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Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves

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Abstract

The possible role of Zn and Cr as catalytic inducers of free radicals in wheat leaves was investigated. Treatment of excess heavy metals decreased the chlorophyll and carotenoid content in wheat leaves with the increase in time of excision. A sharp increase in proline accumulation was marked with the increase in metal concentration. Total peroxide content and lipid peroxidation measured as malondialdehyde content showed uniform increase under metal treatment in excised leaves. At almost all concentrations, catalase, guaiacol peroxidase and superoxide dismutase activities decreased with a minor increase in the earlier days of excision. Though glutathione content decreased ascorbate content showed significant increase in wheat leaves under heavy metal treatment.

Additional key words: catalase, chromium, guaiacol peroxidase, superoxide dismutase, *Triticum aestivum*, zinc.

Introduction

Native and cultivated plants exist in soil environment that can contain phytotoxic levels of metals including Pb, Cd, Zn, Cr, Cu, Hg, Ni, *etc.* Though some heavy metals are essential as micronutrients for plants, at higher concentrations they are toxic. Chromium in nature exists in the form of Cr^{VI} and Cr^{III} of which Cr^{VI} is more toxic. Chromium is known to affect seed germination, seedling growth, pigment content, nutrient content and enzyme activities of various crop plants (Poschenrieder *et al.* 1991, Barcelo *et al.* 1996, Barcelo and Poschenrieder 1997, Panda and Patra 1997, 1998, 2000). Zinc as an essential micronutrient has a role in several metabolic processes of plants (Cakmak and Marschner 1993). Zinc deficiency inhibits plant growth and development (Welch *et al.* 1991, Takkar and Walker 1993, White and Zasoski 1999). However excess of zinc is indicated by a decrease in growth and development, metabolism and an induction of oxidative damage in various plant species (Cakmak and Marschner 1993, Chaney 1993, Bhattacharjee and Mukherjee 1994, Chaoui *et al.* 1997, Weckx and Clijsters 1997, Prasad *et al.* 1999, Fargašová 2001).

The presence of oxygen in the cellular environment posses a constant oxidative threat to cellular structure and processes. Univalent reduction of oxygen as a consequence of spin restriction results in the formation of toxic active oxygen species (AOS) such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), alkoxyl radical ($RO^{\cdot}$), *etc.* (Elstner 1982, Halliwell and Gutteridge 1988). The half life of $O_2^{\cdot-}$ is less than a second and is usually rapidly dismutated to H_2O_2, which is relatively stable. Protonation of $O_2^{\cdot-}$ can produce the hydroperoxyl radical ($HO_2^{\cdot}$) which can convert fatty acids to toxic lipid peroxides, destroying biological membranes. In the presence of divalent metal ions such as Fe^{2+}, H_2O_2 undergoes the Fenton reaction, producing the hydroxyl radical ($OH^{\cdot}$), the most reactive species (Grant and Loake 2000). These AOS have the capacity to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids (Hendry *et al.* 1993).

The AOS produced in the young leaf cells are removed by complex non-enzymic (ascorbate, glutathione, α-tocepherol) and enzymic (CAT, APX,

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Abbreviations: AOS - active oxygen species; APX - ascorbate peroxidase; CAT - catalase; GPX - guaiacol peroxidase; GR - glutathione reductase; MDA - malondiadehyde; TBA - thiobarbituric acid; TCA - trichloroacetic acid; SOD - superoxide dismutase.

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GPX, SOD, GR, *etc.*) antioxidant systems (Asada and Takahashi 1987, Salin 1988, Scandalios 1993). Heavy metals are known to induce free radical formation (Aust *et al.* 1985) and a consequent oxidative damage (Dietz *et al.* 1999). The present study was carried out to understand the implication of Cr and Zn treatment to leaf cells in inducing lipid peroxidation and causing oxidative stress in wheat.

