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The Induction of Antioxidant Enzyme Activities in Cabbage Seedlings by Heavy Metal Stress

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***Abstract*—**Cabbage seedlings grown in vitro were exposed to excess levels of heavy metals, including Cd, Mo, and Zn. High metal levels affected plant growth at cotyledonary stage. Seedlings under Cd, Mo, and Zn treatments could not produce root hairs and true leaves. Under stress conditions, seedlings accumulated a higher amount of anthocyanins in their cotyledons than those in the control. The pigments isolated from Cd and Zn stressed seedling cotyledons appeared as pink, while under Mo stress, was dark pink or purple. Moreover, excess Mo stress increased antioxidant enzyme activities of APX, CAT, SOD. These results suggest that, under excess Mo stress, the induced antioxidant enzyme activity of cabbage seedlings may function as a protective mechanism to shield the plants from toxicity and exacerbated growth.

***Keywords*—**Anthocyanin, antioxidant enzyme activity, heavy metal, growth inhibition.

I. Introduction

MANY agriculture areas of the world are contaminated by heavy metals, such as cadmium (Cd), cobol (Co), chromium (Cr), cupper (Cu), nickel (Ni), lead (Pb), arsenic (As), zinc (Zn) and molybdenum (Mo). These metals are largely found in fertilizers, sewage sludge application, dust from smelters, industrial waste and bad watering practices in agricultural lands for long-term utilization [1]. The heavy metals produced from these activities leached to the soil and irrigation systems and may act as stressors to plants by increasing the production of reactive oxygen species (ROS). This is a common consequence of abiotic stress to plant growth [2]. Mo, an essential element required during the life cycle of plants, is distributed throughout the soil environment because of the use of industrial stainless steel, mining, cast iron, and agricultural activities [3]. The excess Mo accumulated in higher plants was due to increased soil pH to make it alkaline and thus over uptake [4]. Higher plants generally maintain the balance of the essential elements by proper uptake from the soil; however, absorption of excess Mo may result in toxicity symptoms, induce antioxidant activity, and increase anthocyanin accumulation in plant tissues to overcome the excess metal stress [5]. Another metal that may cause abiotic stress is Zn, a minimum essential micronutrient required by plants and potentially toxic for higher plants if in excess. The elevated Zn found in contaminated soils may cause phytotoxicity and inhibit plant metabolic functions, resulting in growth retardation and senescence [6]. Conversely, Cd, a non-essential heavy metal and extremely toxic, affects plant growth and development, such as reduction in photosynthesis, growth inhibition, browning of root tips, and water and nutrient uptake [7].

High level of heavy metals in plant tissues will induce exaggerated amount of free radicals and other ROS. To control the level of ROS and protect cells under stress conditions, plant tissues may produce several enzymes for scavenging ROS, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), and a network of low molecular weight antioxidants such as glutathione, phenolic compounds, carotenoids, and flavonoids [8]. SOD is an important antioxidant enzyme that catalyzes a disproportionate amount of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) [9], while elevated CAT and APX activities help protect plant cell from ROS such as H_2O_2, O_2^- and hydroxyl radicals (OH^-), which were produced in plants under stress conditions [10].

The aim of this study was to evaluate the effect of heavy metals on cabbage seedlings by activating antioxidant enzyme activity and anthocyanin accumulation in plant tissues under metal stress conditions.

II. Materials and Methods

A. Plant Materials and Growth Measurement

The hybrid variety KY Cross of commercial cabbage (*Brassica oleracea* var. *capitata*) was used for all experiments. Seeds were surface sterilized by use of 0.6 % sodium hypochloride for 15 min and washed for three times with sterile water then sown on Murashige and Skoog (MS) medium [11] supplemented with 20 g/L sucrose and 8 g/L Sigma agar. Two days after seed germination, uniformly grown young seedlings were removed carefully from agar medium and transplanted onto both MS medium and MS supplemented cadmium chloride, sodium molybdate and zinc sulfate, respectively, with the concentrations of 0, 0.1, 0.5, 1, 2.5, 5 and 10 mM. The in vitro cultures were kept at 25 ± 2 °C

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under a 16/8 h photoperiod with a light intensity of 35-40 $\mu mol.m^{-2}.s^{-1}$ provided by cool white fluorescent lamps (StarcoatTM F28W/T5/840,170 MA, Hungary). Cotyledons were harvested 8 days after treatment and frozen with liquid nitrogen and stored at -80°C until use. The length of the hypocotyls, root and cotyledons, the cotyledon width and presence of root hairs were recorded respectively. Means of three seedlings for each treatment were collected and subjected to statistical analysis by Fisher's Protected LSD test at $P \leq 0.05$.

