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ORIGINAL ARTICLE

Detection of superoxide radicals in tomato plants exposed to salinity, drought, cold and heavy metal stress using CMC-G-SOD biosensor

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Absract

A novel highly sensitive electrochemical carboxymethylcellulose-gelatin-superoxide dismutase biosensor was used for the determination of superoxide radicals enhancement in tomato plants exposed to salinity, drought, cold and heavy metal stress. The variations in superoxide radicals depending on abiotic stress was determined using biosensor. The superoxide radical production with regard to control rapidly was increased in tomato plants exposed to salinity, drought, cold and heavy metal stress. The superoxide radical enhancement in tomato plants exposed to salinity, drought, cold and heavy metal stress was successfully determined using carboxymethylcellulose-gelatin-superoxide dismutase biosensor.

Keywords: abiotic stress, biosensor, carboxymethylcellulose–gelatin, superoxide dismutase, superoxide radicals, tomato (Lycopersicum esculentum L.)

Introduction

Plants are exposed to a wide array of environmental stress factors such as drought, heat, cold, ultraviolent light or pathogen attacks etc (Mc Kersie and Leshem 1994, Paliyath et al. 1997). These factors affect many genetic, physiological and biochemical responses in plants For example, changes in membrane composition, closure of stoma, photosynthetic efficiency, accumulation of sugar or other compounds, production of small-molecules and free-radicals and activities of antioxidant enzymes and their gene expressions (Beck et al. 2007, Mahajan et al. 2005, Tian et al. 2011, Bowler et al. 1992, Foye et al. 1994).

When stress conditions are perceived by plants, three kind of stress signaling can occur. These are: (a) osmatic stress signaling to reestablishment of cellular homeostasis, (b) detoxification signaling to control or repair stress damage, (c) and signaling to coordinate cell expansion to levels appropriate for the particular stress condition (Zhu 2001).

Abscisic acid (ABA), phytohormone, plays an important role in enhancing tolerance against water, salt and cold stress. It modulates many significant development processes of plant; such as inhibition of germination, maintenance of seed dormancy, regulation of growth and stomatal closure (Finkelstein et al. 2002, Parent et al. 2009, Raghavendra et al. 2010). In consequence of stomatal closure and limited CO_2 availability, chloroplasts mitochondria and peroxisomes generate reactive oxygen species (ROS) such as O_2^-, H_2O_2, $^1O_2$, HO_2^-, OH, ROOH, ROO and RO which tend to act as detoxification signals at cellular level (Smirnoff 1993, Gratao et al. 2005, Mittler 2002).

Under normal growth condition, production of ROS is limited and there is equilibrium between production and scavenging of ROS. Many stress condition such as drought, desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolent radiation, air pollutions etc. disturb the homeostasis and enhance ROS production (Bowler et al. 1992, Allen 1995, Dat et al. 2000). Enhanced production of ROS is critical for plants because overaccumulation of them can result cell death in plants (Asada and Takahashi 1987, Hammond-Kosack and Jones 1996). For scavenging of ROS, plants possess two types of antioxidant mechanism namely nonenzymatic antioxidants and enzymatic antioxidant systems. Glutathione (GSH), proline, carotenoids, tacoppherol etc. are called nonenzymatic antioxidants and monodehydroascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and superoxide reductase (SOD) also called enzymatic antioxidants (Verma et al. 2003, Verma and Dubey. 2003, DalCorso et al. 2008).

SODs have been distinguished into different classes depending on the metal (manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn)) at the active center. All have different localization in plants. For instance; Cu/Zn SODs are generally found in the cytosol or chloroplasts of eukaryotic cells, whereas MnSODs are found in the mitochondria, chloroplasts and peroxisomes. The dimeric FeSODs are found in chloroplasts. SODs play a significant role in detoxifications of toxic effects of oxidative stress by scavenging superoxide radicals,

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providing their conversion into oxygen and hydrogen peroxide. (Van Camp et al. 1994, Fridovich 1995, Salin and Bridges 1980, Del Río et al. 1983, Droillard and Paulin 1990).

