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Screening and detection of biomarkers in chickpea plants exposed to chromium and cadmium

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Abstract

A broad screening protocol, covering the most general phytochemical groups of compounds, was developed on the basis of high performance thin layer chromatography (HPTLC). A total of six TLC systems, comprising three derivatization reagents, two stationary phases and two mobile phases, were included. The screening method was applied for the identification of biomarkers in the chickpea plant exposed to cadmium and chromium. The biomarkers were selected on the basis of significant changes (0.26-4.6 fold) in concentration levels of phytochemicals. Totally, five different amino acids, three organic acids, one sulphur containing compound and one sugar were identified as biomarkers in chickpea exposed heavy metal.

Key words

Screening, Biomarker, Phytochemical, Chickpea, Thin layer chromatography

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Introduction

Heavy metals (HMs) belong to major pollutants that are accumulated in the environment. The accumulation in the soil, however, has become a worldwide problem leading to reduced root and shoots growth, low yield production, low nutrient uptake and impaired homeostasis. Growth inhibition is a general phenomenon associated with most of heavy metals (Peralta *et al.*, 2001; Reichman, 2002), while the tolerance limits for heavy metal toxicity are specific not only for species but also for each variety of crop plants (Liu *et al.*, 1994; Metwally *et al.*, 2005). The heavy metals are frequently accumulated by agriculturally important crops with a significant potential to impair animal and human health (Sanita and Gabbrielli, 1999).

HMs enters the environment mainly from industrial processes, the sewage- sludge and phosphate fertilizers (Davis, 1984) and then transferred to the food chain (Wagner *et al.*, 1993). Of all toxic heavy metals, cadmium (Cd) ranks the highest in terms of damage to plant growth and human health. Daily consumption of Cd-contaminated foods poses a serious risk to human health. While in plants, Cd produces alteration at various stages of physiological processes resulting growth retardation, inhibition of enzymes, and altered stomatal action (Barcelo and Poschenrieder, 1990; Siddhu *et al.*, 2008; Neelima and Reddy, 2003). Photosynthesis is also sensitive to Cd, chlorophyll being one of the targets (Somashekaraiah *et al.*, 1992), as well as the enzymes involved in CO_2 fixation (Greger and Ogren, 1991).

Chromium (Cr^{6+}) is considered the most hazardous to animals and plants due to its high solubility, mobility, toxicity as well as carcinogenic and mutagenic properties (Dixit *et al.*, 2002). It was found that occupational exposure to Cr^{6+} compounds lead to a variety of clinical problems. The biotoxicity of Cr^{6+} is largely a function of its ability to cross biological membranes, its powerful oxidizing capabilities and its interference with electron transport in respiration and photosynthesis (Losi *et al.*, 1994).

Traditional screening methods have used to study the pharmacological effects of phytochemical compounds. Nomura *et al.* (2002) investigated the chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and screened for their estrogenic and cytotoxic activities. Koleva *et al.* (2002, 2003) used different methods to screen antioxidant activity of extracts from *Sideritis* species (Labiatae) while Couladis *et al.* (2003) screened Greek aromatic plants for antioxidant activity. Screening methods, using high performance liquid chromatography (HPLC) with mass spectrometer (MS) assay, also have been used to detect phytochemical compounds and phenylpropanoids in cereals (Bily *et al.*, 2004). Raharjo and Verpoorte (2004) evaluated four different methods, of which one is gas chromatography (GC) that have been used for analysis of cannabinoids in bioactive molecules. Ravn *et al.* (2005) developed a new TLC-based method of phytochemical screening for biomarkers in plants exposed to herbicides and pesticides.

In this study, we are presenting a novel, rapid and simple phytochemical screening method using high performance thin layer chromatography (HPTLC) to detect biomarkers belonging to different groups of phytochemicals in crude ethanol extracts of chickpea plants exposed to chromium and cadmium.