Materials and methods

Uniform wheat (*Triticum aestivum* L. cv. Sonalika) seeds were surface sterilized with 0.1 mercuric chloride for 10 min followed by three rinses in sterile distilled water and then germinated in Petri plates containing *Whatman No. 1* filter paper moistened with distilled water and kept in dark for two days at 25 ± 2 °C. Further germinated seeds were transferred to plastic glasses containing distilled water and kept in the growth chamber under continuous light. White light was provided by cool fluorescent tubes (36 W *Philips TLD*) giving photon flux density of 52 μmol m^{-2} s^{-1} (PAR) under a 16-h photoperiod. After 8 d, primary leaves are excised and kept in Petri plates containing zinc chloride and potassium dichromate (0, 0.1, 1.0, 10, 100 mM) and kept under continuous light. Leaves are sampled every two days.

The extraction of chlorophyll and carotenoid using 80 % cold alkaline acetone was done following the Arnon method. The chlorophyll and carotenoid content was measured using UV-visible spectrophotometer (*Systronics 106*, Mumbai, India). Leaves were homogenized with 3 % aqueous sulfosalicylic acid and centrifuged at 3 000 *g* for 10 min. Proline from the supernatant was estimated by the method of Bates *et al.* (1973). Total peroxide content was estimated with the homogenate of 0.5 g of leaves in 5 % trichloroacetic acid according to Sagisaka (1976). Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Leaf disc (0.3 g) were homogenized in 2 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 *g* for 20 min. To 1 cm^3 of supernatant, 1 cm^3 of TCA (20 %) containing (0.5 % m/v) of TBA and 0.01 cm^3 butylated hydroxytoluene (BHT) (4 % solution in ethanol) was added. The mixture was heated at 95 °C for 30 min and then cooled in ice. The contents were centrifuged at 10 000 *g* for 15 min and the absorbance was measured at 532 nm and corrected for 600 nm.

For enzyme extraction and assay leaves were homogenized with phosphate buffer (pH 6.8) in a pre-chilled glass morter and pestle. The extract was centrifuged at 4 °C for 15 min at 14 000 *g* in a cooled centrifuge. The supernatant was used for the assay of catalase (CAT), guaiacol peroxidase (GPX), and superoxide dismutase (SOD). The catalase (CAT) and the guaiacol peroxidase (GPX) activities were assayed according to Chance and Maehly (1955). The 5.0 cm^3 of assay mixture of catalase comprised of 3.0 cm^3 phosphate buffer (pH 6.8), 1 cm^3 30 mM H_2O_2 and 1 cm^3 enzyme extract. The reaction was stopped by adding 10 cm^3 2 % H_2SO_4 after 1 min incubation at 20 °C. The acidified reaction mixture was titrated against 0.01 M $KMnO_4$ to determine the quantity of H_2O_2 utilised by the enzyme. The 3.0 cm^3 reaction mixture for the assay of GPX comprised of 0.1 M phosphate buffer (pH 6.8), 30 mM guaiacol, H_2O_2 (30 mM) and 0.3 cm^3 enzyme extract. The rate of change in absorbance at 420 nm was measured using UV-visible spectrophotometer (*Systronics*). The assay of SOD was done according to Giannopolitis and Ries (1977). 3 cm^3 assay mixture for SOD contain 79.2 mM Tris HCl buffer (pH 8.9) containing 0.12 mM EDTA and 10.8 mM tetraethylenediamine, bovine serum albumin (0.0033 %), 6 mM nitroblue tetrazolium (NBT), 600 μM riboflavin in 5 mM KOH and 0.2 cm^3 enzyme extract. Reaction mixture was illuminated by placing the glass test tubes in between two fluorescent tubes (*Philips* 20 W) and by switching the light off the reaction was terminated. The increase in absorbance due to formazan formation was read as 560 nm.

For determination of ascorbate and glutathione, 0.5 g of leaves were homogenized in 5 % (m/v) sulfosalicylic acid and the homogenate was centrifuged at 10 000 *g* for 10 min. The supernatant was used for estimation of ascorbate according to Oser (1979) and glutathione according to Griffith (1980).

Results

There is a sharp decrease in chlorophyll content after 2 d of treatment induced by both metals and higher concentrations resulted in a greater decrease. Then chlorophyll content almost remained to the day 8 (Table 1). Carotenoid content at first increased at low concentrations of metals, however, decreased with increase in time period. In case of higher concentrations, there was clear decrease after 2 d (Table 1). The

Table 1. Changes in chlorophyll, carotenoid, proline, total peroxide and malondialdehyde content (MDA) in excised wheat leaves under Zn and Cr treatments. Data presented are means of three separate experiments ± standard errors.