Superoxide radicals ($O_2^{\bullet -}$), the primary species of the ROS, produced in biological systems, play a central role in physiological processes (Auchere and Rusnak 2002, Dean et al. 1997, Halliwell and Gutteridge 1984). Superoxide radicals are generated at a rate that is matched by the capacity of tissue to catabolize them under normal metabolic conditions (Van Lente 1993). When its level exceeds the body's natural ability to deal with the potentially cytotoxic species, various pathological situations such as cancer, stroke, neurodegeneration and aging may result (Halliwell and Gutteridge 1986, Mannino et al. 1999, Pinzino et al. 1999, Hensley and Floyd 2002, Youdim and Joseph 2011). In plants, $O_2^{\bullet -}$ is commonly generated in illuminated chloroplasts by the occasional transfer of an electron from an excited Chl molecule or Photosystem I components under conditions of high NADPH/NADP ratios to molecular O_2 (Scandalios 1993). In most aerobic organisms, different environmental complications, like hyperoxia, pathogens, ozone, temperature fluctuations, and other stresses are known to stimulate $O_2^{\bullet -}$ formation (Scandalios 1993). Therefore, in order to understand the role of $O_2^{\bullet -}$ in pathology and physiology and the relationship between $O_2^{\bullet -}$ and environmental stresses, the quantitative determination of $O_2^{\bullet -}$ in a variety of *in vitro* and *in vivo* models is essential. A major challenge of the quantification of superoxide radical in biological models is of its short duration due to its spontaneous dismutation to oxygen and hydrogen peroxide and low concentration (Fridovich 1972, Beissenhirtz et al. 2006). Determination of free radicals is usually performed by ESR (Carey and Sundberg 1984), chemiluminescence (Prasad et al. 1989) or certain semiquantitative colorimetric tests (Halliwell and Gutteridge 1981, Fridovich 1970). These indirect methods are *ex situ* detection techniques with low sensitivity and poor selectivity. However, among the analytical systems, electrochemical techniques have proven to be powerful tools with the advantage of simplicity, portability, cost, fast response, high selectivity and real-time measurements (Tammeveski et al. 1998a, Land and Swallow 1971, Rigo et al. 1992, Tanaka and Muto 1992, Moscone and Mascini 1988, McNeil et al. 1989, Oshaka et al. 1995, Tammeveski et al. 1998b).

Superoxide dismutases (SODs) which protect the organism against the toxic effects are ubiquitous metalloenzymes in oxygen-tolerant organisms and catalyze the dismutation of $O_2^{\bullet -}$ to O_2 and H_2O_2 via a cyclic oxidation–reduction electron-transfer mechanism (Rotilio et al. 1972, Klug et al. 1972, Lawrence and Sawyer 1979). SOD, a selective scavenger of $O_2^{\bullet -}$, has been the best approach for the electrochemical detection of $O_2^{\bullet -}$ compared to cyt c (Cooper et al. 1993, Lisdat et al. 1999). Therefore, SOD, a specific enzyme for $O_2^{\bullet -}$ dismutation, offers great potential for the sensitive and selective quantification of $O_2^{\bullet -}$ in electrochemical biosensors. Mostly, copper, zinc–SOD-immobilized electrodes have paved an elegant way to detect $O_2^{\bullet -}$.

The development of a highly sensitive superoxide dismutase biosensor for the simultaneous determination of superoxide radicals by immobilization of SOD within carboxymethylcelulose-gelatin on a Pt electrode surface was performed (Kocabay et al. 2012). The parameters affecting the performance of the biosensor were investigated in detail. The response of the CMC–G–SOD biosensor was proportional to $O_2^{\bullet -}$ concentration and the detection limit was 1.25×10^{-3} mM with a correlation coefficient of 0.9994 at a signal-to-noise ratio of 3. The response time of the developed biosensor was 2 seconds (Kocabay et al. 2012). In this study, the applications of developed CMC–G–SOD biosensor to agriculture were studied. For this purpose, tomato plants were exposed to heavy metal, cold, salinity and drought stress to examine the $O_2^{\bullet -}$ enhancement. The effect of heavy metal, cold, salinity and drought stress in tomato plants on $O_2^{\bullet -}$ enhancement was investigated using developed CMC–G–SOD biosensor.

Materials and methods

Materials

SOD (EC 1.15.1.1, 4733 U mg^{-1}, from bovine erythrocytes), xanthine oxidase (XOD; EC 1.1.3.22, 0.3 U mg^{-1}, from milk), xanthine (2,6-dihydroxypurine) sodium salt, gelatin, carboymethylcelulose and chemicals used for preparation of buffers and cross-linking agents were purchased from Sigma (St Louis, MO). The current was measured with a Gamry Framework Version 5.50 Amperometry**.** Aspirin (500 mg acetylsalicylic acid per tablet) and aspirin containing vitamin C (400 mg acetylsalicylic acid and 240 mg ascorbic acid per tablet) were purchased from a pharmacy.