B. Anthyocyanins Extraction and Determination

Cotyledons of 0.12-0.2 g were extracted with 1% HCl in methanol, and the crude extracted was left overnight at 4ºC. Five hundred µl chloroform was then added and centrifuged at 13000 rpm (Centrifuge 5415R) at 4ºC for 5 min at 4 °C. The supernatant was used for anthocyanin measurement by a spectrophotometer (Hitachi U-2900 UV-Vis Double Beam System) at 510 and 700 nm after the differential pH method and the total anthocyanin content in mg g^{-1} FW followed Giusti and Wrolstad [12] calculation method.

C. Antioxidant Enzyme Extraction and Activity Assay

Two days after seed germination, young seedlings were removed carefully from agar medium and transplanted onto both MS medium and MS with sodium molybdate at 0, 0.1, 2.5 and 10 mM. Cotyledons were harvested at 0, 6, 12, 24, 48 h and frozen with liquid nitrogen and stored at -80°C until use. The cotyledons were extracted with 50 mM sodium phosphate buffer (pH 7.0) [13]-[8].

Protein content was determined at 595 nm according to Bradford [14]. The mixture of 1 µl crude extraction with 299 µl Protein Assay Dye Reagent Concentrate (Bio-Rad Protein Assay) and 700 µl distilled water were incubated at room temperature for 5 min.

SOD activity was measured as adapted from Minami and Yoshikawa [15] and Paoletti et al [16]. The extraction buffer consisted of 0.055 µM nitro blue tetrazolium (NBT), 50 mM Tris-HCl, 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 16 µM pyrogallol, 1.4% Triton X-100, 778 µl distilled water, and 200 µl of enzyme extract in a final total volume of 1 ml. The mixtures were reacted at 37ºC for 10 min and then absorption measured spectophotometrically at 540 nm. One unit of SOD activity was expressed as SOD unit mg $protein^{-1}$ min^{-1} and defined as enzyme activity, which caused the inhibition of NBT by 50% (w/v).

APX activity measurement was as described by Nakano and Asada [17], with the reaction mixture containing 150 µM sodium phosphate buffer pH 7.0, 1.5 µM ascorbate, 6 mM H_2O_2, 0.75 mM EDTA, and 50 µl enzyme in a total volume of 1.5 ml. The reaction was allowed to occur for 1 min at room temperature, and the absorbance at 290 nm obtained. The enzyme activity was expressed as µmole of ascorbate oxidized mg $protein^{-1}$ min^{-1}.

CAT activity was measured according to the method of Kato and Shimizu [18], with a mixture containing 0.5 M sodium phosphate buffer pH 7.0, 10 mM H_2O_2 and 100 µl enzyme extract. The final volume was 1.6 ml. The reaction occurred for 1 min at 240 nm. The enzyme activity was expressed as µmole of H_2O_2 decomposed per mg $protein^{-1}$ min^{-1}.

III. Results

A. The Effect of Cd, Mo and Zn Levels on Plant Growth

Supra-optimal levels of Cd, Mo, and Zn were added to Murashige and Skoog (MS) medium, and all significantly decreased seedling growth by the 8th day. Root length ranged between 4.01 to 83.04 mm for all treatments, and 0.5 mM Zn induced the highest root length and was significantly different with control and other treatments. The hypocotyl lengths between 2.93 to 41.44 mm. Hypocotyl growths were inhibited the highest at low concentrations of Cd and Mo (0.1 mM), and Zn (0.5 mM), whereas a high level of Cd completely inhibited its growth. The cotyledon width and length of 0.1 mM Mo treatment was the highest, than other treatments and control. Root hair growth was inhibited at high levels of 5 mM Zn, 2.5 mM Mo, and 0.1 mM Cd (see Table I and Fig. 1)

B. The Influence of Heavy Metal Stress on Accumulation of Anthocyanin

Total anthocyanin content of the cotyledons under the metal stress condition markedly increased, ranging from 0.013 to 0.043 mg g^{-1} fresh weight (FW). The anthocyanin content at 0.1 mM Zn and 1 mM Cd were higher than that in other treatments. In the treatments of Mo, anthocyanin content was slightly increased from at higher concentration, while the treatment of Cd and Zn, the anthocyanin content also increased from low levels to 5 mM and moderated at the highest concentration (Fig. 2). The anthocyanin accumulated at high levels under high heavy metal concentrations, and the pigment extract showed deep pink to purple coloration, whereas in the control and treatments with low level heavy metals resulted in less anthocyanin and the pigment solution appeared as light pink coloration (Fig. 3).