Parameter	Metals	Conc.	Time [d] 0	2	4	6	8
Chlorophyll	Zn	0	19.9 ± 8	12.0 ± 4	13.7 ± 6	15.2 ± 7	8.0 ± 1
[μg g^{-1}(f.m.)]		0.1	19.9 ± 8	10.1 ± 4	10.5 ± 5	13.6 ± 4	4.4 ± 3
		1.0	19.9 ± 8	9.4 ± 3	7.6 ± 5	11.4 ± 3	1.2 ± 3
		10.0	19.9 ± 8	7.1 ± 3	7.4 ± 4	11.2 ± 4	1.6 ± 2
		100.0	19.9 ± 8	4.0 ± 1	10.1 ± 2	7.6 ± 2	0.6 ± 1
	Cr	0	19.9 ± 8	12.0 ± 4	13.7 ± 6	15.2 ± 7	8.0 ± 1
		0.1	19.9 ± 8	8.0 ± 2	9.0 ± 1	12.0 ± 2	8.9 ± 2
		1.0	19.9 ± 8	8.0 ± 2	6.0 ± 1	9.0 ± 2	8.1 ± 1
		10.0	19.9 ± 8	5.0 ± 1	10.0 ± 2	5.4 ± 2	5.9 ± 1
		100.0	19.9 ± 8	2.0 ± 1	4.0 ± 1	1.8 ± 2	0.7 ± 1
Carotenoids	Zn	0	27.6 ± 5	53.0 ± 8	57.2 ± 7	50.6 ± 7	49.8 ± 7
[μg g^{-1}(f.m.)]		0.1	27.6 ± 5	53.1 ± 7	60.3 ± 6	32.1 ± 5	19.4 ± 4
		1.0	27.6 ± 5	37.5 ± 4	47.4 ± 5	23.7 ± 4	7.4 ± 2
		10.0	27.6 ± 5	39.8 ± 3	75.1 ± 5	32.1 ± 4	7.6 ± 2
		100.0	27.6 ± 5	14.4 ± 3	23.9 ± 4	19.9 ± 5	4.8 ± 2
	Cr	0	27.6 ± 5	52.7 ± 8	57.2 ± 7	50.6 ± 7	6.7 ± 7
		0.1	27.6 ± 5	36.5 ± 4	52.5 ± 6	8.4 ± 3	5.9 ± 3
		1.0	27.6 ± 5	27.9 ± 4	48.4 ± 4	16.6 ± 3	5.1 ± 2
		10.0	27.6 ± 5	19.4 ± 3	31.2 ± 3	6.5 ± 2	3.8 ± 2
		100.0	27.6 ± 5	15.4 ± 3	26.7 ± 2	6.7 ± 2	3.0 ± 1
Proline	Zn	0	76.0 ± 8	149.0 ± 18	389.0 ± 55	627.0 ± 61	987.0 ± 110
[μmol g^{-1}(f.m.)]		0.1	76.0 ± 8	211.0 ± 41	541.0 ± 91	932.0 ± 121	1081.0 ± 198
		1.0	76.0 ± 8	263.0 ± 86	627.0 ± 146	1081.0 ± 219	1132.0 ± 169
		10.0	76.0 ± 8	287.0 ± 79	809.0 ± 186	1441.0 ± 226	1451.0 ± 201
		100.0	76.0 ± 8	1087.0 ± 140	1541.0 ± 126	1825.0 ± 251	1852.0 ± 224
	Cr	0	76.0 ± 8	149.0 ± 18	389.0 ± 55	627.0 ± 61	987.0 ± 110
		0.1	76.0 ± 8	190.0 ± 31	512.0 ± 56	731.0 ± 72	748.0 ± 69
		1.0	76.0 ± 8	630.0 ± 42	724.0 ± 71	1101.0 ± 921	1077.0 ± 990
		10.0	76.0 ± 8	789.0 ± 56	1094.0 ± 96	1309.0 ± 121	1421.0 ± 189
		100.0	76.0 ± 8	828.0 ± 86	1427.0 ± 112	1712.0 ± 141	1764.0 ± 196
H_2O_2	Zn	0	982.5 ± 25	1971.2 ± 29	2240.0 ± 31	2419.2 ± 30	2352.0 ± 22
[μmol g^{-1}(f.m.)]		0.1	982.5 ± 25	1489.6 ± 28	2564.8 ± 34	2587.2 ± 29	2587.2 ± 24
		1.0	982.5 ± 25	1579.2 ± 28	3729.6 ± 33	2856.0 ± 30	2587.2 ± 25
		10.0	982.5 ± 25	1355.2 ± 27	2352.0 ± 35	3012.8 ± 28	2867.2 ± 25
		100.0	982.5 ± 25	1489.6 ± 23	1892.8 ± 35	2352.0 ± 30	3494.4 ± 26
	Cr	0	982.5 ± 25	1971.2 ± 29	2240.0 ± 31	2419.2 ± 30	2352.0 ± 22
		0.1	982.5 ± 25	1915.2 ± 32	1848.0 ± 29	2027.2 ± 28	2116.8 ± 31
		1.0	982.5 ± 25	1825.6 ± 33	2094.4 ± 30	2150.4 ± 32	2083.2 ± 33
		10.0	982.5 ± 25	1881.6 ± 33	1912.0 ± 32	1736.0 ± 29	2430.4 ± 29
		100.0	982.5 ± 25	1254.4 ± 30	1467.2 ± 38	2352.0 ± 29	2307.2 ± 30
MDA	Zn	0	402.6 ± 19	1409.0 ± 27	1524.0 ± 28	1629.0 ± 28	1719.0 ± 27
[μmol g^{-1}(f.m.)]		0.1	402.6 ± 19	2121.3 ± 30	2246.0 ± 30	2420.0 ± 32	2568.0 ± 31
		1.0	402.6 ± 19	2461.9 ± 33	2518.0 ± 34	2626.0 ± 34	2805.0 ± 34
		10.0	402.6 ± 19	2512.8 ± 34	2645.0 ± 33	2821.0 ± 33	2958.0 ± 32
		100.0	402.6 ± 19	2662.8 ± 37	2781.0 ± 33	2898.0 ± 34	3151.0 ± 31
	Cr	0	402.6 ± 19	1409.0 ± 27	1524.0 ± 28	1629.0 ± 28	1719.0 ± 27
		0.1	402.6 ± 19	2219.6 ± 30	2301.0 ± 29	2421.0 ± 29	2545.0 ± 29
		1.0	402.6 ± 19	2356.0 ± 29	2412.0 ± 29	2561.0 ± 29	2712.0 ± 31
		10.0	402.6 ± 19	2512.0 ± 30	2656.0 ± 33	2745.0 ± 34	2898.0 ± 32
		100.0	402.6 ± 19	2789.0 ± 32	2912.0 ± 35	3089.0 ± 33	3236.0 ± 34