Electrolysis cell and electrodes

A three-electrode electrochemical cell comprising working, counter and reference electrodes was used for all experiments. The working (area 0.5 cm^2) and counter electrodes were platinum; their surfaces were polished using 0.05 μm alumina slurry. The reference electrode was Ag/AgCl, introduced into the cell with a Luggin capillary.

Preparation of SOD biosensor

Polymer blends (CMC and gelatin) were prepared to obtain a final weight of 3,75 mg polymer in final solution by dissolving them in phosphate buffer (0.05 M, pH 7.4). SOD (4733 U, 1 mg) and glutaraldehyde (0.005 M) were added to the support system solutions to obtain a final volume of 50 μL at 32°C. The platinum foil electrode was covered with a total of 50 μL immobilization gel, 25. μL on each surface. The CMC–G–SOD electrodes were left at room temperature for 48 hour. Enzyme leakage tests were performed for all enzyme electrodes by washing with phosphate buffer (0.05 M, pH 7.4), three times, for 1 hour each, at 25°C. These tests were performed for all CMC–G–SOD biosensors obtained (Kocabay et al. 2012).

Principle of method

The biosensor used to determine the $O_2^{\bullet -}$ was obtained by coupling an amperometric electrode for hydrogen peroxide with the SOD enzyme immobilized on CMC–G support system (Kocabay et al. 2012).

The capacity of the SOD biosensor was determined as follows: the $O_2^{\bullet -}$ is produced in aqueous solution by oxidation of xanthine to uric acid in the presence of xanthine oxidase:

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{XOD}} \text{Uricacid} + 2H^+ + O_2^{\bullet -} \quad (1)$$

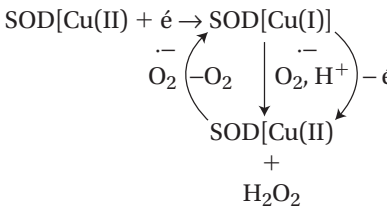
SOD immobilized on the Pt electrode catalyzes the dismutation reaction of the $O_2^{\bullet -}$ with release of oxygen and hydrogen peroxide according to equation

$$2H^+ + O_2^{\bullet -} \xrightarrow{\text{SOD}} H_2O_2 + O_2 \quad (2)$$

The H_2O_2 can be detected at the electrode surface in accordance with equation

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2\acute{e} \quad (3)$$

The current generated by oxidation of hydrogen peroxide at the working electrode held at 650 mV relative to the Ag/AgCl electrode is proportional to the concentration of $O_2^{\bullet -}$ in solution. The reaction scheme on the electrode surface is as follows:



Measurement of procedure

All measurements were performed in a standard three-electrode thermostated Pyrex cell containing 15 mL 0.05 M phosphate buffer, pH 7.4, at 25°C. The working electrode was placed in the cell and allowed to stabilize with a constant stirring rate of 1,000 rpm.

The biosensor response was used to examine the $O_2^{\bullet -}$ enhancement in heavy metal, cold, salinity and drought stress-treated tomato plants. The stress-treated tomato plants (totally leaves, roots and stems) (0.5 g) were homogenized in distilled water (3 mL) using a Bandalin homogenizer. The biosensor was left to stabilize in a cell containing phosphate buffer (0.05 M, pH 7.4) with magnetic stirring, until the response became constant. A solution of homogenized stress-treated tomato plants (500 µL) was added and the biosensor response was recorded. The stress-treated tomato plants were stored at −20°C before use. All experiments were performed five times in this work.

