Acta Bot. Croat. 66 (1), 57–65, 2007

CODEN: ABCRA 25
ISSN 0365–0588

Effect of 4-Cl-indole-3-acetic acid on the seed germination of *Cicer arietinum* exposed to cadmium

BARKET ALI, INDU RANI, SHAMSUL HAYAT*, AQIL AHMAD

Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh-202 002, India

The specific pattern of seed germination in *Cicer arietinum*, in the presence of 4-chloroindole-3-acetic acids (4-Cl-IAA) and/or cadmium was studied in petriplates. Soaking the seeds in an aqueous solution of 4-Cl-IAA favoured the relative water content, moisture content, the activities of nitrate reductase and peroxidase and finally the rate of germination. Treatment of the seeds with cadmium decreased the values for most of the above characteristics, but favoured the activity of peroxidase and produced an increase in the level of proline. Moreover, cadmium followed by 4-Cl-IAA, in its treatment pattern, partially neutralized the toxic effects of the metal, where 10^{-8} M of 4-Cl-IAA proved the best.

Key words: Indole-acetic acid, cadmium, nitrate reductase, peroxidase, proline

Abbreviations: 4-Cl-IAA – 4-chloroindole-3-acetic acids, RWC – relative water content, NR – nitrate reductase, NED-HCL – N-1-naphthyl ethylene diamine dihydrochloride, ProDH – proline dehydrogenase, $\triangle^1$P5CS – $\triangle^1$ pyrroline-5-carboxylate synthetase, $\triangle^1$5CR $\triangle^1$ – pyrroline-5-carboxylate reductase

Introduction

The toxic heavy metal cadmium enters the food chain after release from industrial activity and the application of phosphate fertilizers to the soil (WAGNER 1993). Cadmium damages cellular membranes (TU and BROVILLETE 1987). Moreover, soil loaded with a sufficient quantity of cadmium inhibits seed germination (AL-YAMENI 2001), enzyme level (DITOPPI and GABBRIELLI 1999, GOUIA et al. 2003) and root growth (WILKINS 1978). This results in water stresses (KASTORI et al. 1992) and restricted uptake of essential nutrients (GREGER and LINDBERG 1987). The induced changes lead to an inhibition of chlorophyll biosynthesis and a consequent reduction in photosynthetic rate (SINGH and SINGH 1987).

Of the auxins, indole-3-acetic acid is of universal occurrence in plants but others are restricted to certain groups of plants. Chloro-substituted auxins are of rare occurrence in plants. Their distribution is largely restricted to various tribes of *Vicicae* (ENGVILD 1994, REINECKE 1999) and *Pisum sylvestris* (ERNSTSEN and SANDBERG 1986). Strong auxin-like activity of these chlorinated auxins has been demonstrated in several bioassays: they stimulate rooting and ethylene production in leafy cuttings (AHMAD et al. 1987), seed germina-

* Corresponding author: shayat@lycos.com

tion (AHMAD et al. 2001) and also affect enzyme activities (HIRASAWA 1989, AHMAD and HAYAT 1999, AHMAD et al. 2001a, b).

It is quite evident that the action of cadmium and 4-Cl, IAA on seed germination is diverse. It was, therefore, decided to explore the ameliorative action of 4-Cl-IAA on the metal-induced effects on the selected processes, leading to seed germination in *Cicer arietinum*.

Materials and methods

Growth condition

The seeds of *Cicer arietinum* L. cv. Avarodhi were obtained from the Indian Agricultural Research Institute, New Delhi. The healthy seeds were surface sterilized with 0.01% mercuric chloride solution followed by repeated washing with deionized water. The seeds were soaked for 8 hours in double distilled water (control) or aqueous solution of 4-Cl-IAA (10^{-6}, 10^{-8} or 10^{-10} M) or of cadmium (0.01, 0.1 or 1.0 μM) and for 4 hours in aqueous solution of cadmium (0.01, 0.1 or 1.0 μM) followed by washing with deionized water to remove adhering metal and further soaked for 4 hours in the aqueous solution of 4-Cl-IAA (10^{-6}, 10^{-8} or 10^{-10} M). Treated seeds were rinsed with deionized water to remove the adhering solution and allowed to germinate in sterilized petriplates lined with moistened absorbent cotton, in a BOD incubator run at 25±2°C. These germinating seeds were sampled after 24, 48 and 72 hours of soaking (this also included the treatment duration) to assess various parameters. Protrusion of radicle by 5 mm, outside the testa, was taken as scale for germination.