Materials and Methods

We tested one terrestrial plant species of chickpea (*Cicer arietinum* L.) plants exposed to heavy metals at mM concentrations in 6 different TLC-systems with 2 stationary phases, 2 mobile phases and 3 different derivatization reagents in different combinations. The TLC-systems were classified according to the group of phytochemical compounds. The derivatization reagent for carbohydrates: Thymol-sulphuric acid (Adachi, 1965); for organic acids, N-containing compounds and terpenoids: Anisaldehyde-sulphuric acid (Lisboa, 1963: modified); for amino acids: Ninhydrin (copper [II] nitrate) (Kawerau and Wieland, 1951, modified). This was done to test the variability of a large number of different biomarkers.

All chemicals and solvents used were of analytical grade and obtained from Merck, SD-fine and Qualigens. Standards and reagents were procured from Sigma-Aldrich (Germany), SRL (Mumbai, India), and Loba Chemie (Mumbai, India).

Chickpea plants were grown in 2 lit. pots in a potting mixture consisting of soil, sand and peat (2:2:1 w/w %). Plants were watered as needed and when applicable, fertilized twice in a week with 100 ml Hoagland's nutrient solution (Hoagland and Arnon, 1950). The pots were maintained in growth chamber with a photoperiod of 16 hr light/8 hr dark and light intensity of 60,000 Lux at 22°C. Cd and Cr exposed chickpea were found to show inhibition at 7.5 mM concentration. Thus, to grow the plants under heavy metals, a sub-MIC value was taken into consideration and treatment was given at the four leaf stage. 5 mM of Cd ($CdCl_2$) and 5 mM of Cr (K_2CrO_7) were applied in deionised water. The plants were harvested after 48 hr of the treatment and immediately freeze-dried and kept dry protected to light before phytochemical screening.

Three groups of replicates of control and exposed plants were prepared using crushed freeze-dried plant materials. 0.3 g of plant material was extracted in three ml of 75% ethanol for 2 hr in an ice-cooled ultrasonic bath (UC-250W IMECO, India). The extracts were centrifuged in a micro centrifuge at 5000 rpm for 10 min and immediately used for analysis. 2 µl of the plant extracts were spotted on pre-coated TLC (20 X 10 cm) plates, using a semiautomatic spotter under a nitrogen stream. Plates were developed in CAMAG twin-trough chamber previously saturated with 50 ml of mobile phase for 40 min. One-dimensional TLC was performed until the solvent front was 1.0 cm from the top of the TLC-plate. Several TLC systems were used. Each TLC system is composed of a TLC plate (20 X 10 cm), a chromatographic solvent and a derivatization reagent. Two combinations of TLC plates and mobile phases were used while the derivatization reagents varied between the all test systems. The stationary phases were, plate A: made of silica gel 60 on HPTLC aluminium sheets (No. 1.05547 Merck) and plate B: made of silica gel 60 on Thin Layer Chromatography (TLC) aluminium sheets (No. 1.05554 Merck). The mobile phases were: Solvent 1; consisted of 1-butanol: acetic acid: water in a ratio of 4:1:5 (upper phase after shaking for 5 min) and Solvent 2; consisted of 1-propanol: 25% ammonia/water in the ratio of 11:9. All the derivatization reagents (except for thymol at plate type A) were used in each TLC-System, and hereby 8 different combinations of the screening methods were used. The screening method was developed for quantification, as the separation was optimal for all compounds in the plant extract. Different derivatization reagents were used to detect different functional group of biomarkers. Rf-values (Ganshirt, 1969) described the location of the biomarkers on the TLC-plates.

For the detection of biomarkers, the plates were photographed using CAMAG winCATS 3.00 software (Switzerland),

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Table - 1: Biomarkers detected in HPTLC system with the stationary phase: TLC aluminium sheets coated with silica Merck 1.05547 and the mobile phase: 1-propanol: 25% ammonia (11:9) using derivatizing reagent Ninhydrin and scanned at 500 nm