Growth condition

Tomato (*Lycopersicum esculentum* L. 'Falcon') seeds were germinated and grown hydroponically in pots containing 0.2 L of modified 1/10 Hoagland's solution. Hoagland solution includes macronutrients (K_2SO_4, KH_2PO_4, $MgSO_4.7H_2O$, $Ca(NO_3)_2.4H_2O$ and KCl) and micronutrients (H_3BO_3, $MnSO_4$, $CuSO_4.5H_2O$, NH_4Mo, $ZnSO_4.7H_2O$) with final concentrations of 2 mM Ca, 10-6 M Mn, 4 mM NO3, 2.10-7 M Cu, 1 mM Mg, 10-8 M NH4, 2 mM K, 10-6 M Zn, 0.2 mM P, 10-4 M Fe ve10-6 M B. Six plants were grown in each pot in a controlled environmental growth chamber with light of 250 mmol m–2 s–1 photosynthetic photon flux at 25°C, 70% relative humidity. Twenty-five-days-old plants grown in controlled media were used for stress treatments.

Stress treatment

Salinity and drought treatment

Twenty-five-days-old plants grown in controlled media were used for stress treatments. Treatment of the plants with salt was imposed by the addition of NaCl to the nutrient solution to a final concentration of 100 and 150 mM. Osmotic pressures of the salt solutions were determined by Vapor Pressure Osmometer 5520. The average osmotic pressure of 100 mM NaCl solution was estimated as 190 mmol/kg while 150 mM NaCl solution was found approximately as 290 mmol/kg. For the drought treatment (Verslues et al. 2006), PEG was added to the nutrient solution until the osmolality of the solution, as measured using a vapor pressure osmometer, was the same as that of the salt solutions. In this way, an iso-osmotic level of stress was applied to both the salt and PEG-treated plants, with the goal of matching the stem water potentials of the plants exposed to these treatments. These applications were named as 100 and 150 mM drought treatment. Six plants in a single pot were harvested at 0, 3, 6, 12, 24 and 48 hours after the plants were exposed to these treatments.

Cold treatment

For application of cold stress, plants were exposed to 4°C and samples were collected in different time periods (control, 2 days, 4 days, 6 days, 8 days and 10 days).

Heavy metal treatment

Twenty-five-days-old plants grown in controlled media were used for stress treatments. For application of heavy metal stress Cd^{2+}, Pb^{2+}, Cu^{2+}, Mn^{1+} were added to the hydroponic solution for 24 hours at concentrations of 0 (control), 80, 160, 320, 640 and 1280 mM.

Results and discussion

The development of highly sensitive SOD biosensor for the simultaneous determination of superoxide radicals by immobilization of superoxide dismutase within CMC–G on a Pt electrode surface was performed. The parameters affecting the performance of the biosensor were investigated in detail (Kocabay et al. 2012). The response of the CMC–G–SOD biosensor was proportional to $O_2^{\bullet -}$ concentration and the detection limit was 1.25×10^{-3} mM with a correlation coefficient of 0.9994 at a signal-to-noise ratio of 3. The response time of the developed biosensor was 2 seconds (Kocabay et al. 2012). In this study, the application of developed CMC–G–SOD biosensor to the agriculture was studied. For this purpose, tomato plants were exposed to heavy metal, cold, salinity and drought stress to examine the $O_2^{\bullet -}$ enhancement. The effect of heavy metal, cold, salinity and drought stress in tomato plants on $O_2^{\bullet -}$ enhancement was investigated using the developed CMC–G–SOD biosensor.

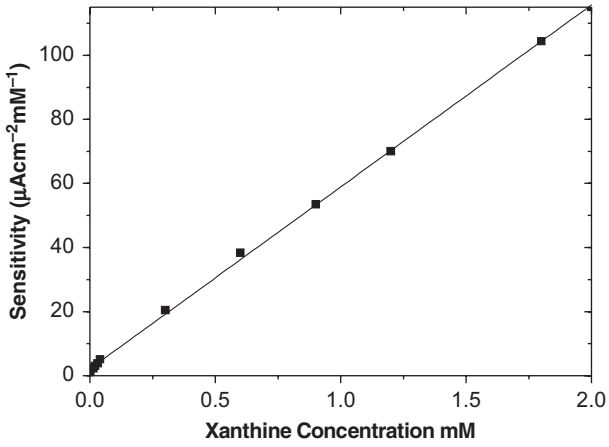


Figure 1. Calibration graph for the CMC–G–SOD biosensor.