C. Antioxidative Enzyme Activity Induced by Mo Excess Stress

The protein contents decreased at high concentrations Mo treatments (Fig. 4a). SOD activity was the highest at 10 mM Mo at 48 h followed by 2.5 mM at 24 h, 10 mM at 12 and 6 h (Fig. 4b). In addition, APX activity increased under Mo excess after 12 h treatments and other treatments decreased at 48 h (Fig. 4c). CAT activity increased under the highest Mo level, 10 mM at 48 h (Fig. 4d). In general, the enzyme activities of SOD, APX, and CAT increased in seedlings under higher Mo concentrations (Fig. 4).

TABLE I
THE EFFECT OF CADMIUM, MOLYBDENUM AND ZINC ON CABBAGE SEEDLING GROWTH AFTER 8 DAYS

Treatments concentration (mM)		Growth parameters (mm)			
		Root length	Hypocotyl length	Cotyledon width	Cotyledon length
0	Control	54.02±3.31^{d}	31.33±1.71^{c}	12.92±2.30^{bcd}	7.46±0.50^{cd}
0.1	Cd	60.35±0.22^{c}	41.24±1.80^{a}	11.27±1.51^{cdef}	7.11±1.07^{cd}
	Zn	55.29±3.61^{d}	36.01±3.23^{b}	12.43±2.03^{bcde}	8.90±0.64^{ab}
	Mo	74.04±1.14^{b}	41.44±1.37^{a}	16.06±0.55^{a}	9.28±0.91^{a}
0.5	Cd	10.44±2.84^{h}	10.02±4.82^{g}	10.70±1.12^{efg}	5.51±0.31^{fg}
	Zn	83.04±3.42^{a}	38.74±4.21^{ab}	13.69±1.30^{b}	7.97±1.52^{bc}
	Mo	46.60±2.61^{e}	28.87±1.96^{cd}	10.19±1.44^{fgh}	6.58±0.51^{def}
1	Cd	6.37±1.64^{hi}	9.24±0.83^{gh}	8.55±0.17^{hi}	4.19±0.12^{hij}
	Zn	26.45±3.70^{f}	24.22±2.82^{e}	13.36±2.47^{bc}	6.76±1.81^{cde}
	Mo	48.23±2.81^{e}	27.69±2.49^{de}	11.38±1.38^{cdef}	7.29±0.65^{cd}
2.5	Cd	4.99±0.99^{i}	6.31±2.02^{hij}	8.88±0.49^{ghi}	4.06±0.12^{ij}
	Zn	16.49±1.08^{g}	13.85±1.40^{f}	13.92±1.83^{b}	6.25±0.78^{def}
	Mo	10.39±0.69^{h}	7.93±0.70^{gh}	9.93±0.77^{fghi}	5.36±0.61^{fgh}
5	Cd	4.01±0.97^{i}	4.99±0.62^{ij}	8.56±0.34^{hi}	4.06±0.16^{ij}
	Zn	9.69±2.61^{h}	9.75±0.92^{gh}	10.97±0.96^{defg}	5.62±0.61^{efg}
	Mo	10.23±0.60^{h}	7.60±0.10^{gh}	10.38±0.21^{efgh}	4.93±0.33^{ghi}
10	Cd	4.04±1.17^{i}	2.93±0.69^{j}	8.05±0.82^{i}	3.68±0.11^{j}
	Zn	4.63±0.32^{i}	6.81±1.15^{ghi}	9.50±0.72^{fghi}	4.76±0.22^{ghij}
	Mo	10.03±0.43^{h}	7.21±0.61^{gh}	9.40±0.34^{fghi}	4.75±0.23^{ghij}

The values represent means ± standard deviation. Means followed by the same letter within a column are not significantly different by LSD test at $P \leq 0.05$ (n=3)