Table 2. Effect of Zn and Cr on the activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and glutathione and ascorbate content in excised wheat leaves. Others same as Table 1.

Parameter	Metals	Conc.	Time [d] 0	2	4	6	8
SOD	Zn	0	4.94 ± 0.98	3.54 ± 0.56	3.62 ± 0.52	3.42 ± 0.42	3.62 ± 0.40
[U g^{-1}(f.m.)]		0.1	4.94 ± 0.98	3.16 ± 0.51	3.18 ± 0.50	3.12 ± 0.48	3.84 ± 0.46
		1.0	4.94 ± 0.98	3.88 ± 0.54	3.56 ± 0.52	3.76 ± 0.46	3.26 ± 0.44
		10.0	4.94 ± 0.98	3.48 ± 0.S2	3.66 ± 0.39	3.80 ± 0.36	4.20 ± 0.34
		100.0	4.94 ± 0.98	3.36 ± 0.42	4.02 ± 0.33	4.02 ± 0.31	4.40 ± 0.30
	Cr	0	4.94 ± 0.98	3.54 ± 0.56	3.62 ± 0.52	3.42 ± 0.42	3.62 ± 0.40
		0.1	4.94 ± 0.98	4.07 ± 0.51	4.06 ± 0.42	4.10 ± 0.40	2.06 ± 0.21
		1.0	4.94 ± 0.98	3.18 ± 0.28	5.10 ± 0.43	4.02 ± 0.44	2.18 ± 0.23
		10.0	4.94 ± 0.98	3.00 ± 0.24	3.52 ± 0.26	3.12 ± 0.24	1.16 ± 0.24
		100.0	4.94 ± 0.98	2.94 ± 0.28	3.58 ± 0.21	2.68 ± 0.29	1.38 ± 0.21
CAT	Zn	0	1000 ± 28.5	2667 ± 56.6	2512 ± 58.2	1754 ± 59.2	1185 ± 51.3
[U g^{-1}(f.m.)]		0.1	1000 ± 28.5	2215 ± 49.2	2000 ± 46.2	1545 ± 48.5	1000 ± 39.3
		1.0	1000 ± 28.5	1778 ± 41.6	1500 ± 40.9	1254 ± 40.22	615 ± 36.5
		10.0	1000 ± 28.5	985 ± 29.7	798 ± 28.6	571 ± 26.6	364 ± 29.5
		100.0	1000 ± 28.5	712 ± 22.5	667 ± 24.5	465 ± 21.6	181 ± 26.6
	Cr	0	1000 ± 28.5	2667 ± 56.6	2512 ± 58.2	1754 ± 59.2	1185 ± 51.3
		0.1	1000 ± 28.5	2000 ± 50.6	1777 ± 51.2	1333 ± 50.0	916 ± 49.4
		1.0	1000 ± 28.5	1618 ± 48.5	1200 ± 41.3	1091 ± 42.7	810 ± 41.6
		10.0	1000 ± 28.5	908 ± 46.9	652 ± 42.1	501 ± 31.3	303 ± 38.2
		100.0	1000 ± 28.5	667 ± 41.6	514 ± 40.1	394 ± 34.5	121 ± 29.6
GPX	Zn	0	50.38 ± 8.21	26.02 ± 7.52	19.69 ± 6.99	18.19 ± 6.44	18.19 ± 5.22
[U g^{-1}(f.m.)]		0.1	50.38 ± 8.21	26.92 ± 7.02	21.35 ± 6.46	20.60 ± 6.82	16.39 ± 5.92
		1.0	50.38 ± 8.21	26.12 ± 7.54	23.02 ± 7.01	21.20 ± 6.24	19.69 ± 5.49