Amperometric response of the G–SOD biosensor to $O_2^{\bullet-}$

The amperometric response of the CMC–G–SOD biosensor resulted from superoxide anion was investigated in the stirred buffer solution. An efficient and simple method was utilized for generation of $O_2^{\bullet-}$ by oxidation of xanthine to uric acid in the presence of xanthine oxidase at an applied potential of 650 mV. The measurement solution should be operated at a constant and high stirring speed (1,000 rpm) to acquire reproducible results. A peak-like response was observed, which is in agreement with a report by other worker (Lisdat et al. 1999, Di et al. 2004, Fujita et al. 2009, Di et al. 2007, Wang et al. 2009). A calibration plot as a function of increasing xanthine concentration for the CMC–G–SOD biosensor was obtained previously by ourself (Kocabay et al. 2012) (Figure 1). The amperometric response of the CMC–G–SOD biosensor increases with ascending xanthine concentration.

Salinity and drought treatment

The effect of drought stress in tomato plants on $O_2^{\bullet-}$ enhancement was investigated using developed CMC–G–SOD biosensor. The measurements were performed for a period of 48 hours. As can be seen from Figure 2, with regard to control, the $O_2^{\bullet-}$ production rapidly increased for a 12 hour-period of both 100 and 150 mM drought-treated tomato plants. After this time period, profiles of $O_2^{\bullet-}$ production were differentiated between 100 and

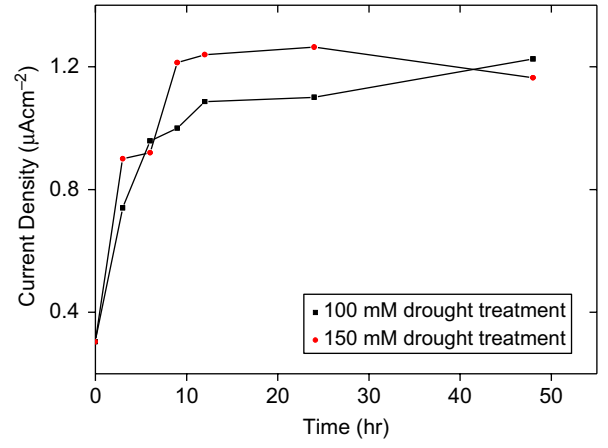


Figure 2. Effect of drought on $O_2^{\bullet-}$ production in tomato plants seedling after 48 hours treatment.

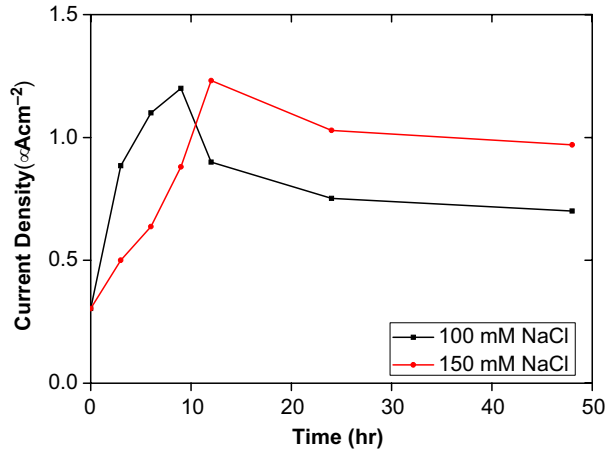


Figure 3. Effect of salt on $O_2^{\bullet-}$ production in tomato plants seedling after 48 hours treatment.

150 mM drought-treated samples. $O_2^{\bullet-}$ production continued to increase and reached maximum level after 48 hours in 100 mM drought-treated tomato plants. On the other hand, 150 mM drought-treated tomato plants reached maximum $O_2^{\bullet-}$ production after 24 hours and then decreased, while $O_2^{\bullet-}$ enhancement was obtained by CMC–G–SOD biosensor for 100 mM drought-treated tomato plants.

Also, the $O_2^{\bullet-}$ productions were studied with the CMC–G–SOD biosensor for 100 and 150 mM salt-treated tomato plants (Figure 3). The $O_2^{\bullet-}$ production significantly increased up to 9 days for 100 mM and 12 days for 150 mM salt-treated tomato plants. After this time period, decrease orientations were monitored in the $O_2^{\bullet-}$ productions. Profile of decrease was similar for all salt treatment but minimum $O_2^{\bullet-}$ level was recorded in 100 mM salt-treated tomato plants for 48 hours. At the same time, all recorded $O_2^{\bullet-}$ levels for time period of salt treatments were above control level. These results are compatible with the idea that productions of ROS are low under normal growth condition (as control in the current study) and there is equilibrium between ROS production and defense pathways (such as SOD). Stress conditions such as salt, drought, cold, heavy metals, ultraviolent etc. disturb this cellular homeostasis and enhance the production of ROS (Wang et al. 2009) and APX, CAT and SOD are substantial components of