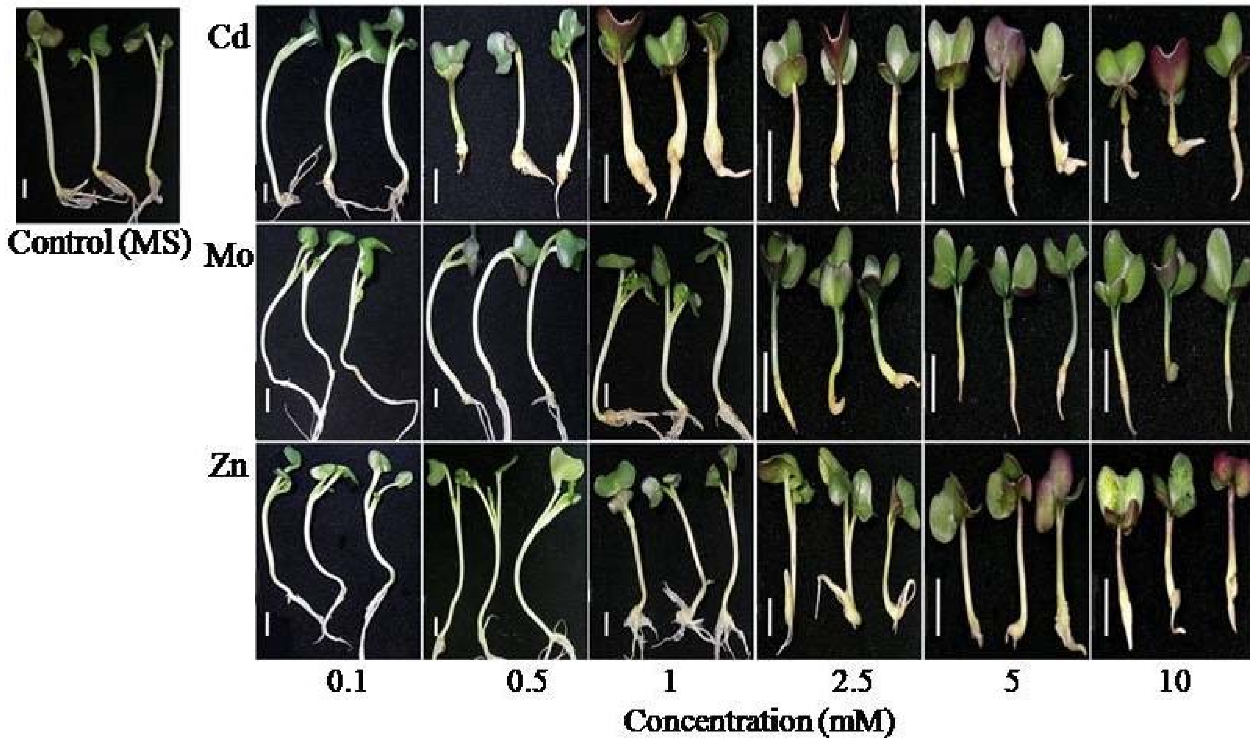

Fig. 1 Eight-day-old cabbage seedlings grown in vitro in control (MS) and MS supplemented with Cd, Mo and Zn at concentrations 0.1, 0.5, 1, 2.5, 5 and 10 mM, respectively (bar 5 mm)

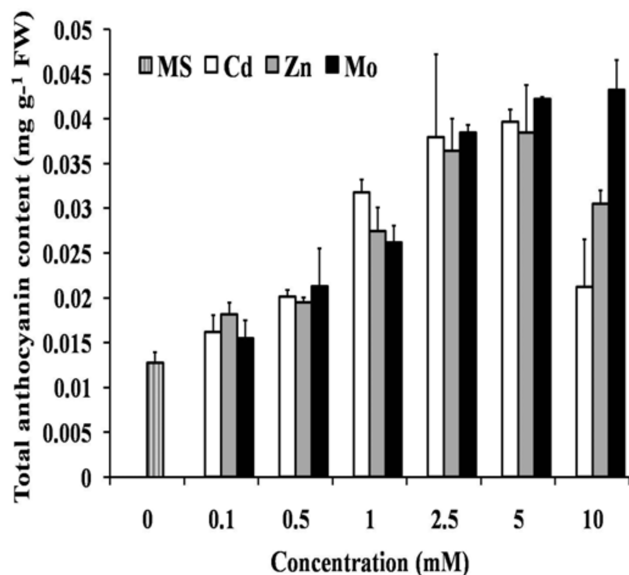

Fig. 2 Total anthocyanin content in cabbage seedlings under control (MS) and MS supplemented with Cd, Mo and Zn at concentrations 0, 0.1, 0.5, 1, 2.5, 5 and 10 mM for 8 days. Values represent means of three replications ± standard deviation