Relative water content

The relative water content (RWC) was calculated by putting the values in the formula proposed by JONES and TURNER (1978).

$$RWC = [(\text{fresh mass} - \text{dry mass}) / (\text{turgor mass} - \text{dry mass})] \times 100$$

The turgor mass was obtained by dipping the seed samples in double distilled water (DDW) for 8 hours, thereby saturating them.

Moisture content

The moisture content in fresh seed samples was calculated by employing the formula:

$$\text{Moisture content} = [(\text{fresh mass} - \text{dry mass}) / \text{fresh mass}] \times 100$$

Peroxidase activity

The activity of peroxidase was determined by the method described by CHANCE and MAEHLY (1955). The fresh seeds were homogenized in 50 mM of phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone. The samples were centrifuged at 15000 g for 15 minutes, at a temperature of 4°C. The supernatant was transferred to spectrophoto-

metric vials, followed by the addition of 0.05 M pyrogallol and 0.1 mM each of EDTA and H_2O_2. The change in colour was noted at 430 nm for 2 minutes on a spectrophotometer and the activity was calculated on the standard curve by using purpurogallin.

Nitrate reductase activity

The activity of NR was measured following the procedure used by JWORSKI (1971). The germinating seeds were homogenized in phosphate buffer (pH 7.0) to which was added 0.2 M potassium nitrate and 5% isopropanol solutions. This reaction mixture was incubated at 30±2°C for two hours. Sulphanilamide (1%) and 0.02% N-1-naphthyl ethylene diamine dihydrochloride (NED-HCl) solution were added to this mixture. The absorbance was read at 540 nm in a spectrophotometer.

Proline content

Proline content was determined following the method given by BATES et al. (1973). The germinating seeds were homogenized with 3% aqueous sulphosalicyclic acid and centrifuged. To the supernatant, glacial acetic acid and acid ninhydrin solutions were added and this reaction mixture was heated in a water bath at 60°C for 1 hour and then cooled. Toluene was added to this reaction mixture and the colour of the toluene layer was read at 520 nm on a spectrophotometer.

Statistical analysis

Each observation was repeated five times and the difference among the treatments was calculated by following the procedure described by GOMEZ and GOMEZ (1984). The significance was tested at the 5% level.

Results

Seed germination

Treating the seeds with 4-Cl-IAA favoured per cent germination but was inhibited by the presence of cadmium in the soaking medium (Tab. 1). Out of the three concentrations tested, the two lower concentrations (10^{-10} and 10^{-8} M) of auxin proved better and the values generated were equal. However, in case of cadmium the degree of inhibition increased as the metal content was enhanced from 0.01 to 1 μM. The ill effect generated by cadmium was to some extent overcome by the follow-up treatment with 4-Cl-IAA. The auxin, irrespective of its concentrations, completely neutralized the damage caused by the lowest concentration (0.01 μM) of the metal and the values were comparable with those of the control.

Relative water content

The relative water content (RWC) of the seeds increased as the germination progressed (Tab. 1). Treating the seeds with either of the concentrations of 4-Cl-IAA increased the values significantly over the control. However, cadmium treatment significantly decreased the

Tab. 1. Effect of 4-Cl-Indole-3-acetic acid and cadmium on germination (%) and relative water content (%) of chickpea seeds.

Treatment	Per cent germination (%)	Relative water content (%)		
		Sampling stage		
		24 hrs	48 hrs	72 hrs
Control (water soaked)	88.6	66.5	71.3	76.5
4-Cl-IAA (10^{-6} M)	92.0	66.2	70.6	74.8
4-Cl-IAA (10^{-8} M)	94.6	71.5	77.3	86.9
4-Cl-IAA (10^{-10} M)	94.0	70.4	75.6	78.6
Cd (0.01 μM)	82.3	57.3	59.3	60.7
Cd (0.1 μM)	76.6	53.5	55.2	57.8ž
Cd (1.0 μM)	49.9	51.6	53.6	54.2
Cd (0.01 μM)+4-Cl-IAA (10^{-6}M)	87.4	59.4	61.2	61.5
Cd (0.01 μM)+4-Cl-IAA (10^{-8}M)	89.0	58.5	61.6	62.3
Cd (0.01 μM)+4-Cl-IAA (10^{-10}M)	88.6	57.3	60.9	61.8
Cd (0.1 μM)+4-Cl-IAA (10^{-6}M)	78.3	54.2	57.8	59.3
Cd (0.1 μM)+4-Cl-IAA (10^{-8}M)	79.6	55.8	59.4	63.0
Cd (0.1 μM)+4-Cl-IAA (10^{-10}M)	79.0	55.2	58.1	62.5
Cd (1.0 μM)+4-Cl-IAA (10^{-6}M)	52.0	52.2	54.8	56.5
Cd (1.0 μM)+4-Cl-IAA (10^{-8}M)	54.0	54.6	56.0	57.8
Cd (1.0 μM)+4-Cl-IAA (10^{-10}M)	53.3	53.9	55.6	56.9
C.D. at 5%	1.65	1.4	1.8	1.7