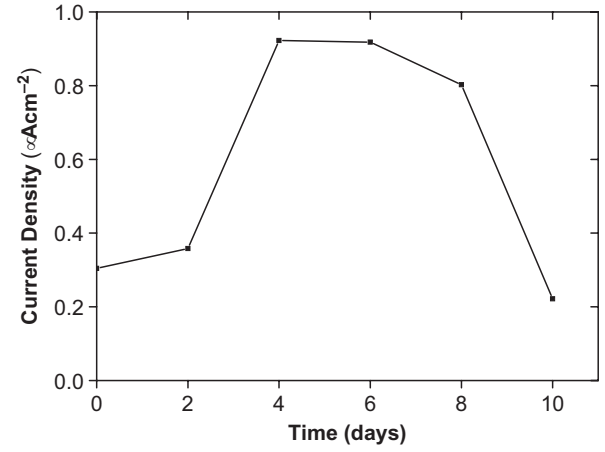


Figure 4. Effect of cold on $O_2^{\bullet-}$ production in tomato plants seedling after 10 days treatment.

ROS-scavenging mechanism of plants (Bowler et al. 1992, Asada and Takahashi 1987, Polle 2001).

Accumulation of ROS including $O_2^{\bullet -}$ could act as an oxidative damaging factor at cellular level. A protective or signaling factor depends on the equilibrium between ROS production and scavenging or defense mechanisms, the significant ones being SODs, a family of metalloenzymes catalyzing the dismutation of O_2^- to H_2O_2. Plants can scavenge altered formation of ROS by activation of SOD (Smirnoff 1993, Mittler 2002, Dat et al. 2000). $O_2^{\bullet -}$ productions were altered depending on stress application and exposure time of the stress. In the view of this information, decreased orientations of $O_2^{\bullet -}$ productions in salt treated plants and stationary orientations of $O_2^{\bullet -}$ productions in drought-treated plants can conclude as plants can successfully cope with long time period of salt but not the drought stress. Therefore, the decreased production of $O_2^{\bullet -}$ after long time period of salt stress could indicate the activated SOD isoenzymes as a component of antioxidant defense mechanism.

Cold treatment

Tomato plants were exposed to 4°C for the cold stress and samples were collected in different time periods (control, 2 days, 4 days, 6 days, 8 days and 10 days). The $O_2^{\bullet -}$ concentrations in cold-treated tomato plants are presented in Figure 4. Throughout the time period of cold stress $O_2^{\bullet -}$ production increased except for other 10 days. While $O_2^{\bullet -}$ production had slightly increased in the first 2 days, it reached maximum level after 4 days. This level became constant for 6 days. $O_2^{\bullet -}$ production was gradually declined and it was under the control level after 10 days of cold exposure. These results are agreed with the accumulation of ROS under cold stress which has been reported in several plants (Prasad et al. 1995, Gonzales-Meler et al. 1999). The production of $O_2^{\bullet -}$ after 10 days cold exposure which is under the control level can be explained as cell death and interrupted stress perception in tomato tissues.

Heavy metal treatment

Heavy metals are significant environmental contaminants, and their presence in the atmosphere, soil and water can cause serious problems to all organisms. Inhalation and ingestion are the primary routes for heavy metals to enter the human body. Ingestion is the main route of exposure to these heavy metals. Human populations' intake of heavy metals is through food chain. Vegetables uptake metals by absorbing them from contaminated atmosphere, soil and water (Ellen et al. 1990). The bioaccumulation of heavy metal in the food chain can be highly dangerous to human health. Unlike organic compounds, they cannot be degraded or destroyed under biotic conditions. Since there is no good mechanism for their elimination, the chronic low-level intakes of these elements have damaging effects on human beings and other animals. These metals cause environmental hazards and are reported to be exceptionally toxic (Conte et al. 1998).