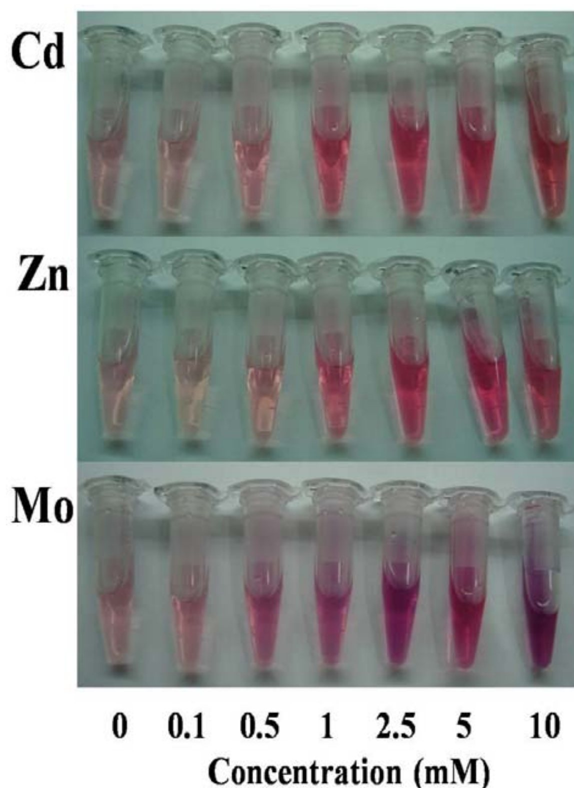

Fig. 3 Mapping appearance of anthocyanin extracts from cabbage seedling cotyledons using 1% HCl in methanol as solvent

IV. Discussion

Excess heavy metals in the soil environment cause damage to plant via growth retardation. Therefore, plant may produce more antioxidant enzyme activities to overcome cellular damages under this stress condition [19]. The experiments on cabbage seedlings exposed to Mo stress, also showed elevated antioxidant activities Lower concentrations of Mo and Zn at minimum levels are necessary for normal plant growth, as both are essential micronutrients [20]-[21]. However, too much Mo may decrease shoot growth and yields in tomato, oilseed rape, red clove, and ryegrass [22]-[23]. As also revealed in our results in retarding hypocotyl length, and shoot and root growths of cabbage seedlings by heavy metals.

According to Bia et al [20], high concentration of Zn was toxic and inhibited plant growth, as observed in plants grown in soil near smelting areas rich in Zn. Cabbage seedlings growth was apparently inhibited under much lower Cd concentration than Mo and Zn, which suggests that Cd is the more dangerous heavy metal to plants because of its high mobility and toxicity, which affects growth and yield [24]. Under stress conditions such as presence of heavy metals, light, temperature, etc., tolerant plant evolved mechanisms to protect itself by increasing the antioxidative properties such as that of flavonoids, including anthocyanin, and antioxidative enzymes to overcome metal toxicity [25].

Anthocyanin accumulation also has been implied to confer tolerance to diverse environmental stressors [26]. The high anthocyanin content of bilberry calli is caused by high Zn in the medium [27]. In addition, Cd induced anthocyanin accumulation in *Azolla imbricata* [28] and pine [29]. Moreover, it also increased anthocyanin level in the leaves of *Brassica juncea* [30]. While Cd+NaCl treated *Myriophyllum heterophyllum* Michx. and *Potamogeton crispus* increased anthocyanin level [31].

In this study and other reports, enzyme activity of SOD, APX, and CAT increased under heavy metal stress. This suggests that antioxidants induced in plants act to protect against stress conditions. According to Tewari et al [32], plants response to Zn toxicity by increasing the activity of antioxidative enzymes SOD, CAT, and APX. Groppa et al [33] reported that under Cd stress, the activities of the enzymes, SOD, CAT, APX, GR, and DHAR were increased in both the shoots and roots of radish [34] and pea plants [35]. In addition, Mo and B increased the antioxidant enzyme activities of CAT, SOD, POD and protein content in soybean seeds [36].