Rf-values	Detected compounds	% Variation in concentration as compared to control plants	
		Cadmium treated plants	Chromium treated plants
0.75 ± 0.03	Isoleucine	(+)56.26 ± 2.98	(+)29.53 ± 1.38
0.70 ± 0.03	Tyrosine	(+)77.83 ± 2.30	(-)8.31 ± 0.55
0.57 ± 0.02	Alanine	(+)10.36 ± 0.88	(+)14.70 ± 0.27
0.31 ± 0.01	Arginine	(+)125.02 ± 8.30	(+)57.10 ± 2.68
0.38 ± 0.02	Lysine	(+)126.26 ± 8.98	(-)5.62 ± 1.08
0.63 ± 0.03	Threonine	(+)115.33 ± 4.22	(+)30.61 ± 1.34
0.50 ± 0.02	Valine	(+)41.26 ± 3.53	(+)50.62 ± 2.30
0.54 ± 0.02	Proline	(+)28.04 ± 1.88	(+)26.18 ± 1.56

(+) denotes increase in concentration while (-) denotes decrease in concentration, Each values represent mean ± SE (n=3)

Table - 2: Biomarkers detected in HPTLC system with the stationary phase: TLC aluminium sheets coated with silica Merck 1.05554 and the mobile phase: 1-butanol: acetic acid: water in a ratio of 4:1:5 (upper phase after shaking for 5 min) detected at 254 nm

Rf-values	Detected compounds	% Variation in concentration as compared to control plants	
		Cadmium treated plants	Chromium treated plants
0.83 ± 0.04	Abscisic acid	(+)55.62 ± 6.22	(+)72.06 ± 8.15
0.48 ± 0.02	Ascorbic acid	(+)468.69 ± 65.84	(+)172.85 ± 28.56
0.56 ± 0.03	Maleic acid	(+)307.48 ± 39.25	(+)62.29 ± 8.45
0.08 ± 0.009	Glutathione	(+)331.07 ± 43.65	(+)166.29 ± 20.75
0.22 ± 0.01	Citric acid	(+)50.15 ± 6.02	(+)13.74 ± 1.42

(+) denotes increase in concentration, Each values represent mean ± SE (n=3)

Table - 3: Biomarkers detected in HPTLC system with the stationary phase: TLC aluminium sheets coated with silica Merck 1.05554 and the mobile phase: 1-butanol: acetic acid: water in a ratio of 4:1:5 (upper phase after shaking for 5 minutes) using derivatizing reagent anisaldehyde-sulphuric acid and detected at 550 nm

Rf-values	Detected compounds	% Variation in concentration as compared to control plants	
		Cadmium treated plants	Chromium treated plants
0.34 ± 0.02	Sarsasaponin	(+)49.91 ± 2.78	(-)21.45 ± 1.85
0.87 ± 0.03	Coumarin	(+)14.59 ± 1.75	(+)9.91 ± 1.54

(+) denotes increase in concentration while (-) denotes decrease in concentration, Each values represent mean ± SE (n=3)

Table - 4: Biomarkers detected in HPTLC system with the stationary phase: TLC aluminium sheets coated with silica Merck 1.05554 and the mobile phase: 1-butanol: acetic acid: water in a ratio of 4:1:5 (upper phase after shaking for 5 min) using derivatizing reagent thymol- sulphuric acid and detected at 550 nm

Rf-values	Detected compounds	% Variation in concentration as compared to control plants	
		Cadmium treated plants	Chromium treated plants
0.18 ± 0.01	Sucrose	(+)54.61 ± 1.28	(+)79.22 ± 2.02

(+) denotes increase in concentration while (-) denotes decrease in concentration, Each values represent mean ± SE (n=3)

CAMAG Ultraviolet (UV) lamp (254 nm, 366 nm and white light), a CAMAG Reprostar 3 with a Canon PSG2 digital camera. CAMAG winCATS programme version 1.3.2 software was used for computer analysis. The identification and quantification of biomarkers was performed using standards, Rf-values, colour reaction in different TLC systems and UV-VIS spectra of both standard and the biomarkers from CAMAG TLC Scanner 3.00.