This study was performed to detect the effects of the heavy metal Cd^{2+},Pb^{2+}, Cu^{2+}, Mn^{1+} on the tomato plants utilizing the CMC–G–SOD biosensor to compare the alterations in $O_2^{\bullet -}$ concentration. Different metals and concentrations were used to determine the impact of heavy metal treatment on $O_2^{\bullet -}$ in tomato plants. For this purpose, 25-day-old plants grown in controlled media were used for stress treatments. The heavy metal stress was performed with Cd^{2+}, Pb^{2+}, Cu^{2+}, Mn^{1+} with different concentrations for a period of 24 hours. The concentrations for all heavy metals were 0 (control), 80, 160, 320, 640 and 1280 mM. Each metal had a different impact on $O_2^{\bullet -}$ enhancement in tomato plants. The

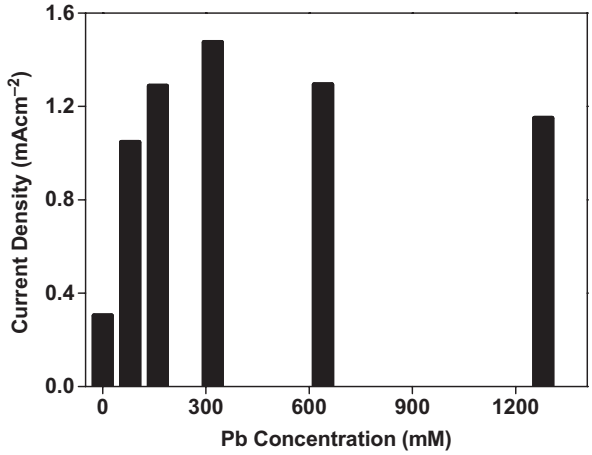


Figure 6. Effect of Pb^{+2} concentration on $O_2^{\bullet -}$ production in tomato plants after 24 hours treatment.

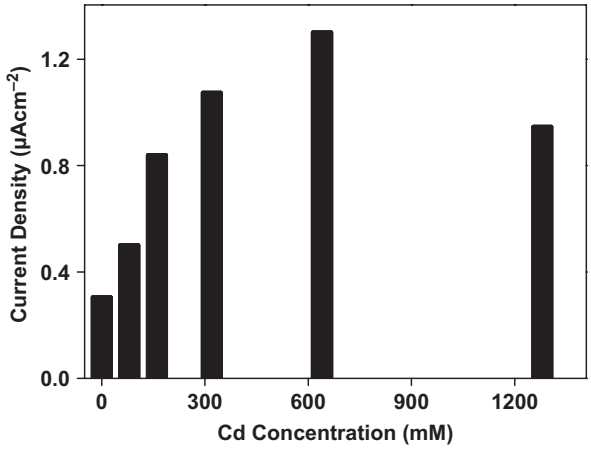


Figure 5. Effect of Cd^{+2} concentration on $O_2^{\bullet -}$ production in tomato plants after 24 hours treatment.

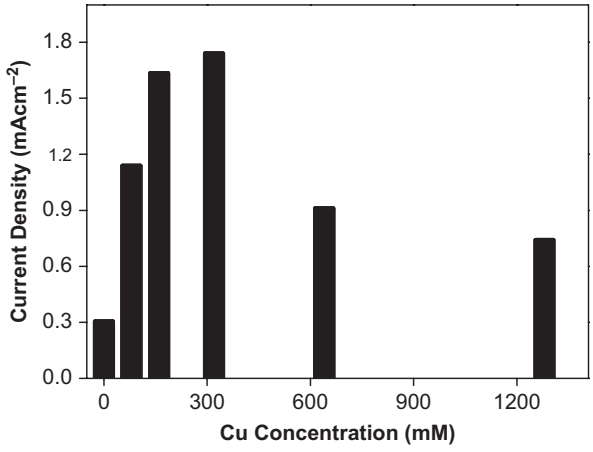


Figure 7. Effect of Cu^{+2} concentration on $O_2^{\bullet -}$ production in tomato plants after 24 hours treatment.

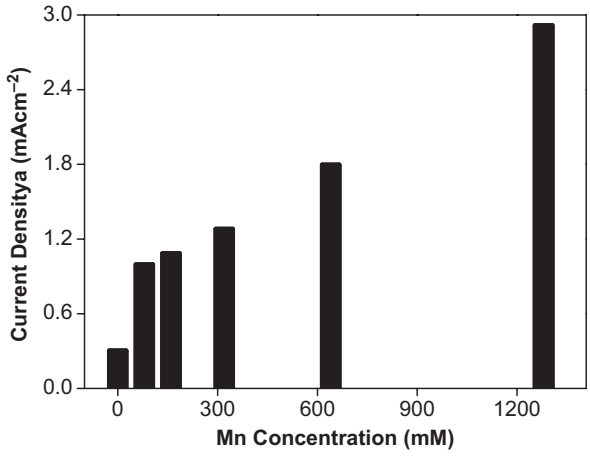


Figure 8. Effect of Mn^{+1} concentration on $O_2^{\bullet-}$ production in tomato plants after 24 hours treatment.