relative water content and the loss was in proportion to the concentration of metal. The ill effect generated by cadmium could not be counteracted by the auxin.

Seed moisture content

The pattern adopted by relative water content in the seeds was closely followed by seed moisture content (Tab. 2). Irrespective of the concentration of chloroindole auxin, the values for moisture content increased significantly at all the three stages of sampling and 10^{-8}M proved best enhancing it by 14, 29 and 30% over the control, at 24, 48 and 72 hours of sampling, respectively. However, the presence of cadmium in the soaking medium decreased the moisture content whose values were proportionate to the concentration of the metal. The follow-up treatment with auxin had no impact on the damage caused by cadmium.

Nitrate reductase activity

As the seed germination progressed, the activity of nitrate reductase increased consistently (Tab. 2). The activity of enzyme in the seeds fed with 4-Cl-IAA was significantly higher than the control/any other treatment and the two lower concentrations (10^{-10} M and 10^{-8} M) proved best. The medium concentration (10^{-8} M) of the auxin increased the activity of the enzyme by 43, 48 and 50%, more than the control, at 24, 48 and 72 hours of germina-

Tab. 2. Effect of 4-Cl-Indole-3-acetic acid and cadmium on moisture content and nitrate reductase (NR) activity of chickpea seeds.

Treatment	Moisture content (%)			NR activity (nm NO_3 $g^{-1}h^{-1}$ FW)		
	Sampling stage			Sampling stage		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control (water soaked)	39.9	40.3	40.9	456.50	532.50	730.50
4-Cl-IAA (10^{-6} M)	39.3	42.8	45.3	579.54	666.50	730.50
4-Cl-IAA (10^{-8} M)	45.5	52.3	53.4	707.91	791.84	841.32
4-Cl-IAA (10^{-10} M)	44.6	46.5	51.3	686.50	777.32	800.93
Cd (0.01 μM)	28.6	28.6	29.9	434.51	501.43	530.50
Cd (0.1 μM)	28.4	29.8	31.4	357.68	431.50	472.40
Cd (1.0 μM)	24.8	25.4	27.9	313.32	386.05	425.20
Cd (0.01 μM)+4-Cl-IAA (10^{-6}M)	28.4	31.1	32.5	446.25	512.15	560.25
Cd (0.01 μM)+4-Cl-IAA (10^{-8}M)	29.3	32.4	36.0	471.42	546.20	593.75
Cd (0.01 μM)+4-Cl-IAA (10^{-10}M)	27.1	31.3	33.0	468.67	547.15	589.56
Cd (0.1 μM)+4-Cl-IAA (10^{-6}M)	29.4	30.5	31.6	418.04	489.43	530.20
Cd (0.1 μM)+4-Cl-IAA (10^{-8}M)	32.6	33.8	35.7	486.32	525.23	555.25
Cd (0.1 μM)+4-Cl-IAA (10^{-10}M)	31.5	32.9	34.9	479.5	509.5	539.0
Cd (1.0 μM)+4-Cl-IAA (10^{-6}M)	21.9	26.2	27.8	381.6	420.3	446.5
Cd (1.0 μM)+4-Cl-IAA (10^{-8}M)	25.2	27.9	30.7	407.8	441.40	460.50
Cd (1.0 μM)+4-Cl-IAA (10^{-10}M)	23.8	27.4	30.3	405.6	439.30	461.43
C.D. at 5%	0.85	1.1	0.90	15.76	19.45	23.65

tion, respectively. However, NR activity decreased significantly with the treatment with cadmium and was proportionate to the concentration of the metal. Moreover, the inhibitory action by 0.01 μM and 0.1 μM of the metal was to some extent overcome by the two lower concentrations of auxin and more efficiently by 10^{-8} M, which increased the values by 96, 142 and 83% higher than the seeds soaked in 1.0 μM of cadmium, at 24, 48 and 72 hours, respectively.