The results of this study demonstrated detrimental effects of Cd, Mo, and Zn stressors on growth inhibition in cabbage seedlings. High Mo condition increased antioxidant enzyme activity and anthocyanin accumulation, probably to scavenge ROS in plant cells under stress and unlike that in the control under normal conditions.

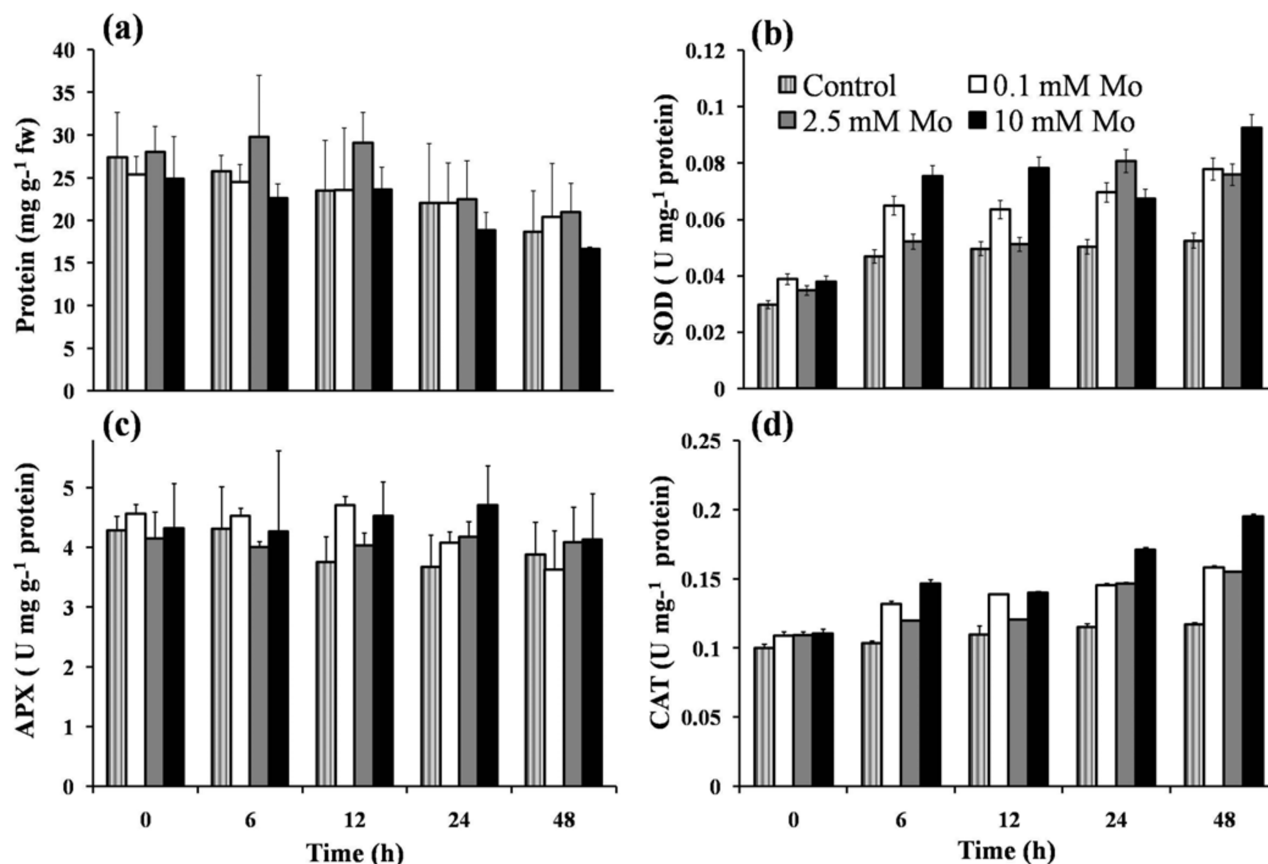

Fig. 4 Protein content (a) and the kinetics of the antioxidant enzymes, superoxide dismutase (SOD) (b), ascorbate peroxidase (APX) (c) and catalase (CAT) (d). The activity is presented for cabbage seedlings cotyledons exposed to MS with molybdenum at 0, 0.1, 0.5, 1, 2.5, 5 and 10 mM for 8 days. Values represent mean of three replications ± standard deviation

REFERENCES

[1] B. Passariello, V. Giuliano, S. Quaresima, M. Barbaro, S. Caroli, G. Forte, G. Carelli, and I. Iavicoli, “Evaluation of the environmental contamination at an abandoned mining site. *Microchem. J.*, vol. 73, pp. 245-250. 2002.