		10.0	50.38 ± 8.21	25.56 ± 6.99	30.23 ± 7.21	23.30 ± 5.81	15.94 ± 6.01
		100.0	50.38 ± 8.21	24.21 ± 6.48	31.43 ± 6.26	32.93 ± 6.78	8.72 ± 2.82
	Cr	0	50.38 ± 8.21	26.02 ± 7.52	19.69 ± 6.99	18.19 ± 6.44	18.19 ± 5.22
		0.1	50.38 ± 8.21	37.74 ± 7.96	38.65 ± 6.86	11.73 ± 5.12	25.71 ± 5.68
		1.0	50.38 ± 8.21	24.96 ± 4.82	40.00 ± 4.56	14.21 ± 4.02	27.61 ± 4.92
		10.0	50.38 ± 8.21	27.51 ± 6.56	32.03 ± 4.98	13.83 ± 4.01	18.19 ± 4.22
		100.0	50.38 ± 8.21	33.68 ± 6.22	41.65 ± 5.61	26.01 ± 5.00	20.15 ± 4.52
Glutathione	Zn	0	3010 ± 59.2	4905 ± 65.3	4251 ± 60.4	2252 ± 48.2	1012 ± 46.6
[μmol g^{-1}(f.m.)]		0.1	3010 ± 59.2	3210 ± 50.5	2230 ± 50.6	1801 ± 46.2	1010 ± 40.6
		1.0	3010 ± 59.2	5201 ± 61.3	2240 ± 44.4	1821 ± 41.5	1000 ± 39.6
		10.0	3010 ± 59.2	4250 ± 60.6	2180 ± 45.5	1758 ± 39.2	989 ± 34.3
		100.0	3010 ± 59.2	4248 ± 41.5	2180 ± 42.5	1755 ± 39.4	976 ± 34.8
	Cr	0	3010 ± 59.2	4905 ± 65.3	4251 ± 60.4	2252 ± 48.2	1012 ± 46.6
		0.1	3010 ± 59.2	4000 ± 60.2	1741 ± 39.6	3415 ± 58.5	1469 ± 34.6
		1.0	3010 ± 59.2	4002 ± 60.5	2960 ± 38.5	2000 ± 40.5	1476 ± 38.2
		10.0	3010 ± 59.2	5180 ± 59.0	2451 ± 39.3	2891 ± 41.3	1470 ± 36.6
		100.0	3010 ± 59.2	5175 ± 59.9	3000 ± 51.6	1605 ± 42.1	1472 ± 36.0
Ascorbate	Zn	0	512 ± 20.2	649 ± 21.6	668 ± 24.5	660 ± 22.6	595 ± 21.6
[μmol g^{-1}(f.m.)]		0.1	512 ± 20.2	697 ± 20.6	972 ± 29.6	601 ± 24.6	649 ± 22.2
		1.0	512 ± 20.2	712 ± 21.6	1023 ± 29.5	632 ± 21.9	697 ± 22.8
		10.0	512 ± 20.2	701 ± 20.8	1348 ± 29.0	915 ± 20.4	1105 ± 21.2
		100.0	512 ± 20.2	1081 ± 20.5	1521 ± 29.9	998 ± 22.6	1177 ± 24.0
	Cr	0	512 ± 20.2	649 ± 21.6	668 ± 24.5	660 ± 22.6	595 ± 21.6
		0.1	512 ± 20.2	889 ± 20.9	804 ± 18.1	612 ± 19.0	601 ± 19.5
		1.0	512 ± 20.2	897 ± 21.6	1064 ± 24.6	553 ± 19.2	616 ± 21.2
		10.0	512 ± 20.2	982 ± 22.0	576 ± 21.9	657 ± 20.0	734 ± 21.6
		100.0	512 ± 20.2	804 ± 20.5	1119 ± 22.5	735 ± 20.9	812 ± 20.9