$O_2^{\bullet-}$ concentration was gradually increased with increasing Cd^{2+} concentrations (Figure 5). Maximum production of $O_2^{\bullet-}$ was observed at 640 mM Cd^{2+} treatment. It can be seen from Figure 5 that this production was decreased with the treatment of 1280 mM Cd^{2+}, but still significantly upon control level.

Tomato plants exposed to several concentrations of Pb^{2+} and Cu^{2+} contamination resulted in increased $O_2^{\bullet-}$ production up to 320 mM treatments of these metal. After 320 mM concentration a gradual decline was observed in $O_2^{\bullet-}$ production for both heavy metals (Figures 6 and 7). All patterns of current densities in $O_2^{\bullet-}$ production were similar among Cd^{2+}, Pb^{2+}, Cu^{2+} contaminated tomato plants. Initially the production of $O_2^{\bullet-}$ increased with increasing metal concentration and was followed by a decrease in $O_2^{\bullet-}$ production, still protecting their levels at 1280 mM concentration.

On the other hand, the $O_2^{\bullet-}$ production showed alteration with Mn^{1+} contamination. The $O_2^{\bullet-}$ production increased with increasing concentration of Mn^{1+} up to 1280 mM (Figure 8). No decreased orientation with the exposure of Mn^{1+} to the tomato plants was observed. We concluded that with all $O_2^{\bullet-}$ production data obtained from tomato plants contaminated with different concentrations of Cd^{2+}, Pb^{2+}, Cu^{2+} and Mn^{1+} treatment antioxidant defense mechanism is not stimulated with low concentration of metal contamination. Antioxidant defense mechanisms start to prevent $O_2^{\bullet-}$ production and scavenge $O_2^{\bullet-}$ at 320 mM concentration for Pb^{2+} and Cu^{2+} contamination and 640 mM concentration for Cd^{2+} contamination. However it could not prevent or scavenge $O_2^{\bullet-}$ production in tomato plants exposed to Mn^{1+} solutions or the rate of $O_2^{\bullet-}$ production was significantly higher than scavenge capacity of antioxidant defense mechanism. The other conviction of us about data of $O_2^{\bullet-}$ production under Mn^{1+} contaminations is that the tomato plants is principally sensitive to contamination of this metal solution. The $O_2^{\bullet-}$ production increase of 89.6%, 82.5%, 79.4%, 76.6% for Mn^{1+}, Cu^{2+}, Pb^{2}, Cd^{2+}, respectively, was observed by increasing the heavy metal concentration.

In the current study, $O_2^{\bullet-}$ production resulting under different concentrations of Cd^{2+}, Pb^{2+}, Cu^{2+}, Mn^{1+} indicate that these heavy metals can substantially intensify the accumulation of ROS in leading to oxidative stress followed by superoxide anion in mitochondria and other organelles of plants (Prasad et al. 1999, Cuypers et al. 1999).

Conclusion

A highly sensitive SOD biosensor for the simultaneous determination of superoxide radicals was developed by immobilization of superoxide dismutase within CMC–G on a Pt electrode surface (Kocabay et al. 2012). The response of the CMC–G–SOD biosensor was proportional to $O_2^{\bullet-}$ concentration and the detection limit was 1.25×10^{-3} mM with a correlation coefficient of 0.9994 at a signal-to-noise ratio of 3. The response time of the developed biosensor was 2 seconds. The developed biosensor exhibited high analytical performance with wider linear range, high sensitivity and low response time (Kocabay et al. 2012). In this study, the application of developed CMC–G–SOD biosensor to agriculture was studied. For this purpose, tomato plants were exposed to heavy metal, cold, salinity and drought stress to examine the $O_2^{\bullet-}$ enhancement. The effect of heavy metal, cold, salinity and drought stress in tomato plants on $O_2^{\bullet-}$ enhancement was investigated using developed CMC–G–SOD biosensor.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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