Peroxidase activity

Unlike other parameters, the activity of peroxidase was enhanced both by auxin and the cadmium, irrespective of their individual concentrations (Tab. 3). Moreover the values increased further if the cadmium treatment was followed with that of the 4-Cl-IAA.

Proline content

The proline content exhibited a linear increase as the seed germination progressed (Tab. 3). Treating the seeds with 4-Cl-IAA decreased the proline content. However cadmium increased its values significantly in proportion to the concentration of the metal. This effect of cadmium could be nullified by the auxin to a limited extent, at all concentrations of the metal.

Tab. 3. Effect of 4-Cl-Indole-3-acetic acid and cadmium on peroxidase activity and proline content in germinating chickpea seeds

Treatment	Peroxidase activity mg purpurogalline g^{-1} protein (min^{-1})			Proline content (μ mol g^{-1})		
	Sampling stage			Sampling stage		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control (water soaked)	0.141	0.159	0.173	8.88	12.96	20.43
4-Cl-IAA (10^{-6} M)	0.156	0.181	0.195	7.93	11.56	18.23
4-Cl-IAA (10^{-8} M)	0.172	0.198	0.221	7.65	10.93	16.58
4-Cl-IAA (10^{-10} M)	0.164	0.188	0.204	7.43	10.65	16.25
Cd (0.01 μM)	0.170	0.203	0.220	10.97	15.69	26.23
Cd (0.1 μM)	0.183	0.217	0.242	14.35	35.30	48.45
Cd (1.0 μM)	0.198	0.133	0.263	35.56	62.29	89.45
Cd (0.01 μM)+4-Cl-IAA (10^{-6}M)	0.201	0.226	0.238	9.23	13.30	22.50
Cd (0.01 μM)+4-Cl-IAA (10^{-8}M)	0.213	0.252	0.276	9.45	13.00	24.00
Cd (0.01 μM)+4-Cl-IAA (10^{-10}M)	0.208	0.240	0.260	9.65	13.15	23.90
Cd (0.1 μM)+4-Cl-IAA (10^{-6}M)	0.210	0.242	0.262	12.15	29.14	41.00
Cd (0.1 μM)+4-Cl-IAA (10^{-8}M)	0.225	0.261	0.285	12.90	27.85	39.45
Cd (0.1 μM)+4-Cl-IAA (10^{-10}M)	0.216	0.243	0.264	13.25	29.30	40.65
Cd (1.0 μM)+4-Cl-IAA (10^{-6}M)	0.221	0.250	0.272	28.65	46.50	68.00
Cd (1.0 μM)+4-Cl-IAA (10^{-8}M)	0.238	0.276	0.290	29.45	49.43	69.32
Cd (1.0 μM)+4-Cl-IAA (10^{-10}M)	0.223	0.264	0.284	28.93	47.85	67.00
C.D. at 5%	0.024	0.019	0.026	0.95	1.14	1.65

Discussion

Various hydrophilic groups (–NH_3, –OH and/or –COOH) of proteins and carbohydrates located in the seed coat attract dipolar molecules of water to form a hydrated shell around the macromolecules facilitating the existing imbibitional force, resulting in water swelling the seed. The germinating seeds, therefore, exhibited an increase in relative water content and moisture content, with the lapse of time. The values for the said parameters increased further in the seeds fed with 4-Cl-IAA. The auxin possibly increased the permeability of the membrane for the diffusing water (HOPKINS 1995). Moreover, hydration of the seed is also associated with the activation of the existing proteins and/or *de novo* synthesis of a specific hydrolytic enzyme (BEWLEY and BLACK 1985). The level of such proteins is determined by various hormones (HOPKINS 1995) including auxins (HIRASAWA 1989, AHMAD et al. 2001). A cumulative response to increased water content and enzyme activity speeded up the process of germination. In other crops also the seed germination was stimulated under the influence of 4-Cl-IAA (AHMAD et al. 2001a). In contrast, cadmium treatment decreased water uptake, moisture content and relative content in the germinating seeds. The seedlings of *Brassica juncea* also exhibited a similar response to cadmium treatment, even though the response was species-specific (SINGH and TEWARI 2003). The net result of the metal was expressed in the inhibition of seed germination. Moreover, in the case of *Arachis hypogea* cadmium is said to act at some additional point i.e. the function of various biomolecules of the cell in inhibiting seed germination (SATAKOPAN and RAJENDRAN 1989).