osmoprotectant proline accumulated due to both the metal treatments and a higher concentration of metals resulted in higher content of proline (Table 1).

There was almost regular increase in H_2O_2 content due to Zn and Cr. Both the metals also induced regular increase of MDA with increasing concentration (Table 1).

At lower concentration of metals, catalase activity firstly increased (after 2 d) and subsequently decreased (after 4, 6 and 8 d). At high metal concentrations (10 and 100 mM) CAT activity decreased (Table 2). Guaiacol peroxidase activity decreased sharply after 2 d under both metals treatment and with the increase in time changed very slowly. When treated with 100 mM Zn decrease in GPX activity after 8 d was maximum. Under Cr treatment, the activity was increased after 4 d compared to 2 d, decreased after 6 d and again increased after 8 d (Table 2). SOD activity mostly decreased. However, on day 4 when treated with 1 mM Cr SOD activity increased (Table 2).

Ascorbate concentration significantly increased when treated with Zn at concentration of 10 and 100 mM. In other concentrations also, an increase in ascorbate content was observed. When treated with 10 mM Cr increase was maximum after 2 d and at 100 mM after 4 d. Glutathione content showed a minor increase in first days of treatment, however, decreased with increase in time of treatment and concentration of metals as compared to control (Table 2).

Discussion

Treatment of leaf segments with Zn and Cr for 0, 2, 4, 6, 8 d in continuous light diminished significantly chlorophyll and carotenoid content similarly as reported for other metals (Luna *et al.* 1994, Gallego *et al.* 1996, Fargašová 2001, Zeid 2001). Proline was found to accumulate with the increase in time and concentration of the metals. The precise mechanism and the significance of proline accumulation in plants under heavy metal stress have not been elucidated yet. Exposure to Cd is known to deteriorate the plant water balance (Barcelo and Poschenrieder 1990) and decrease in water potential might induce this proline accumulation to overcome the osmotic stress (Schat *et al.* 1997). However, several authors have implicated a role for proline in the detoxification of active oxygen species (Alia *et al.* 1995, Pardha Saradhi *et al.* 1995, Prasad *et al.* 1999). Excess of Zn and Cr promoted lipid peroxidation and production of MDA perhaps as a result of generation of free radicals. Similar responses were observed in other crop plants treated with various heavy metals (Somashekaraiah *et al.* 1992, Gallego *et al.* 1996, Mazhoudi *et al.* 1997, Weckx and Clijsters 1997, Panda and Patra 2000). Total peroxide content also increased under metal treatment in leaves as reported, *e.g.* Dietz *et al.* (1999).

