidate that the treatment of heavy metals induced the activity of antioxidant enzymes in the leaves of tomato, but there was no clearly changes in the roots of tomato compared to control groups. Additional studies can be needed to further define and elucidate the responses of tomato by researching the various enzyme activities and their gene expressions, proteome analysis, nutrient contents and transports in point of the molecular, biochemical and physiological criterion in plants.

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