The activity of the enzyme nitrate reductase not only depends on the presence or absence of substrate (SOLOMONSON and BARBER 1990) but also on some other external and internal factors to which the germinating seeds are exposed. One such factor is the level of phytohormones, where the auxin concentration in the seeds, if it increases, elevates the activity of the enzyme (AHMAD and HAYAT 1999, AHMAD et al. 2001a, Tab. 2). This may be the consequence of the action of auxin to derepress a specific gene, to activate transcription and translation (KEY 1969, HOPKINS 1995). The use of radioactive vanadium that formed the analogue of molybdenum enzyme was proposed as a reason for the said effect of cadmium (LEE et al. 1974).

It is an established fact that phytohormones have an inherent role in derepressing specific genes to activate protein synthesis (KEY 1969). This is expressed as an increase in the activity of peroxidase in the seeds treated with 4-Cl-IAA. However, cadmium-induced activation in peroxidase activity may be a consequence of the stress generated by the metal to which the plant responds by activating its defence system, of which peroxidase is an important component (SCHUTZENDUBEL and POLLE 2002). Such stress-induced increase in peroxidase activity in *Oryza sativa* (CHEN and KAO 1995) and *Glycine max* (BALESTRASSE et al. 2001) was reported earlier. An additive effect of auxin and that of cadmium was, therefore expressed as a further increase in the activity of the enzyme which is in conformity with LI et al. (1998) who observed it in *Zea mays*, grown under water stress.

The synthesis of the proline involves the enzymes $\triangle^1$ pyrroline-5-carboxylate synthetase ($\triangle^1$P5CS) and $\triangle^1$pyrroline-5-carboxylate reductase ($\triangle^1$5CR). However, its subsequent degradation is mediated by proline dehydrogenase (ProDH). It is the proportion of the two sets of enzymes, under stress, that increases the proline content (SUMITHRA and REDDY 2004). Over-expression of the gene/s coding $\triangle^1$P5CS and repression of that responsible for ProDH in response to water stress in transgenic plants has been already noted (KISHOR et al. 1995). The seeds exposed to cadmium, in the present case, possibly experienced water stress (decreased water uptake, lower moisture content and lower relative water content) and therefore possessed more proline. Moreover, cadmium might have increased proline by itself acting on the enzyme responsible for the degradation of the protein. However, 4-Cl-IAA decreased the proline content and also neutralized the effect of cadmium by somehow suppressing the synthesis and/or activating degradation through the involvement of the related genes.

The inherent character of auxin in improving water content, protein synthesis (HOPKINS 1995) and promotion of cell division and elongation (HOPKINS 1995) favoured the process of seed germination. Moreover, the effect was so pinpointed that it also partially overcame the ill effect of cadmium on germination and related metabolic processes.

References

AHMAD, A., ANDERSON, A.S., ENGVILD, K.C. 1987: Rooting growth and ethylene evolution of pea cutting in response to chloroindole auxins. Physiol. Plant. 69, 137–140.

AHMAD, A., HAYAT, S. 1999: Response of nitrate reductase to substituted IAA in pea seedlings In SRIVASTAVA, G.C., SINGH, K., PAL, M. (Eds.), Plant physiology for sustainable agriculture, 252–259. Pointer Publisher, Jaipur, India.

AHMAD, A., HAYAT,0 S., FARIDUDDIN, Q., ALVI S. 2001a: Germination and ?-amylase activity in the grains of wheat, treated with chloroindole acetic acids. Seed Technol. 23, 88–91.

AHMAD, A., HAYAT, S., FARIDUDDIN, Q., AHMAD, I. 2001b: Photosynthetic efficiency of plants of *Brassica juncea* treated with chlorosubstituted auxins. Photosynthetica 39, 565–568.

AL-YEMENI, M.N. 2001: Effect of cadmium, mercury and lead on seed germination and early seedling growth of *Vigna ambacensis* L. Indian J. Plant Physiol. 6, 147–151.

BALESTRASSE, K.B., GARDEY, L., GALLEGO, S.M., TEMARO, M.L. 2001: Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. Aust. J. Plant Physiol. 28, 497–504.

BATES, L. S., WALDRON, R. P., TEARE, I. D. : Rapid determination of free proline for water stress studies. Plant Soil 39, 205–209.