[2] T. J. Bricker, J. Pichtel, H. J. Brown, and M. Simmons, “Phytoextraction of Pb and Cd from superficial soil: effects of amendments and croppings,” *J. Environ. Science. Health. Part A Toxic/Hazardous Substances and Environmental Engineering*, vol. 36, pp. 1597-1610, 2001.

[3] R. R. Mendel, “Molybdenum: biological activity and metabolism,” *Dalton Trans*. vol. 21, pp. 3404-3409, Nov. 2005.

[4] K. S. Smith, L. S. Balistrieri, S. M. Smith and R. C. Severson, “Distribution and Mobility of Molybdenum in the Terrestrial Environment,” in Molybdenum in Agriculture, U. C. Gupta, Ed. Cambridge: Cambridge University Press, 1997, pp. 23-46.

[5] J. L. Stroud, F. J. Zhao, P. Buchner, F. Shinmachi, S. P. McGrath, J. Abecassis, M. J. Hawkesford, and P. R. Shewry, “Impacts of sulphur nutrition on selenium and molybdenum concentrations in wheat grain,” *J. Cereal Sci*., vol. 52, pp. 111-113, 2010.

[6] M. S. Warne, D. Heemsbergen, D. Stevens, M. McLaughlin, G. Cozens, M. Whatmuff, K. Broos, G. Barry, M. Bell, D. Nash, D. Pritchard, and N. Penney, “Modeling the toxicity of copper and zinc salts to wheat in 14 soils,” *Environ. Toxicol. Chem*., vol. 27, pp. 786-792, Apr. 2008.

[7] P. Mohanpuria, N. K. Rana, and S. K. Yadav, “Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze,” *Environ. Toxicol*., vol. 22, pp. 368-374, Aug. 2007.

[8] M. M. Posmyk, R. Kontek and K. M. Janas, “Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress,” *Ecotoxicol. Environ. Saf*., vol. 72, pp. 596-602, Feb. 2009.

[9] C. Bowler, M. Vanmontagu and D. Inze, “Superoxide dismutase and stress tolerance,” Ann. Rev. *Plant Physiol. Mol. Biol*., vol. 43, pp. 83-116, June 1992.

[10] S. Srivastava. A. D. Pathak, P. S. Gupta, A. K. Shrivastava, and A. K. Srivastava, “Hydrogen peroxide-scavenging enzymes impart tolerance to high temperature induced oxidative stress in sugarcane,” *J. Environ. Biol*., vol. 33, pp. 657-661, May 2012.

[11] T. Murashige, and F. Skoog, “A revised medium for rapid growth and bioassays with tobacco tissue cultures,” *Physiol. Plant*, vol.15, pp. 473-497, 1962.

[12] M. M. Giusti, and R. E. Wrolstad, “Anthocyanins Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy,” in Current Protocols in Food Analytical Chemistry Hand Book of Food Analytical Chemistry, vol. 12, R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemker, D. Smith, and P. Sporns, Ed. New York: John Wiley & Sons, 2001, pp.19-32,

[13] E. Gajewska, and M. Skłodowska, “Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress,” *Acta Physiol. Plant*, vol. 27, pp. 329-339, 2005.

[14] M. M. Bradford, “A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding,” *Anal. Biochem*., vol. 72, pp. 248-254, May 1976.

[15] M. Minami, and H. Yoshikawa, “A simplified assay method of super oxide dismutase,” *Clin. Chim. Acta*, vol. 29, pp. 337-342, 1979.

[16] F. Paoletti, D. Aldinucci, A. Mocali, and A. Capparini, “A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts,” *Anal. Biochem*., vol. 154, pp. 536-541, May 1986.