Results and Discussion

The phytochemical screening method was developed to provide a simple, rapid and sensitive method to detect phytochemical compounds, present in high concentration in ethanolic extracts of heavy metal exposed plants. To find the best solvents, the combination of polarity of the solvents was used according to Snyder (Snyder, 1974). The screening method was developed using ethanolic extract of chickpea plants, exposed to two heavy metals (Cd^{2+} and Cr^{6+}). The extracts using 75% ethanol/water represented the best view of phytochemical compounds, since both partly polar and non-polar compounds were extracted. In the present study, all compounds were identified and quantified by comparing with standards. The Rf-values, colour reactions and UV-VIS spectra of these compounds and standards facilitated their identification. All the biomarkers were

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selected on the basis of remarkable changes of their content level in Cd and Cr exposed plants. The stationary phases with silica gel 60 and mobile phases of 1-butanol: acetic acid: water (4:1:5) and 1-propanol: 25% ammonia (11:9) gave the best separation of the detectable phytochemical compounds after derivatization with the different reagents. The amino acids were detected as red, yellow and violet spots on a white plate in visual light using ninhydrin. All the biomarkers detected and identified in ethanolic extract of chickpea plants exposed to Cd and Cr are presented in Table 1 to 4.

Metabolites of primary metabolism can act as signal molecules. A well-known example is sucrose (Koch, 1996; Chiou and Bush, 1998; Roitsch, 1999; Smeekens, 2000; Rolland *et al.*, 2002), whose content was found to increase in response to Cd and Cr exposure to plants. Thus, sucrose could be a candidate signalling molecule for Cd and Cr exposed plants based on accumulation in response to Cd and Cr stress. In addition to its signalling role, sucrose and other soluble sugars (maltose, Glc, and Fru) act as compatible solutes during abiotic stresses, such as cold, drought, desiccation, salt, and osmotic stress (Guy *et al.*, 1992; Fan *et al.*, 1993; Rizhsky *et al.*, 2004). The biomarker proline has previously been described as a compound present at increased concentration following various types of stress exposure in plants, *viz.* water stress (Irigoyen *et al.*, 1992), drought stress (Good and Zaplanchinski, 1994), heavy-metal stress (Mehta and Gaur, 1999) and NaCl stress (Lee and Liu, 1999). In our study, proline content increased by 28.04±1.88 and 26.18±1.56%, respectively, in Cd and Cr exposed plants. Arginine, lysine and threonine levels were also increased by 125.02±8.30, 126.26±8.98 and 115.33±4.22%, respectively, in Cd exposed plants after 48 hr of the treatment and, in Cr treated plants, the level of arginine (57.10±2.68%), threonine (30.61±1.34%) and valine (50.62±2.30%) were found to increased after 48hr of the treatment. The biomarkers lysine, threonine, valine, isoleucine and leucine have previously been described as compounds present at increased concentration in *Apera spica-venti* L. plants exposed to herbicides (Ravn *et al.*, 2005).

Saponins and coumarins are constitutively produced in many plant species. The level of sarsasaponin increased in Cd and Cr exposed chickpea plants. They have been suggested to constitute part of some plant defense systems. Increased levels of glutathione, citric acid, maleic acid and ascorbic acid in Cd and Cr exposed plants were also observed in this study, which may protect these plants from heavy metal induced oxidative stress (Gajewska and Sklodowska, 2005).

Abscisic acid (ABA) plays important role during many phases of the plant life cycle, including seed development and dormancy, and in plant responses to various environmental stresses. Some studies reported that the abscisic acid content increased when the plants were exposed to copper, cadmium, mercury and nickel pollution (Munzuroglu *et al.*, 2008; Monni *et al.*, 2001). In this study, also ABA was found to accumulate at higher levels in both Cd and Cr exposed chickpea plants.

Distinctive phytochemical differences were found between the control and heavy metal exposed samples of this crop. Out of 10 different biomarkers detected five were amino acids (arginine, isoleucine, proline, threonine and valine), three organic acids (abscisic acid, ascorbic acid and maleic acid), one sulphur compound (glutathione) and one sugar (sucrose). This screening method, may possibly be used as a tool to monitored heavy metal stress not only in chickpea, but also in other crops.

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References

Adachi, S.: Thin-layer chromatography of carbohydrates in the presence of bisulphite. *J. Chromatog.,* **17**, 295-299 (1965).