With the increase in Zn and Cr concentration and time of treatment, a decrease in activities of antioxidative enzymes, CAT, GPX and SOD was found with a minor increase in the early days of treatment. An increase in peroxide content and decrease in enzyme activity suggested an imposition of oxidative stress (Luna *et al.* 1994, Gallego *et al.* 1996, Patra and Panda 1998, Dietz *et al.* 1999, Cakmak 2000, Shah *et al.* 2001). A decrease in glutathione content with a concomitant decrease at higher Zn and Cr concentration also indicated oxidative stress (Gallego *et al.* 1996). However, ascorbate content showed an increase in almost all concentrations, especially under zinc treatment which suggested its participation in detoxification of AOS (Rennenberg 1982, Asada and Takahashi 1987, Prasad *et al.* 1999).

References

Alia, Prasad, K.V.S.K., Pardha Saradhi, P.: Effect of zinc on free radical and proline in *Brassica juncea* and *Cajanus cajan*. - Phytochemistry **39**: 45-47, 1995.

Asada, K., Takahashi, M.: Production and scavenging of active oxygen in photosynthesis. - In: Kyle, D.J., Osmond, C.J., Artzen, C.J. (ed.): Photoinhibition: Topics in Photosynthesis. Pp. 227-287. Elsevier, Amsterdam 1987.

Aust, S.D., Morehouse, L.A., Thomas, C.E.: Role of metals in oxygen radical reactions. - J. free Radical Biol. Med. **1**: 3-25, 1985.

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

Barcelo, J., Poschenrieder, C.: Chromium in plants. - In: Carati, S., Tottarelli, F., Seqmi, P. (ed.): Chromium Environmental Issues. Pp. 101-129. Francotangati Press, Milan 1997.

Barcelo, J., Poschenrieder, C., Vazquez, M.D., Gunse, B.: Aluminium phytotoxicity. A challange for plant scientists. - Fertil. Res. **43**: 217-223, 1996.

Bates, L.S., Waldren, R.P., Teare, L.D.: Rapid determination free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Bhattacharjee, S., Mukherjee, A.K.: Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L.) Walp. seedlings. I. Germination behaviour, total protein and proline content and protease activity. - Poll. Res. **13**: 269-277, 1994.

Cakmak, I.: Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. - New Phytol. **146**: 185-205, 2000.

Cakmak, I., Marschner, H.: Effect of zinc nutritional status on superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. - In: Barrow, N.J. (ed.): Plant Nutrition - From Genetic Engineering to Field Practice. Pp. 133-137. Kluwer Academic Publishers, Dordrecht 1993.

Chance, B., Maehly, A.C.: Assay of catalase and peroxidases. - Methods Enzymol. **2**: 764-775, 1955.

Chaney, R.L.: Zinc phytotoxicity. - In: Robson, A.D. (ed.): Zinc in Soil and Plants. Pp. 135-150. Kluwer Academic Publishers, Dordrecht 1993.

Chaoui, A., Mazhoudi, S., Ghorbal, M.H., Ferjani, E.E.L: Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzymes activities in bean (*Phaseolus vulgaris* L.). - Plant Sci. **127**: 139-147, 1997.

Dietz, K.J., Baier, M., Kramer, U.: Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 73-79. Springer-Verlag, Berlin 1999.

Elstner, E.F.: Oxygen activation and oxygen toxicity. - Annu. Rev. Plant Physiol. **33**:78-96, 1982.

Fargašová, A.: Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapsis alba* L. seedlings and their accumulation in roots and shoots. - Biol. Plant. **44**: 471-473, 2001.

Gallego, S.M., Benavioes, M.P., Tomaro, M.L.: Effect of heavy metal ion excess on sunflower leaves - evidence for involvement of oxidative stress. - Plant Sci. **121**: 151-159, 1996.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutase. I. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Grant, J.J., Loake, G.J.: Role of reactive oxygen intermediate and cognate redox signalling in disease resistance. - Plant Physiol. **124**: 21-29, 2000.