[17] Y. Nakano, and K. Asada, “Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts,” *Plant Cell Physiol*., vol. 22, pp. 867-880, 1981.
[18] M. Kato, and S. Shimizu, “Chlorophyll metabolism in higher plants VI. Involvement of peroxidase in chlorophyll degradation,” *Plant Cell Physiol*., vol. 26, pp. 1291-1301, 1985.
[19] S. P. McGrath, C. Micó, R. Curdy, and F. J. Zhao, “Predicting molybdenum toxicity to higher plants: Influence of soil properties,” *Environ. Poll*., vol.158, pp. 3095-3102, Oct. 2010.
[20] X. Bia, X. Fenga, Y. Yanga, G. Qiua, G. Lia, F. Lia, T. Liua, Z. Fua, and Z. Jina, “Environmental contamination of heavy metals from zinc smelting areas in Hezhang County, western Guizhou, China,” *Environ. Inter*., vol. 32, pp. 883-890, Sep. 2006.
[21] R. R. Mendel, “Biology of the molybdenum cofactor,” *J. Exp. Bot*., vol. 58, pp. 2289-2296, Mar. 2007.
[22] S. S. Sharma, and K. J. Dietz, “The significance of amino acids and amino acid derived molecules in plant responses and adaptation to heavy metal stress,” *J. Exp. Bot*., vol. 57, pp. 711-726, Mar. 2006.
[23] S. Chutipaijit, S. Cha-um, and K. Sompornpailin, “High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. spp. *indica*,” *Aust. J. Crop Sci*., vol. 5, pp. 1191-1198, 2011.
[24] S. L. di Toppi, and R. Gabrielli, “Response to cadmium in higher plants,” *Environ. Exp. Bot*., vol. 41, pp. 105-130, Apr. 1999.
[25] I. Sperdouli and M. Moustakas, “Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress,” *J. Plant Physiol*., vol. 169, pp. 577-585, Apr. 2012.
[26] K. S. Gould, “Nature’s Swiss army knife: The diverse protective roles of anthocyanins in leaves,” *J. Biomed. Biotechnol*., vol. 2004, pp. 314-320, Dec. 2004.
[27] V. V. Bolda, D. Botau1, R. Szôllôsi, A. Petô, A Gallé, and I. Tari, “Studies on elemental composition and antioxidant capacity in callus cultures and native plants of *Vaccinium myrtillus* L. local populations,” *Acta Biol. Szeged*., vol. 55, pp. 255-259, 2011.
[28] L. P. Dai, X. J. Dong, and H. H. Ma, “Molecular mechanism for cadmium-induced anthocyanin accumulation in *Azolla imbricate*,” *Chemosphere*, vol. 87, pp. 319-325, Jan. 2012.
[29] V. Pasqualini, C. Robles, S. Garzino, S. Greff, A. Bousquet-Melou, and G. Bonin, “Phenolic compounds content in *Pinus halepensis* Mill. needles: a bioindicator of air pollution,” *Chemosphere*, vol. 52, pp. 239-248, Jul. 2003.
[30] M. Mobin, and N. A. Khan, “Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress,” *J. Plant Physiol*., vol. 164, pp. 601-610, Apr. 2007.
[31] A. Sivaci, and E. Elmas, “The combined effects of cadmium and salinity on some pigments and total phenolic compounds of *Myriophyllum heterophyllum* Michx. and *Potamogeton crispus* L.,” *Afr. J. Agric. Res*., vol. 7, pp. 3813-3818, Jul. 2012.
[32] R. K. Tewari, S. Y. Kim, E. J. Hahn, and K. Y. Paek, “Involvement of nitric oxideinduced NADPH oxidase in adventitious root growth and antioxidant defense in *Panax ginseng*,” *Plant Biotechnol. Rep*., vol. 2, pp. 113-122, June 2008.
[33] M. D. Groppa, M. L. Tomaro, and M. P. Benavides, “Polyamines as protector against cadmium or copper-induced oxidative damage in sunflower leaf discs,” *Plant Sci*., vol. 161, pp. 481-488, Aug. 2001.
[34] E. Lombi, F. J. Zhao, S. J. Dunhan, and S. P. McGrath, “Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*,” *New Phytol*., vol. 145, pp.11-20, Jan. 2000.
[35] M. C. Romero-Puertas, I. McCarthy, L. M. Sandalio, J. M. Palma, F. J. Corpas, M. Gomez, and L. A. del Rio, “Cadmium toxicity and oxidative metabolism of pea leaf peroxisomes,” *Fee Rad. Res*., vol. 31, pp. 21-31, Dec. 1999.
[36] P. Liu, Y. S. Yang, G.D. Xu, Y. H. Fang, and Y. A. Yang, “The response of antioxidant enzymes of three soybean varieties to molybdenum and boron in soil with a connection to plant quality,” *Plant Soil Environ*., vol. 51, pp. 351-359, 2005.