Barcelo, J. and C. Poschenrieder: Plant water relations as affected by heavy metal stress: A review. *J. Plant Nutr.*, **13**, 1-37 (1990).

Bily, A.C., A.J. Burt, A. Ramputh, J. Livesey, C. Regnault-Roger, B.R. Philogene and J.T. Arnason: HPLC-PAD-APCI/MS assay of phenylpropanoids in cereals. *Phytochem, Anal.,* **15**, 9-15 (2004).

Chiou, T.Z. and D.R. Bush: Sucrose is a signal molecule in assimilate partitioning. *Proc. Natl. Acad. Sci.,* USA, **95**, 4784-4788 (1998).

Couladis, M., O. Tzakou, E. Verykokidou and C. Harvala: Screening of some Greek aromatic plants for antioxidant activity. *Phytother. Res.,* **17**, 1443-1445 (2003).

Davis, R.D.: Cadmium-a complex environmental problem: Cadmium in sludge used as fertilizer. *Experientia,* **40**, 117-126 (1984).

Dixit, V., V. Pandey and R. Shyam: Chromium ions inactivate electron transport and enhance superoxide generation *in vivo* in pea (*Pisum sativum* L.cv. Azad) root mitochondria. *Plant Cell Environ.,* **25**, 687-693 (2002).

Fan, T.W.M., T.D. Colmer, A.N. Lane and R.M. Higashi: Determination of metabolites by ^{1}H NMR and GC: Analysis for organic osmolytes in crude tissue extracts. *Anal. Biochem.,* **214**, 260-271 (1993).

Gajewska, E. and M. Sklodowska: Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress. *Acta Physiol. Plant.,* **27**, 329-339 (2005).

Ganshirt, H.G.: Documentation of thin layer chromatograms. *In*: Thin Layer chhromatography (*Ed.*: E. Stahl). 2nd Edn., Springer-Verlag, New York. p. 127 (1969).

Good, A.G. and S.T. Zaplachinsky: The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus. Physiol. Plant.,* **90**, 9-14 (1994).

Greger, M. and E. Ogren: Direct and indirect effect of Cd on photosynthesis in sugar beet (*Beta vulgaris). Physiol. Plant.*, **83**, 129-135 (1991).

Guy, C.L., J.L.A. Huber and S.C. Huber: Sucrose phosphate synthase and sucrose accumulation at low temperature. *Plant Physiol.,* **100**, 502-508 (1992).

Hoagland, D.R. and D.I. Arnon: The water culture method for growing plants without soil. *Calif. Agric. Exp. Stu. Circ.*, **347**, 1-39 (1950).

Irigoyen, J.J., D.W. Emerich and M. Sanchez-diaz: Water stress induced changes in concentrations of proline and total soluble sugars in

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nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant.*, **84**, 55-60 (1992).

Kawerau, E. and T. Wieland: Conservation of amino acid chromatograms. *Nature*, **168**, 77-78 (1951).

Koch, K.E.: Carbohydrate-modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **47**, 509-540 (1996).

Koleva, I.I., J.P.H. Linsswn, A. de Groot and L.N. Evstatieva: Screening of plant extracts for antioxidant activity: A comparative study on tree testing methods. *Phytochem. Anal.*, **13**, 8-17 (2002).

Koleva, I.I., J.P.H. Linsswn, A. de Groot, L.N. Evstatieva, V. Kortenska and V. Handijeva: Antioxidant activity screening of extracts from Sideritis species (Labiatae) grown in Bulgaria. *J. Sci. Food Agric.*, **83**, 809-819 (2003).

Lee, T.M. and C.H. Liu: Regulation of NaCl-induced proline accumulation by calmodulin via modification of proline dehydroganase activity in *Ulva fasciata* (Chlorophyta). *Aus. J. Plant Physiol.*, **26**, 595-600 (1999).

Lisboa, B.P.: Characterization of Δ^4-3-oxo-C_{21}-steroids on thin-layer chromatograms by *"in situ"* colour reactions. *J. Chromatogr.*, **16**, 136-151 (1963).