Griffith, O.W.: Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. - Anal. Biochem. **10**: 207-211, 1980.

Halliwell, B., Gutteridge, J.M.C.: Free Radicals in Biology and Medicine. - Clarendon Press, Oxford 1988.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Hendry, G.A.F.: Oxygen, free radical process and seed longevity. - Seed Sci. Res. **3** :141-153, 1993.

Luna, C.M., Gonzalez, C.A., Trippi, V.S.: Oxidative damage caused by an excess of copper in oat leaves. - Plant Cell Physiol. **35**: 11-15, 1994.

Mazhoudi, S., Chaoui, A., Ghorsab, M.W., Ferjani, E.E.: Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum* Mill.). - Plant Sci. **127**: 129-137, 1997.

Oser, B.L.: Hawks Physiological Chemistry. - McGraw-Hill, New York 1979.

Panda, S.K., Patra, H.K.: Physiology of chromium toxicity. A review. - Plant Physiol. Biochem. **24**: 10-17, 1997.

Panda, S.K., Patra, H.K.: Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. - Indian J. agr. Biochem. **11**(2): 56-57, 1998.

Panda, S.K., Patra, H.K.: Does chromium (III) produce oxidative damage in excised wheat leaves ? - J. Plant Biol. **27**: 105-110, 2000.

Pardha Saradhi, P., Alia, Arora, S., Prasad, K.V.S.K.: Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. - Biochem. biophys. Res. Commun. **209**: 1-5, 1995.

Poschenrieder, C., Vazquez, M.D., Bonet, A., Barcelo, J.: Chromium III iron interaction in iron suffcient and iron-deficient bean plants. II Ultrastructural aspects. - J. Plant Nutr. **14**: 415-428, 1991.

Prasad, K.V.S.K., Pardhasaradhi, P., Sharmila, P.: Concerted action of antioxidant enzyme and curtailed growth under zinc toxicity in *Brassica juncea*. - Environ. exp. Bot. **42**: 1-10, 1999.

Rennenberg, H.: Glutathione metabolism and possible roles in higher plants. - Phytochemistry **21**: 2771-2781, 1982.

Sagisaka, S.: The occurrence of peroxide in a perennial plant *Populus gelrica*. - Plant Physiol. **57**: 308-309, 1976.

Salin, M.L.: Toxic oxygen species and protective systems of chloroplast. - Physiol. Plant. **72**: 681-728, 1988.

Scandalios, J.G.: Oxygen stress and superoxide dismutase. - Plant Physiol. **101**: 7-12, 1993.

Schat, H., Sharma, S.S., Vooijs, R.: Heavy metal induced accumulation of free proline in a metal tolerant and a non tolerant ecotype of *Silene vulgaris*. - Physiol. Plant. **101**: 477-482, 1997.

Shah, K., Kumar, R.G., Verma, S., Dubey, R.S.: Effect of cadmium on lipid peroxidation, superoxide anion, germination and activities of antioxidant enzymes in rice seedlings. - Plant Sci. **161**: 1135-1144, 2001.

Somashekaraiah, B.V., Padmaja, K., Prasad, A.R.K.: 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.

Takkar, P.N., Walker, C.D.: The distribution and correction of zinc deficiency. - In: Robson, A.D. (ed.): Zinc in Soils and Plants. Pp. 151-166. Kluwer Academic Publishers, Dordrecht 1993.

Weckx, J.E.J., Clijsters, H.M.M.: Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. - Plant Physiol. Biochem. **35**: 405-410, 1997.

Welch, R.M., Allaway, W.H., House, W.A., Kubota, J.: Geographic distribution of trace element problems. - In: Mortdvedt, J.J., Cox, F.R., Shumen, L.M., Welch, R.M. (ed.): Micronutrients in Agriculture. Pp. 31-57. Soil Science Society of America, Madison 1991.

White, J.G., Zasoski, R.H.: Mapping soil micronutrients. - Field Crops Res. **60**: 11-26, 1999.

Zeid, I.M.: Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. - Biol. Plant. **44**: 111-115, 2001.