BEWLEY, J.D., BLACK, M. 1985: Seed physiology of development and germination. Plenum Publishing Corporation, New York.

CHANCE, B., MAEHLY, A.C. 1955: Assay of catalase and peroxidases. Meth. Enzymol. 2, 764–775.

CHEN, S.L., KAO, C.N. 1995: Cadmium induced changes in proline level and peroxidase activity in roots of rice seedlings. Plant Growth Regul. 17, 67–71.

DITOPPI, L.S., GABBRIELLI, R. 1999: Response to cadmium in higher plants. Environ. Exp. Bot. 41, 105–130.

ENGVILD, K.C. 1994: The chloroindole auxins of pea, strong plant growth hormones or endogenous herbicides? PhD. Thesis, Riso National Laboratory, Denmark.

ERNSTSEN, A., SANDBERG, G. 1986: Identification of 4-chloroindole-3-acetic acid and indole-3-aldehyde in seeds of *Pinus sylvestris*. Physiol. Plant. 68, 511–518.

GOMEZ, K.A., GOMEZ, A.A. 1984: Statistical procedure for agricultural research. John Wiley and Sons, New York.

GOUIA, H., SUZUKI, A., BRULFERT, J., GHORBAL, M.H. 2003: Effect of cadmium on the co-ordination of nitrogen and carbon metabolism in bean seedlings. J. Plant Physiol. 160, 367–376.

GREGER, M., LINDBERG, S. 1987: Effect of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris*). II. Net uptake and distribution of Mg^{2+}, Ca^{2+} and Fe^{2+}/Fe^{3+}. Physiol. Plant. 69, 81–86.

HIRASAWA, E. 1989: Auxins induce α-amylase activity in pea cotyledons. Plant Physiol. 91, 484–486.

HOPKINS, W.G. 1995: Introduction to plant physiology. John Wiley and Sons, Inc. New York.

JAWORSKI, E.G. 1971: Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43, 1274–1279.

JONES, M.M., TURNER, T.C. : Osmotic adjustment in leaves of sorghum in response to water deficit. Plant Physiol. 25, 591–597.

KASTORI, R., PETROVIC, M, PETROVIC, N. 1992: Effect of excess lead, cadmium, copper and zinc on water relations in sunflower. J. Plant Nutr. 15, 2427–2439.

KEY, J.L. 1969: Hormones and nucleic acid metabolism. Ann. Rev. Plant Physiol. Mol. Biol. 20, 449–474.

KISHORE, P.B.K., HONG, Z., MIAO, G.H., HU, C.A.A., VERMA, D.P. 1995: Overexpression of $\triangle^1$-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108, 1387–1394.

LEE, K.Y., ERISKSON, R., PAN, S.S., JONES, G., MAY, F, NASON, A. 1974: Effect of tungsten and vanadium on the *in vitro* assembly of assimilatory nitrate reductase utilizing *Neurospora* mutant nit-1. J. Biol. Chem. 249, 3953–3959.

LI, L., STADEN, V., JAGER, A.K. 1998: Effect of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjected to water stress. Plant Growth Regul. 25, 81–87.

REINECKE, D.M. 1999: 4-chloroindole-3-acetic acid and plant growth. Plant Growth Regul. 27, 3–13.

SATAKOPAN, V.N., RAJENDRAN, L. 1989: Cd^{2+} interaction with RNA in germinating *Arachis hypogea*. Indian J. Plant Physiol. 32, 129–132.

SCHUTZENDUBEL, A., POLLE, A. 2002: Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53, 1351–1365.

SINGH, D.P., SINGH, S.P. 1987: Action of heavy metals on hill activity and O_2 evolution *Anacystis nidulans*. Plant Physiol. 83, 12–14.

SINGH, P.K., TEWARI, R.K. 2003: Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. J. Environ. Biol. 24, 107–112.

SOLOMONSON, L.P., BARBER, M.J. 1990: Assimilatory nitrate reductase. Functional properties and regulation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 41, 225–253.

SUMITHRA, K., REDDY, A.R. 2004: Changes in proline metabolism of cowpea seedlings under water deficit. J. Plant Biol. 31, 201–204.

TU, S.I., BROVILLETTE, J.N. 1987: Metal ion inhibition of cotton root plasma membrane ATPase. Phytochemistry 26, 65–69.

WAGNER, G.J. 1993: Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron., 51, 173–212.

WILKINS, D.A. 1978: The measurement of tolerance to edaphic factors by means of root growth. New Phytol. 80, 623–633.