Liu, D., W. Jiangm, F.M. Zhaom and C. Lum: Effects of lead on root growth, cell division and nucleolus of *Allium cepa. Environ. Pollut.,* **86**, 1-4 (1994).

Losi, M.E., C. Amrhein and E.T. Frankenberger: Environmental biochemistry of chromium. *Rev. Environ. Contam. Toxicol.*, **136**, 91-121 (1994).

Mehta, S.K. and J.P. Gaur: Heavy-metal induced proline accumulation and its role in ameliorating metal toxicity in *Chorella vulgaris*. *New Phytologist*, **143**, 253-259 (1999).

Metwally, A., V.I. Safronova, A.A. Belimov and K.J. Dietz: Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *J. Exp. Bot.*, **56**, 167-178 (2005).

Monni, S., C. Uhling, E. Hansen and E. Magel: Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. *Environ. Pollut.*, **112**, 121-129 (2001).

Munzuroglu, O., F.K. Zengin and Z. Yahyagil: The abscisic acid levels of wheat (*Triticum aestivum* L. cv. Çakmak 79) seeds that were germinated under heavy metal (Hg^{++}, Cd^{++}, Cu^{++}) Stress. G.U. *J. Sci.,* **21**, 1-7 (2008).

Neelima, P. and K.J. Reddy: Differential effect of cadmium and mercury on growth and metabolism of *Solanum melongena* L. *J. Environ. Biol.,* **24**, 453-460 (2003).

Nomura, T., T. Fukai and T. Akaiyama: Chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and their estrogenic and cytotoxic activities. *Pure Appl. Chem.*, **74**, 1199-1206 (2002).

Peralta, J.R., J.L. Gardea-Torresdey, K.J. Tiemann, E. Gomez, S. Arteaga, E. Rascon and J.G. Parsons: Study of the effects of heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.) grown in solid media. *Bull. Environ. Contam. Toxicol.*, **66**, 727-734 (2001).

Raharjo, T.J. and R. Verpoorte: Methods for the analysis of Cannabinoids in biological materials: A review. *Phytochem. Anal.*, **15**, 79-94 (2004).

Ravn, H.W., M. Hjorth, L. Lauridsen, P. Kudsk, S.K. Mathiassen and L. Mondolot: New phytochemical screening method for biomarkers in plants exposed to herbicides. *Bull. Environ. Contam. Toxicol.*, **75**, 236-245 (2005).

Reichman, S.M.: The responses of plants to metal toxicity: A review focusing on copper, manganese and zinc. The Australian Minerals and Energy Environment Foundation, Melbourne, Australia. *Occasional Paper*, **14**, 1-54 (2002).

Rizhsky, L., H. Liang, J. Shuman, V. Shulaev, S. Davletova and R. Mittler: When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiol.*, **134**, 1683-1696 (2004).

Roitsch, T.: Source-sink regulation by sugar and stress. *Curr. Opin. Plant Biol.*, **2**, 198-206 (1999).

Rolland, F., B. Moore and J. Sheen: Sugar sensing and signalling in plants. *Plant Cell*, **14**, 185-205 (2002).

Sanita' di Toppi, L. and R. Gabbrielli: Response to cadmium in higher plants. *Environ. Exp. Bot.*, **41**, 105-130 (1999).

Siddhu, G., D.S. Sirohi, K. Kashyap, I.A. Khan and M.A. Khan: Toxicity of cadmium on the growth and yield of *Solanum melongena* L. *J. Environ. Biol.*, **29**, 853-857 (2008).

Smeekens, S.: Sugar-induced signal transduction in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **51**, 49-81 (2000).

Snyder, L.R.: Classification of the solvents properties of common liquids. *J. Chromatog.*, **92**, 223-230 (1974).

Somashekaraiah, B.V., K. Padmaja and A.R.K Prasad: Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*) involvement of lipid peroxides in chlorophyll degradation. *Physiol. Plant*, **85**, 85-89 (1992).

Wagner, G.J.: Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.*, **51**, 173-212 (1993).

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